Tumor-Infiltrating Immune Cells Promoting Tumor Invasion and Metastasis: Existing Theories

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Received: 2012.11.04; Accepted: 2012.12.20; Published: 2013.01.05

Abstract

It is a commonly held belief that infiltration of immune cells into tumor tissues and direct physical contact between tumor cells and infiltrated immune cells is associated with physical destructions of the tumor cells, reduction of the tumor burden, and improved clinical prognosis. An increasing number of studies, however, have suggested that aberrant infiltration of immune cells into tumor or normal tissues may promote tumor progression, invasion, and metastasis. Neither the primary reason for these contradictory observations, nor the mechanism for the reported diverse impact of tumor-infiltrating immune cells has been elucidated, making it difficult to judge the clinical implications of infiltration of immune cells within tumor tissues. This mini-review presents several existing hypotheses and models that favor the promoting impact of tumor-infiltrating immune cells on tumor invasion and metastasis, and also analyzes their strength and weakness.

Key words: immune cell, tumor progression, invasion, metastasis

Background

Cancer has been detected in all epithelium-derived tissues of the human body. Carcinogenesis in different organs is believed to share a similar process, i.e, sequential progression from normal to hyperplasia, to in situ, and then, to invasive or metastatic cancer [1-4]. A vast majority of in situ cancer can be cured by surgical resection alone, while invasive and metastatic cancer accounts for over 90% of can-
cer-related mortality [5-8]. The significant difference in clinical prognosis between in situ and invasive or metastatic cancer results predominantly from the presence or absence of the surrounding basement membrane (BM). All normal or pre-invasive tumor epithelia are normally devoid of lymphatic ducts and blood vessels and are also physically segregated from vascular structures within the stroma by the BM. The BM consists of mainly type IV collagen, laminins, and other molecules that form a continuous sheet (more commonly called the tumor capsule), surrounding the epithelial cells [9-12]. In human breast, prostate, and major salivary glands, the capsule is further reinforced by a single layer of elongated cells, which are named “myoepithelial” cells in the breast and salivary glands, and “basal cells” in the prostate. The basal or myoepithelial cell layer lies between the epithelial cells and the BM. In the gastrointestinal tract, the normal mucosa and in situ cancer are further separated from the submucosa by the muscularis mucosa, a dense band comprised of two layers of smooth muscle cells [13]. Due to these structural relationships, the disruption of the tumor capsule and its associated physical barriers is an absolute pre-requisite for tumor cell invasion or metastasis.

It is a commonly held belief that progression from in situ to invasive or metastatic cancer is caused by proteolytic enzymes produced by tumor cells that increase linearly in concentration with tumor progression, reaching their highest level at the in situ cancer stage. It has been proposed that these proteolytic enzymes cause degradation or disruption of the tumor capsule and allow the in situ cancer cells to migrate into the adjacent stroma or to disseminate to distant organs [14-17]. The above model of tumor invasion and metastasis is consistent with results obtained from tissue culture and animal model studies [18-20]; however, it is hard to reconcile with a number of well-established observations: (1) although a vast majority of tumor cells express high levels of proteolytic enzymes, only 10-30% of untreated in situ cancers progress to invasive or metastatic cancer [21-25]; (2) the outcomes of world-wide clinical trials with proteolytic enzyme-targeted inhibitors have yielded very disappointing results [26,27]; (3) prostate tissues from many cancer-free men harbor a DNA phenotype identical to that of invasive prostate cancer [28,29]; and (4) cancer of unknown primary site is one of the ten most frequent cancers and the 4th highest cause of cancer-related mortality, despite the lack of an identifiable primary tumor to serve as a source of metastatic tumor cells [30]. Together, these facts argue that alternative pathways may exist for tumor progression and subsequent invasion or metastasis.

Existing hypotheses of tumor infiltrating immune cells promoting tumor invasion and metastasis

A great number of studies have shown that infiltration of the immune cells into tumor tissues and direct physical contact between infiltrating immune cells and tumor cells are associated with the physical destruction of tumor cells, reduction of the tumor burden, and an improved clinical prognosis [31-36]. On the other hand, a significant and steadily increasing number of studies have shown that increased infiltration of immune cells may promote tumor progression and invasion. For example, several studies have documented that stage- and histopathologically-matched pre-invasive prostate and esophageal tumors with increased immune cell infiltration have a significantly higher frequency of subsequent progression to invasive cancer than their counterparts without aberrant immune cell infiltration [37-39]. Unfortunately, the primary reasons for these contradictory observations remain elusive, making it difficult to judge the clinical implications of the infiltration of immune cells within tumor tissues. To address these issues, numerous studies [40-47] have been conducted, and a number of hypotheses [48-55] have been presented to explore the primary impact of tumor infiltrating immune cells on associated tumor tissues. Again, the outcomes of these studies are highly contradictory and the primary impact of infiltrating immune cells on associated tumors remains elusive [56-57]. In this mini-review, we present several existing hypotheses that favor the promoting impact of tumor-infiltrating immune cells on tumor invasion and metastasis and analyze their strengths and weakness. These specific hypotheses were selected for a number of reasons, including (1) they directly address the impact of tumor infiltrating immune cells on tumor cell behavior, and (2) they appear to be applicable to multiple, or perhaps all, epithelial-derived tumors.

I. Tumor-educated macrophages (paracrine loop signaling)

The main concept of this hypothesis was introduced in 2004 [58] and was based on findings of a chemotaxis-based in vivo invasion assay and multiphoton-based intravital imaging on transgenic mice. The study shows that interactions between breast tumor cells and macrophages facilitates the migration of cancer cells into the primary tumor and also that tumor cell intravasation occurs in association with perivascular macrophages [59,60]. According to this hypothesis, “macrophages are recruited to the inva-
sive front by expression of tumor-derived chemotactic factors and in response to the disruption of the basement membrane. At this invasive site, macrophages enhance tumor cell migration and invasion through their secretion of chemotactic and chemokinetic factors including epidermal growth factor (EGF). They promote angiogenesis by the synthesis of angiogenic factors including vascular endothelial growth factor (VEGF), and they remodel the extracellular matrix and in particular, regulate collagen fibrillogenesis. A combination of these factors provides a triple-whammy, as the more mobile and invasive tumor cells track along collagen fibers that are also anchored to blood vessels, which are fabricated at sites of invasion and through which macrophages potentiate tumor cell intravasation” [61].

The main strength of this hypothesis is that the assay system used in these studies allows direct visualization of macrophage-assisted tumor cell migration and intravasation in mammary tumors. The primary weakness of this hypothesis is that it may not truly reflect the intrinsic events in humans for four important reasons. (1). Previous studies have shown that human macrophages are significantly different from mouse macrophages not only in their relative ratio to other immune cell types, but also in their use of arginine for production of nitric oxide (NO), which is the most important component of the macrophage arsenal against intracellular pathogens [62-69]. The mouse macrophages produce large amounts of NO and L-citrulline from L-arginine via induction of the inducible form of NOS (iNOS), and also synthesize the obligatory cofactor tetrahydrobiopterin (BH4), essential for stabilization and function of the iNOS enzyme protein [63,64]. The human macrophages, however, do not have NO activity nor do they synthesize BH4 [65-69]. (2). The human immune system is also fundamentally distinct from the mouse immune system in development, activation, and response to challenge, in both innate and adaptive immunity [70-73]. The human peripheral blood is neutrophil rich (50-70% neutrophils, 30-50% lymphocytes), whereas the mouse peripheral blood is predominantly lymphocytes (75-90% lymphocytes, 10-25% neutrophils) [70]. The putative human hematopoietic stem cells (HSC) express predominantly c-kit[low], flt-3+, whereas the mouse HSC express predominantly c-kit[high], flt-3− [71]. Similarly, the human neutrophils are a rich source of leukocyte defensins, whereas defensins are not expressed by neutrophils in mice [72-74]. (3). It has been well documented that the primary function of the human macrophages is to remove cell debris and infiltrated microorganisms after tissue injury [75-79]. It has also been documented that aberrant accumulation of macrophages often has destructive impact on their associated tissues [80]. (4). In the adult organs, only stem cells retain the potential for unlimited proliferation and multi-lineage differentiation, and, consequently, stem cells have been considered as the primary source for invasive and metastatic lesions [81-85]. The paracrine loop signaling hypothesis, however, has failed to address the role of tumor stem cells in invasion or metastasis. Collectively, these weaknesses make it difficult to determine whether, or to what extent, this hypothesis truly reflects the intrinsic events of human tumor invasion or metastasis.

2. Immune cell-based mediation

This hypothesis is based on studies using the polyoma-middle-T-antigen (PyMT) transgenic mouse model of mammary carcinogenesis [86]. According to this hypothesis, “IL-4-expressing CD4+ T lymphocytes indirectly promote invasion and subsequent metastasis of mammary adenocarcinomas by directly regulating the phenotype and effector function of tumor-associated CD11b+Gr1 F4/80+ macrophages that in turn enhance metastasis through activation of epidermal growth factor receptor signaling in malignant mammary epithelial cells”. More recently, the same concept and similar pathways, have been extended to lymphocytes and their subtypes [87-90]. This includes the recruitment of macrophages through expression of colony-stimulating factor 1 (CSF1) by mouse mammary epithelium [90].

The main strength of this hypothesis is that it presents a broader view of the potential impact of interactions among different immune cell types on tumor progression. The primary weakness of this hypothesis is that it is based on studies using mouse models and thus raises the same issues with applicability to human tumor tissues as discussed above. In addition, the model focuses predominately on pathways that facilitate late-stage promotion of tumor progression to metastasis.

3. Cancer cell-leukocyte fusion

This hypothesis is the extension of a century-old theory introduced by a German pathologist, Aichel O, in 1911 [91]. Based on this hypothesis, macrophages ingest tumor cells leading to the fusion of genetic materials from the two cell types, resulting in the creation of a hybrid phenotype that exhibits chemotactic migration in vitro toward fibronectin and shows high frequencies of metastasis when implanted in mice [92-97].

The main strength of this hypothesis is its relevance to human carcinogenesis as both cancer cell- and macrophage-specific molecules are detectable in a
subset of human clinical tumor samples [92-97]. The mechanism upon which this hypothesis is based, however, is not likely to represent a major route of metastatic cancer for a simple reason: if the fusion of macrophages and cancer cells is indeed the precursor of metastatic cancer, all metastatic cancer cells from different organs and histological origins should share the same or similar morphology, specifically a giant cell population with polyplody. However, it is well established that a majority of metastatic cancer cells are morphological and immunohistochemically similar to their primary tumors, which are highly heterogeneous in morphology.

4. Regulatory T cells (Treg) induced immune suppression

This hypothesis is based on both clinical observations of ovarian cancer patients and laboratory findings from studies of the syngeneic ID8 ovarian cancer model [98,99]. According to this hypothesis, “recruitment of Treg cells to the tumor supports disease progression through a dual mechanism: (1) the canonical subversion of antitumor immunity, and (2) through the establishment of a proangiogenic reprogramming of the tumor microenvironment”. The authors of this hypothesis believe that the recruitment of regulatory T cells to the tumors are mediated primarily through the CCL28-CCR10 interaction [99].

The main strength of this hypothesis is that it is based on both clinical and laboratory data and that Treg-cell induced immune suppression has been well documented in multiple types of human cancer and diseases [100-104]. The main weakness of the hypothesis is that CCL28 is not a widely distributed molecule; consequently, the applicability of this hypothesis to other tumor types has yet to be established. In addition, this hypothesis fails to address the impact of immune cells on tumor stem cells, which are now believed to serve as the “seeds” for both invasive, metastatic, and recurrent cancer.

5. Monocyte-mediated protection against natural killer cell lysis of cancer stem cells

This recently introduced hypothesis is based on the findings that “increased NK cell cytotoxicity was seen when they were cultured with primary oral squamous carcinoma stem cells (OSCSCs) and Glioblastoma (GBM) stem cells and not with their more differentiated counterparts. In addition, human embryonic stem cells (hESCs), human mesenchymal Stem Cells (hMSCs), and human dental pulp stem cells (hDPSCs) and human induced pluripotent stem cells (hiPSCs) were significantly more susceptible to NK cell-mediated cytotoxicity than their differentiated counterparts or parental cells from which they were derived, suggesting that NK cells were preferentially targeting and lysing stem cells and not their differentiated counterparts. It was also found that inhibition of differentiation or reversion of cells to a less-differentiated phenotype by blocking NF-kB or targeted knockdown of COX2, significantly augmented NK cell functions”. In addition, it was also found that “total population of monocytes and those depleted of CD16+ subsets were able to substantially prevent NK cell-mediated lysis of OSCSCs, MSCs and DPSCs” [105]. Furthermore, it was suggested that NK cells played a significant role in differentiation of the cells by providing critical signals via secreted cytokines as well as direct cell-cell contact after the induction of split anergy which conditioned NK cells to lose cytotoxicity and gain the ability to secrete cytokines. To be conditioned to drive differentiation, NK cells had to first receive signals through their key surface receptors either from healthy stem cells or those which had been transformed. In addition, NK cells by targeting other inflammatory cells or fibroblasts in the tumor microenvironment may become conditioned to lose cytotoxicity and gain cytokine producing phenotype before they can aid in differentiation of stem cells. These alterations in NK cell effector function could ultimately aid in driving differentiation of a population of surviving healthy as well as transformed stem cells. In cancer patients since the majority of NK cells have lost cytotoxic activity, they may eventually contribute rather than halt the progression of cancer by allowing the growth and expansion of the pool of cancer stem cells.

The main strength of this hypothesis is that it provides a general mechanism how immune cells may behave in inflammatory microenvironment for the ultimate goal of tissue regeneration and the resolution of inflammation. More importantly, this hypothesis provides a novel concept and approach to study the link between stem cells and carcinogenesis and cancer progression. Indeed, many correlates to this hypothesis has been found in the clinical setting, such as substantially decreased levels of cytotoxicity in NK cells, or increased modulation of NK cell surface antigens in cancer patients [106-114]. The weakness of this hypothesis may be that it is primarily based on findings from human cell cultures, and animal studies, and may not have taken the structural features of certain types of tumors into the consideration: the stem cell population in normal or pre-invasive cancer tissues are normally segregated from the immune cells by a dense fibrous epithelial capsule in certain tumors [9-12]. However, as noted below once the tumor capsule is disrupted conditioned NK cells may get access
to drive differentiation of stem cells. On the other hand, unconditioned, cytotoxic NK cells should aid in the elimination of cancer stem cells [115-118]. Disruption of the extracellular matrix components in the tumor capsule may also be achieved via increased enzymatic digestion since conditioned NK cells and regulatory T cells are likely to secrete increased levels of degrading enzymes such as MMPs. Indeed, the presence of intraepithelial lymphocytes in the healthy and diseased gut mucosa is a good indication that CD8+ lymphocytes have access to both normal and transformed epithelial microenvironment and that their function may be important in the differentiation and maintenance of tissue integrity and repair. The events described in these studies share many common features with those described below and as such they may be complementary to our in vivo morphological observations in humans.

6. Aberrant lymphocyte infiltration-induced focal capsule disruptions

Our research group has attempted to identify more effective approaches to assess the intrinsic impact of tumor-infiltrating immune cells on tumor invasion and metastasis. In our recent studies, we compared the frequency of lymphocyte infiltration in stage- and morphology-matched human breast and prostate tumors with and without focally disrupted capsules. Of 191 breast tumor nests with focally disrupted capsules, 186 (97%) had distinct lymphocyte infiltration, compared to 46 (22%) in 207 morphologically similar counterparts with non-disrupted capsules [119]. Similarly, distinct lymphocyte infiltration was seen in 183 (91%) of 201 prostate tumor nests with focally disrupted capsules, compared to 67 (33%) in 201 morphologically similar counterparts with non-disrupted capsules [120]. Subsequent studies revealed that residual cells within focally disrupted myoepithelial or basal cell layers had a significantly lower expression of tumor suppressor and cell proliferation-related proteins, but showed a significantly higher frequency of degeneration-related changes than their morphologically similar counterparts located in regions devoid of focal disruptions [121-123]. Using double immunohistochemistry to simultaneously elucidate the BM and associated basal cell layers, all the BM-overlying focally-disrupted basal cell layers demonstrated either the presence of correlated disruptions (76 of 89, 85%) or significant attenuation of immunostaining intensity (13 of 89, 15%) [124]. Correlated BM and myoepithelial cell layer alterations were also seen in human breast tissues. In contrast to the basal or myoepithelial cells, tumor cells overlying focally disrupted capsules had a significantly higher level of proliferation and greater expression of tumor stem cell- and growth factor-related genes than their adjacent counterparts distant from the disruptions [125-131].

These findings suggest that focal basal or myoepithelial cell degeneration, aberrant lymphocyte infiltration, and capsule disruptions are likely to be correlated events that contribute to tumor progression and invasion. These findings have led to a novel hypothesis that tumor invasion or metastasis is triggered by focal capsule degeneration-induced lymphocyte infiltration that causes physical disruptions within the capsule, which selectively favors proliferation and dissemination of overlying tumor stem cells [121,122]. Based on this hypothesis, aberrant lymphocyte infiltration promotes capsule disruptions and tumor invasion through the following pathways. (1). Myoepithelial and basal cells belong to a self-renewal population that must constantly undergo both proliferation and differentiation to replace aged or injured basal cells [132-134]. Both internal and external insults, such as a predisposition of genetic defects, exposure to chemicals or radiation, and chronic inflammation, may through chronic or acute mechanisms, damage the normal stem cells in these layers, resulting in a “senescent” cell population that is prone to degeneration. (2). Degradation products of degenerated basal and myoepithelial cells, or diffusible molecules from the overlying epithelial cells, can function as self-epitopes to attract the trafficking and infiltration of immune cells into the affected sites. (3). The direct physical contact of immune cells with degenerated basal or myoepithelial cells results in the discharge of their proteolytic enzymes, leading to the physical degradation of the degenerate myoepithelial or basal cells and the surrounding local basement membrane, resulting in focal disruptions in these structures. (4). As both the basal or myoepithelial cell layers are the sole source of tumor suppressor p63 and maspin [135-138], a focal disruption could lead to several consequences: (a) a localized loss of tumor suppressors and paracrine inhibitory function would confer tumor cells with growth advantages and allow them to escape from programmed cell death [139-143], (b) a localized alteration of permeability for nutrients, growth factors, and oxygen, would selectively favor proliferation of the overlying stem cells [144-146], (c) a localized increase of lymphocyte infiltration would disrupt inter-cellular junctions and cell surface adhesion molecules, facilitating cell “budding” from the tumor core [147-150]; and (d) direct physical contact between epithelial and stromal cells would facilitate the epithelial-mesenchymal transition [151-154]. As epithelial stem cells are believed to be located over-
lying the basal cell layer and the BM, a focal capsule disruption at the site of genetically-altered stem cells may favor their exit from quiescence.

This new hypothesis entitled “aberrant lymphocyte infiltration-induced focal capsule disruptions” differs fundamentally from the linear model of tumor progression and the proteolytic enzyme theory of tumor invasion in the followings. (1) The direct cause of tumor capsule disruptions. According to the proteolytic enzyme theory, the disruption of the tumor capsule is directly caused by tumor cell-produced proteolytic enzymes. The new hypothesis proposes that the disruption of the tumor capsule results from focal basal cell degeneration-induced immune cell infiltration. Although the enzymes of immune cells may belong to the same proteolytic enzyme family, they are discharged only upon physical contact with aged or injured basal or myoepithelial cells. Thus, their impact is focal and independent of the tumor stage and histological grade. (2) The stage of tumor invasion. According to the enzyme theory, the level of proteolytic enzymes increases linearly with tumor progression and reaches the highest level at the in situ cancer stage, in which invasion occurs. Based on the new hypothesis, invasion can take place at any stage of carcinogenesis, requiring only that the focal capsule disruption occur at a site where the overlying epithelial layer contains tumor progenitors or stem cells. (3) The cellular origin of invasive lesions. Based on the proteolytic enzyme theory, all in situ cancer cells could contribute equally to invasive lesions. According to our hypothesis, invasive lesions are predominantly derived from tumor stem cells overlaying focally disrupted tumor capsules. (4) Potential approaches for treatment, prevention, and detection of tumor invasion. According to the proteolytic enzyme theory, administration of corresponding enzyme inhibitors is the only regime for treatment and prevention of tumor invasion. Based on the new hypothesis, there are at least five such approaches: (a) to neutralize the molecules that attract immune cell infiltration, (b) to reduce the specific subtype of immune cells associated with focal capsule disruptions, (c) to develop therapeutic agents to specifically target stem cell clusters overlaying focally disrupted capsules, (d) to develop therapeutic agents to stabilize the BM, and (e) to stimulate basal cell growth.

The main strength of this hypothesis is that: (1) it can reasonably explain all major events involved in metastasis, which includes dissociation from the primary site, intravasation, extravasation, migration, and colonization at distant sites, (2) it is applicable to all epithelium-derived tumors, and (3) it provides, for the first time, a morphologically defined precursor of metastatic cancer. The main weakness is that it is descriptive in nature primarily based on morphological

7. Lymphocyte-mediated cell dissemination and metastasis (the Piggy-back theory)

This new hypothesis for tumor cell dissemination and metastasis is an expansion of the hypothesis presented above (aberrant lymphocyte infiltration-induced focal capsule disruptions), which is based on new findings from our more recent studies of multiple types of human cancer, including those from breast, prostate, lung, cervix, skin, and colorectum [158-160]. These recent studies have detected almost identical frequency and pattern of focal capsule disruptions, immune cell infiltration, and cell “budding” from focally disrupted capsules, as those seen in our previous studies of breast and prostate [119-131]. More importantly, these new studies have consistently shown that aberrant tumor-infiltrating lymphocytes can trigger tumor metastasis through three correlated pathways: (a) the physical movement of infiltrated lymphocytes into the budding tumor cell nest can disrupt intercellular junctions and surface adhesion molecules, causing dissociation of some cells from the tumor core; (b) lymphocytes can conjoin with dissociated tumor cells through cell membrane fusion to form tumor-lymphocyte chimeras (TLCs); and (c) the natural ability to migrate and to cross intercellular barriers allows lymphocytes to physically drag tumor cells to remote sites and to intravasate into blood vessels or lymph ducts [158-160].

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and immunohistochemical findings on tissue sections. In addition, it has not been able to elucidate the molecular and mechanistic pathways or the specific molecules, which account for the formation of TLCs. Furthermore, the clinical significance of the presence of TLCs within the vascular structures remains to be established.

Collectively, there are three central tenants of these new hypotheses. The first is that tumor stem cells are co-localized with normal, benign, and malignant cells within capsules, while they are the only ones retaining the potential for unlimited proliferation and multi-lineage differentiation. The second is that tumor invasion or metastasis may occur at any stage of carcinogenesis if a focal capsule disruption occurs at a site where the overlying epithelium contains tumor stem cells. The third is that the interaction of lymphocytes with tumor stem cells can lead to stable adhesions between the two creating a TLC that facilitates dissemination of tumor stem cells. Based on this new hypotheses, the apparent contradiction in the role of tumor infiltrating immune cells may result primarily from the differences in tumor stages and distributions of infiltrating immune cells. As the primary tumor infiltrating immune cell types, including cytotoxic T-lymphocytes, natural killer and Mast cells, have to physically contact their targets in order to exert their cytotoxic functions [161-164], it is likely that infiltrating immune cells may be involved primarily in the physical destruction of altered epithelial capsules at the pre-invasive stage, and thus, to promote tumor invasion into the stroma. On the other hand, immune cell infiltration into the invasive tumor nests may lead to physical destruction of the tumor cells and reduction of the tumor burden. These hypotheses could reasonably explain the contradictory reports and statements regarding the impact and clinical significance of immune cell infiltration into tumor tissues. More importantly, as the disruption of the tumor capsule is an absolute prerequisite for tumor invasion and metastasis, local or systematic administration of anti-inflammatory agents to prevent immune cell infiltration-induced capsule destruction may be beneficial in preventing tumor progression. A recent report published in Lancet Oncology has revealed that regular use of Aspirin, a non-steroidal anti-inflammatory drug, reduces the long-term risk of CRC and other cancer and the risk of distant metastasis [165].

The main contents of the aberrant lymphocyte infiltration-induced focal capsule disruption and lymphocyte-mediated cell dissemination and metastasis hypotheses are depicted in Figure 1.

Conclusions

The impact of tumor-infiltrating immune cells has been subject of debate for decades. A great number of studies have shown that tumor-infiltrating immune cells are associated with the physical destruction of the tumor cells, reduction of the tumor burden, and improved clinical prognosis. On the other hand, a significant and steadily increasing number of studies have shown that increased infiltration of immune cells may promote tumor progression and invasion. In an effort to elucidate the primary impact and mechanism of tumor infiltrating immune cells on associated tumor tissues, a great number of studies have been conducted and a number of hypotheses have been presented. In this mini-review, we present several existing hypotheses that favor the promoting impact of tumor-infiltrating immune cells on tumor invasion and metastasis, and also analyze their strengths and weaknesses. These hypotheses were selected for a number of reasons, including (1) they specifically address the direct impact of tumor infiltrating immune cells on tumor behavior, and (2) they appear to be applicable to multiple or all epithelial-derived tumors. Each of these hypotheses has their individual strengths and weaknesses and are supported by laboratory findings and/or clinical data, suggesting that tumor-infiltrating immune cells may impact, directly or indirectly, associated tumors through multiple pathways and mechanisms.
The normal breast (B) and prostate (P) epithelium (asterisks) is physically separated from the stroma (stars) by a continuous capsule (arrows).

Focal degeneration of aged or injured tumor capsule (thick arrows) attracts the infiltration of lymphocytes (thin arrows), which causes focal disruptions (circles) within the capsules.

Focal disruptions within the capsule (arrows) selectively favor proliferation of the overlying stem cells (circles).

Lymphocyte infiltration into the proliferating cell clusters causes dissociation of some cells (thick arrows) from the core. Some dissociated cells are conjoined with lymphocytes (thin arrows) forming tumor cell-lymphocyte chimeras (TLCs; circles).

Lymphocytes (thin arrows) of the TLCs physically drag the tumor cells (thick arrows) into vascular structures and distant organs during their migration.

Figure 1. Aberrant lymphocyte infiltration-induced focal capsule disruption and lymphocyte-mediated cell dissemination and metastasis hypotheses.
Competing Interests

The authors have declared that no competing interest exists.

Disclaimer

The views expressed in this manuscript are those of the authors and do not reflect the official policy of the Department of the Army, the Department of Defense or the United States Government or the Henry Jackson Foundation.

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