Review

Cancer Research

The Emerging Role of Immunosurveillance in Dictating Metastatic Spread in Breast Cancer

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Abstract

It is now well known that the immune system can recognize transformed cells and control the initiation and growth of some cancers, a process termed tumor immunosurveillance. Key regulators of this process have been described in the primary tumor setting, where the balance of protumor and antitumor responses dictates tumor initiation and progression. Accumulating evidence suggests that immunosurveillance may also be critical for regulating metastatic spread, the most fatal aspect of cancer, and that mechanisms of overcoming immune control may be quite different from those at the primary site. Our recent findings support loss of type I interferon (IFN) signaling as a tumor-cell intrinsic mechanism of evading metastasis-specific immune responses in breast cancer. We revealed that type I IFN-induced innate (natural killer) and adaptive ($CD8^+$ T cell) responses suppressed bone metastatic growth and this was associated with decreased accumulation of immune suppressor cells (myeloid-derived suppressor cells). This review summarizes recent findings that are in support of tumor-induced immunosurveillance in regulating metastatic spread, including evidence that immune regulation of primary tumors may be distinct from those dictating metastasis. *Cancer Res; 73(19); 5852–7. ©2013 AACR.*

Introduction

Metastasis is the major cause of mortality in patients with breast cancer. Breast cancer cell invasion into the circulation and dissemination to distant tissues such as the bone marrow can occur very early, often before primary tumor diagnosis. The most inefficient step in the metastatic cascade is considered to be the outgrowth of disseminated cells or indolent micrometastases into overt metastases (1, 2). Metastatic outgrowth of tumor cells lying dormant in organs such as the bone can occur up to 20 years after initial cancer diagnosis. It is this outgrowth from dormancy, occurring in less than 20% of patients with breast cancer, that invariably leads to death. Unfortunately, without knowledge of the characteristics of disseminated cells that give rise to metastases or the mechanisms driving outgrowth from dormancy, it is difficult to predict metastatic relapse or prevent it therapeutically.

There is strong evidence that in some cancers, immunosurveillance plays an integral role in tumor initiation, growth, and response to conventional therapeutics (1–4). In models of primary tumor dormancy, it has been shown that tumor cells are held in check by the immune system and that evasion of antitumor immunity is required for primary tumor establishment (3). Considering tumor cell dormancy is a rate-limiting step in the metastatic cascade, it is feasible that overcoming immunosurveillance is a requirement for outgrowth of disseminated cells into overt metastases. This has, however, proven difficult to confirm, as immunocompetent models that mimic the clinical presentation of breast cancer dormancy are very limited. Our recent findings in an aggressive immunocompetent model of metastasis support the existence of metastasis-specific immunosurveillance in breast cancer. We have shown that cross-talk between tumor cells and immune cells, via release of type I IFNs, dictates metastasis to bone (5). Our data suggest that the balance between adaptive and innate antitumor immune response and the accumulation of protumor immune suppressor cells is critical in regulating metastasis. In this review, we will focus on the accumulating evidence supporting a role for immunosurveillance in regulating breast cancer metastasis (summarized in Fig. 1) and emphasize the necessity for further investigations aimed at dissecting the key mechanisms at play.

Cytotoxic T lymphocytes

As stated above, previous work has revealed that immunosurveillance retains tumors in a dormant state (3). This study highlighted the critical function of the adaptive immune response in holding tumor cells in check, as depletion of T cells allowed tumor cells to escape an equilibrium state in the immunogenic methylcholanthrene (MCA)-induced model of sarcoma. However, this work focused on the development of primary tumors and not on metastasis. Our findings support $CD8^+$ T cells as crucial components of metastasis-specific immunosurveillance in breast cancer. In our study using the aggressive 4T1.2 syngeneic mouse model, enforced tumor cell expression of IFN regulatory factor 7 (Irf7) suppressed metastasis to bone via type I IFN signaling. The suppression of

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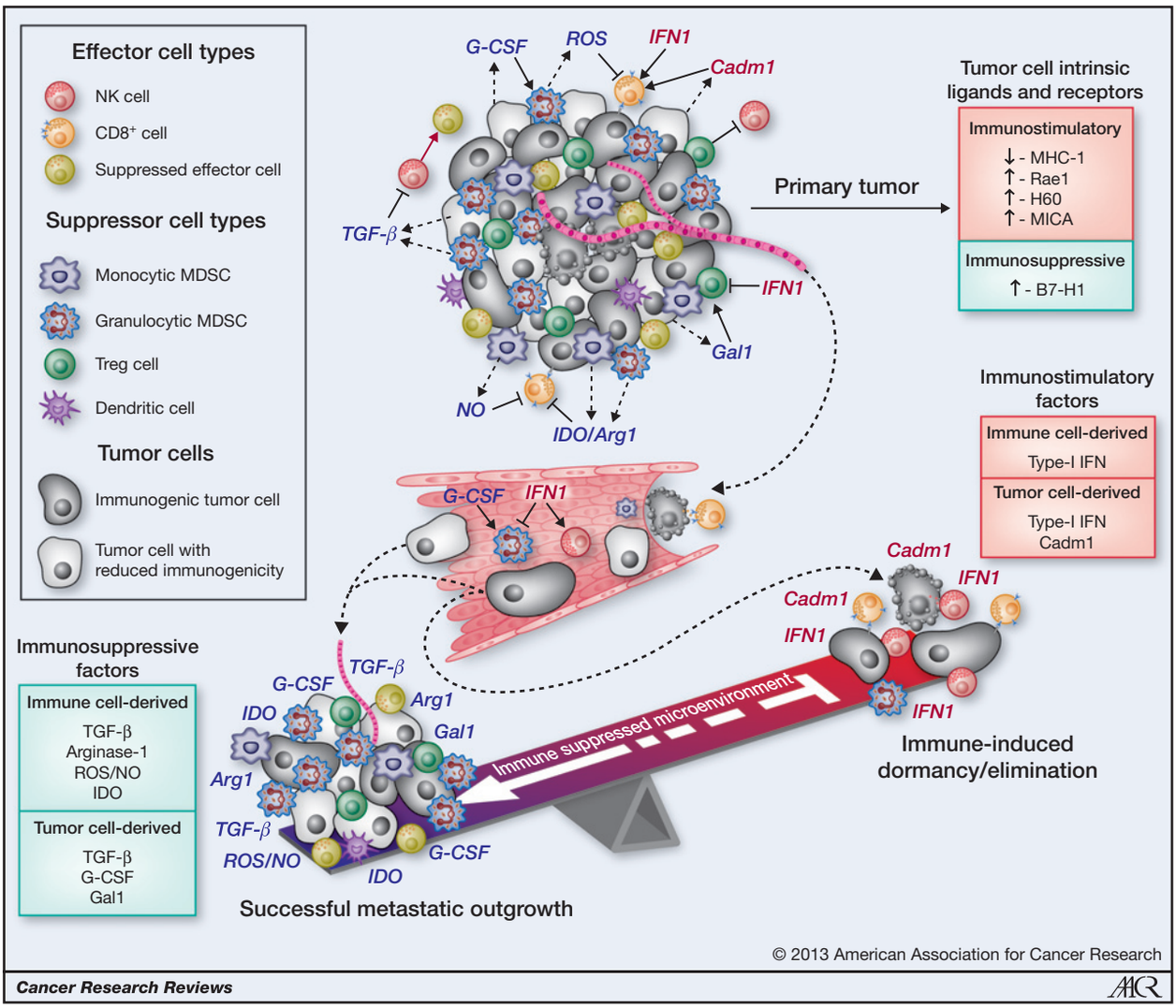

Figure 1. A balancing act between immunosuppression and activation dictates metastatic outgrowth. Upon invasion into the circulation, tumor cells need to escape immune elimination to thrive in distant tissues. Successful survival and outgrowth of tumor cells is governed by the balance of immune cells resident in the metastatic site. The production of immunostimulatory factors [type I IFNs (IFN1), Cadm1] and cell-surface ligands (Rae1, H60, and MICA) promote immune recognition of tumor cells, leading to immune-induced dormancy or elimination. In contrast, reduced tumor cell immunogenicity (such as reduced IFN1 secretion), direct suppression of T-cell function (via expression of the PD-1 ligand, B7-H1), or increased accumulation of suppressor cell types through various factors [TGF-β, G-CSF, and Galectin 1 (Gal1)] tips the balance in favor of an immunosuppressed microenvironment that promotes metastatic outgrowth. The recruitment of immunosuppressive cells blunts antitumor immune responses through various mechanisms [arginase-1 (Arg1), ROS/NO, IDO].

metastasis was at least partially reliant on $CD8^+$ T cells, as depletion of $CD8^+$ T cells together with natural killer (NK) cells restored bone metastasis and shortened metastasis-free survival, without impacting primary tumor growth (5).

$CD8^+$ cytotoxic T lymphocytes (CTL) kill target cells via recognition of peptides presented on MHC class I (MHCI) molecules and are seen as critical mediators of the antitumor immune response (6). Detection of tumor cells by CTLs can occur via recognition of tumor antigens that are aberrantly expressed or altered as a result of genetic mutation or post-translational modification (7, 8). Tumor antigens have been identified in various tumors, including breast cancer and melanoma, and have been reviewed elsewhere (7, 8). Breast cancer cells have also been shown to directly evade antitumor T cells by expressing the programmed death-1 (PD-1) ligand, B7-H1, on their cell surface causing T-cell anergy and hence, blocking T-cell responses (9). It remains unclear whether these tumor antigens and T-cell suppressive factors actually predict and regulate metastatic outgrowth of breast tumor cells.

Indeed, studies into the role of $CD8^+$ T cells in regulating breast cancer metastasis are limited. A recent report has shown that tumor cell expression of the cell surface protein Cadm1 suppresses lung metastasis without impacting primary growth and that metastasis suppression is reliant on $CD8^+$ T-cell function (10). However, it is yet to be determined whether $CD8^+$ T-cell immunosurveillance is important in Cadm1-

negative breast tumors. There is no doubt that more studies are required to prove a role of tumor antigen-specific CTL immune response in regulating metastasis. However, these studies may prove difficult, considering the current lack of clearly defined breast cancer antigens and the likely heterogeneity within and across tumor subtypes and metastases.

The evidence for a role of CTLs in restraining metastases extends beyond mouse models. In humans, tumor-infiltrating CTLs predict favorable prognosis in various cancers, including breast cancer (6, 11) and melanoma (12). However, the prognostic benefit of CTLs is not always observed across breast cancer subtypes. For example, prechemotherapy tumor-infiltrating CTLs predict favorable outcome in basal-like breast cancers but not in other breast cancer subtypes (13, 14). Without knowledge of the phenotype and antigen specificity of CTLs and whether their accumulation is an accurate measurement of activation, it may be difficult to predict prognosis across all subtypes. Another value of measuring lymphocytic infiltrates is predicting response to chemotherapy. Primary tumor lymphocytic infiltration predicts increased chemotherapeutic response in HER2-positive and estrogen receptor–negative patients (14, 15). Overall, this evidence suggests that in some cancer subtypes, CTLs may play an important role in targeting breast cancer cells in the primary tumor or during dissemination. Dissecting the stage and site-specific roles of CTLs in metastasis is required to further evaluate their importance in metastatic outgrowth

Natural killer cells

Along with a role for adaptive immunity in the 4T1.2 model, our studies revealed that innate immunity, particularly the NK cell response, is important in restraining breast cancer metastasis. The depletion of NK cells together with $CD8^+$ T cells greatly accelerated metastasis to multiple organs in mice bearing 4T1.2 tumors (5). Along with a functional role for NK cells in metastasis, we also observed that IFN-induced metastasis suppression was associated with increased accumulation of NK cells in the blood and bone marrow.

Among the many cell types of the innate immune system, NK cells are one of the earliest cell types arriving at an inflamed site where they sense cell-surface stress signals such as retinoic acid early-inducible protein 1 (RAE1) and H60 in mice or MHCI polypeptide–related sequence A (MICA) and B in humans (reviewed in ref. 16). NK cells express a range of inhibitory receptors (such as human killer cell immunoglobulin-like receptors and mouse lectin-like LY49 dimers that recognize MHCI) and activating receptors (such as NKG2D that recognize the stress-inducible proteins such as RAE1, MICA, and MICB). The balance of signaling through activating and inhibitory receptors is particularly important in the antitumor response where a downregulation of MHCI and an increase in stress-induced ligands is commonly observed on tumor cells (16, 17). NK cells have been shown to kill a broad range of mouse and human tumor cells *in vitro* and there is evidence that NK cells can eliminate transplantable and spontaneous mouse tumors *in vivo* (16). NK cells also interact with multiple other cells and are likely to have indirect roles in tumor metastasis through stimulation or killing of other immune cells such as CTLs, dendritic cells, macrophages, and regulatory T cells (Treg). Thus, NK cells are recognized as a key element in the antitumor immune response.

In support of our findings on the role of NK cells in metastasis, other studies have also shown that a deficiency in NK cells accelerates the establishment of metastases. For example, 4T1 tumors metastasize to lung more aggressively after NK cell depletion in SCID mice, which are deficient in adaptive immunity (18, 19). Consistent with this, our findings showed that metastasis is accelerated in NOD/SCID/IL-2r$\gamma^{-/-}$ mice that lack adaptive immunity and have defective NK cell function. Interestingly, in these studies, the deficiency of NK cell function did not impact primary/orthotopic tumor growth (5, 18, 19). Furthermore, support of a metastasis-specific suppressive role of NK cells comes from recent studies showing that surgery-induced NK cell suppression or direct NK cell depletion accelerates metastasis in immunocompetent mouse models (20, 21).

Perhaps the most convincing evidence to date for a role of innate immunosurveillance in metastatic breast cancer comes from a recent study where NK cells were measured and characterized in the peripheral blood and primary tumors of patients with breast cancer. In patients with advanced breast cancer, NK cells from the peripheral blood were shown to have impaired functionality and this was even more prominent in tumor-infiltrating NK cells (22). Interestingly, the altered NK cell function and phenotype were believed to be induced by the tumor microenvironment, as stromal and/or tumor-derived factors including TGF-β were responsible for the reduction of NK cell function (22). Future work that compares the function and characteristics of NK cells in different metastatic organs are important as NK cells usually show tissue-specific phenotypes (23) that may impact cancer cell destruction in a particular microenvironment.

Immune suppressor cells

Tumor cells not only evade immune recognition via reduced immunogenicity, they also recruit immune suppressor cells to the tumor microenvironment. Accumulating evidence suggests that myeloid-derived suppressor cells (MDSC) and Tregs play essential roles in immunosuppression and tumor progression.

MDSCs are a heterogeneous population of bone marrow-derived myeloid cells, including immature macrophages, monocytes, neutrophils, and dendritic cells, which accumulate in a range of tumor models and in patients with cancer. Because of their function as potent inhibitors of T and NK cell responses, MDSCs are considered critical in assisting tumors to escape immune recognition (24) and creating an immunosuppressive microenvironment (20). MDSC recruitment and expansion is promoted when tumor and stromal cells release a variety of cytokines and other soluble factors including matrix metalloproteinases (MMP), granulocyte colony-stimulating factor (G-CSF), VEGF, and TGF-β (25).

In mice, MDSCs are categorized into two major subpopulations, monocytic [$Ly6G^-/Ly6C^{hi}(Gr1^+)CD11b^+$] and granulocytic [$Ly6G^+(Gr1^+)CD11b^+$] MDSCs (24, 26). These subtypes are functionally different and use distinct mechanisms to

suppress immune responses. Monocytic MDSCs preferentially suppress T-cell responses via upregulation of inducible nitric oxide synthase and increased nitric oxide production, whereas granulocytic MDSCs function via an increased production of reactive oxygen species. Both subsets also increase production of arginase-1 that depletes L-arginine required for T-cell proliferation (24, 26).

Our recent work and work by others in the 4T1 model (5, 26) have shown that the accumulation of granulocytic MDSCs in tumor-bearing mice correlates with metastatic progression and potential. Indeed, granulocytic MDSCs derived from 4T1 tumor-bearing mice are potent suppressors of T-cell proliferation *in vitro* (5), suggesting an important role of this population in suppressing antimetastatic immune responses. In our study, decreased metastasis by restored type I IFN signaling was associated with decreased accumulation of granulocytic MDSCs in the blood and bone marrow (5). This was in line with previous reports that type I IFN-induced tumor suppression is associated with decreased MDSCs (27). Thus, our research supports a prometastatic and immunosuppressive role of MDSCs in breast cancer metastasis. However, an immune-specific functional role of MDSCs in promoting metastasis *in vivo* remains to be tested.

Studies directly proving a prometastatic role of MDSCs via immunosuppression are lacking and some studies using the 4T1 model suggest that metastasis-specific functional roles of MDSCs are not limited to immunosuppression. For example, Yang and colleagues reported that MDSCs recruited to the tumor invasive front enhance tumor cell invasion via MMP secretion (28). In this study, coinjection of MDSCs with 4T1 cells greatly increased lung metastasis (28). MDSCs have also been implicated in creating a favorable environment in the lungs to support the impending lodgment and growth of tumor cells (a "premetastatic niche;" ref. 29). It is reported that MDSCs can infiltrate into the lungs before tumor cell arrival and promote metastasis by decreasing the local antitumor immune response (30) and by increasing MMP9 expression to promote vascular remodeling/angiogenesis (31). Combined, these studies support MDSCs as metastasis promoters.

The clinical relevance of these findings has been validated in patients with breast cancer. Circulating MDSCs correlate with clinical cancer stage, with an increase in patients with cancer over healthy individuals and the highest abundance in stage IV patients with metastatic disease (32). In addition, MDSCs isolated from breast cancer tissues have been shown to suppress T-cell response via indoleamine 2,3-dioxygenase (IDO; ref. 33), consistent with findings in mouse models (34). In fact, recent data also support a role for MDSCs in promoting metastasis in other cancers, such as melanoma (35). Together, enhanced numbers of MDSCs are detected in mouse models and patients with advanced breast cancer, and future investigations are required to determine if the accumulation of these cells is associated with a clear prometastatic function.

The other immunosuppressive population associated with tumor progression are Tregs. Tregs are defined as T cells that have a suppressor function (36). Since their discovery in the early 1970s as "suppressive T cells," several phenotypically distinct Tregs have been suggested and reviewed elsewhere (36). Tregs have been recognized to play an essential role in sustaining self-tolerance against autoimmunity and immune homeostasis by suppressing a wide variety of immune responses via multiple mechanisms, including cell–cell contact and soluble factors such as TGF-β and interleukin-10 (36). The importance of Tregs in tumor immunity has been shown using various mouse models. The depletion of Tregs using CD25-specific antibodies suppressed growth of various solid tumors, such as melanoma, colorectal tumor, and MCA-induced fibrosarcoma. In agreement with this, adoptive transfer of Tregs in mouse models greatly reduced tumor immunity. Together, these data indicate that Treg-mediated immunosuppression is important in immune evasion (36). However, most of these studies only address the role of Tregs in the development of the primary tumors.

Although we did not measure Tregs in our study, it has been documented that IFN-α inhibits Treg proliferation and suppressive function (37). Thus, it is feasible that Treg inhibition was associated with type I IFN-induced metastasis suppression. This is supported by other studies in the 4T1 model that have shown establishment of lung metastases requires Treg-mediated NK cell inhibition (18), and depletion of Tregs suppresses lung metastasis (38). In addition, tumor cell expression of Galectin-1 is associated with increased Treg frequency and function and promoting lung metastasis in the 4T1 model (39). As with MDSCs, Tregs also have proinvasive roles that are independent of their immunosuppressive functions. For example, it has been shown that Tregs promote lung metastasis of RANK-expressing mammary tumors via the production of receptor activator of nuclear factor-κB ligand (40).

Tregs have been considered particularly important in human breast cancer immunopathogenesis. In patients with breast cancer, the prevalence of Tregs in the peripheral blood is highly compared with healthy individuals (41). It has also been reported that tumor-infiltrating Treg numbers are significantly higher in breast cancer tissues than in normal breast and that an enhanced infiltrate correlates with poor prognosis (42). A correlation of Treg numbers and poor prognosis has been reported in other cancers. For example, in patients with metastatic melanoma, increased Treg recruitment to lymph node metastases correlates with shorter survival (43) and depletion of Tregs allows the expansion of tumor-specific CTLs and causes metastasis regression (44). Altogether, accumulating evidence suggest Tregs are important in prometastatic immunity in both mouse models and human disease.

Future directions

Mechanisms of metastasis, such as tumor invasion and angiogenesis, differ in primary and metastatic microenvironments. Our recent findings, along with some studies covered in this review, provide enticing evidence that immune recognition and control of metastasis may also differ from that of the primary tumor. To validate immunosurveillance as a critical event in metastatic outgrowth in breast cancer, studies need to be extended to multiple metastasis models to accelerate the identification and validation of key immune regulators and target cells in metastasis. Given the heterogeneity of breast cancer, it will be important to determine if such regulators have

breast cancer subtype and metastatic site-specific roles. Aside from the effector and suppressor immune cells and molecules we have discussed in this review, emergent evidence has suggested the involvement of other immune cells and molecules in immune regulation of metastasis, such as tumor-associated macrophages (24, 45), $CD4^+$ T cells (45), NKT cells (38), and γδT cells (46). Future studies are required that investigate the interaction and signaling between tumor cells and these immune cell lineages, and how such interactions regulate site-specific metastasis.

Here, we summarized the accumulating evidence that suggests evasion of immunosurveillance is a requirement for metastasis in human disease. It is now imperative to validate the prognostic benefit of measuring cytotoxic and suppressive immune cells in patient samples. Identification of tumor-derived factors (such as type I IFNs) that regulate metastasis-specific immune responses is critical for development of biomarkers in primary tumors that predict patient metastatic relapse. Considering there are limited effective therapies for preventing and/or treating metastatic breast cancer, the identification of key steps in the metastatic cascade where immune surveillance is critical may aid the development of novel immunomodulatory therapeutic approaches.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: C.Y. Slaney, J. Rautela, B.S. Parker
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): B.S. Parker

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