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Mechanisms that drive inflammatory tumor microenvironment, tumor heterogeneity, and metastatic progression

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Abstract

Treatment of cancer metastases has been largely ineffective. It is paramount to understand the mechanisms underlying the metastatic process, of which the tumor microenvironment is an indispensable participant. What are the critical cellular and molecular players at the primary tumor site where metastatic cascade initiates? How is tumor-associated inflammation regulated? How do altered vasculatures contribute to metastasis? What is the dynamic nature or heterogeneity of primary tumors and what are the challenges to catch a moving target? This review summarizes recent progress, mechanistic understanding, and options for metastasis-targeted therapy.

Keywords

Tumor metastasis; Microenvironment; Tumor suppressor; Inflammation/Immune; Heterogeneity

1. Introduction

Tumor metastasis accounts for most cancer-associated deaths in patients. Currently, there are very few effective treatment options [1]. During metastasis, tumor cells must disseminate, intravasate into circulation, travel through vascular networks, arrest in vascular beds of target organs, and subsequently extravasate into the organ parenchyma [2]. In a hostile distant organ, they must escape host immune surveillance, adapt to supportive niches, survive as latent tumor-initiating seeds, and eventually break out to grow [3]. Evidence from recent years strongly suggests that the tumor microenvironment (TME) is an indispensable participant in the metastatic process [3, 4], allowing the tumor cells not only to escape from host immune surveillance, but also induce the formation of new blood vessels and invade the

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vasculature [5]. This review will focus on the primary tumor site where the initiating step of metastatic cascade occurs, and provide updates on the critical cellular and molecular players, as well as potential targeting options.

2. Players in the primary tumor microenvironment

Tumor is described as an unhealed chronically inflamed wound. In addition to the tumor cells at a primary tumor site, there are a variety of inflammatory cells including both innate and adoptive immune cells, and myeloid and lymphoid lineages (Fig. 1). They impact diverse biological functions and tumor phenotypes (Fig. 1). Of tumor-infiltrating myeloid cells, there are three major types: Gr-1+CD11b+ immature myeloid cells, tumor-associated macrophages (TAM), and tumor-associated neutrophils (TAN). Gr-1+CD11b+ cells are a heterogeneous set of immature myeloid cells, while TAM and TAN are well-differentiated. It remains to be seen how these three populations of cells are interrelated in phenotype and function in the TME. Gr-1+CD11b+ cells are also called myeloid-derived suppressor cells (MDSCs) for their well-known immune suppressive activities [6, 7]. In addition, MDSCs produce matrix metallopeptidase 9 (MMP9) and transforming factor beta (TGFβ), executing a profound impact on tumor progression and metastasis through modulation of tumor vascularization and tumor cell invasion [8, 9]. TAMs are identified as Mac-1 (CD11b+) and/or F4/80+ myeloid cells. They promote tumor progression and metastasis [10–12] through elevated colony stimulating factor 1 (CSF-1) production and enhanced epithelial growth factor (EGF) signaling in cancer cells [13, 14]. TAN, identified as CD11b+Ly6G+ cells, have only recently been reported to possess tumor-promoting functions [15–17]. However, in early stages of human lung cancer, subsets of TANs exhibit anti-tumor properties with characteristics of both neutrophils and antigen-presenting cells [17]. TGFβ regulates N1–N2 polarization of neutrophils [15]. This N1–N2 polarization of neutrophils may mirror the M1–M2 polarization of macrophages that are defined by interferon-γ and IL-4 production as Th1 and Th2 cells, respectively [18].

Tissue-residing lymphoid cells are very important in tissue repair and integrity [19]. In tumor tissues, a number of lymphoid cells are found, including cytotoxic T lymphocytes (CTL), regulatory T cells (Treg), and B cells. **CTL** proliferation and function are usually suppressed under tumor conditions through various mechanisms including decreased perforin, granzymes, and cytotoxins [20, 21]; lost co-localization of the T cell receptor and CD8 [22]; the development of other T cell types such as gamma/delta T cells that suppress T and dendritic cell functions [23]; aberrant expression of stress-inducible NKG2D ligands; and expansion of immunosuppressive T cells [24]. Importantly, activation of immune inhibitory pathways through programmed cell death protein 1 and its ligand (PDL-1/PD1) impairs cytotoxic T cell functions [25, 26]. **CD4+CD25+ Treg cells** are induced in the TME through TGFβ-promoted Foxp3 expression and possess strong immune suppressive functions [27, 28]. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) is identified as an important inhibitory mediator particularly in CD4+ Treg cells. Drugs targeting CTLA4 unleashes anti-tumor immunity [25, 29]. Chronic activation of **B cells** is indicated in potentiating carcinoma development in the transgenic mouse model K14-HPV16 [30]. In fact, B cells, humoral immunity, and activating FcyRs are required for establishing chronic inflammatory programs that promote *de novo* carcinogenesis [31]. TGFβ (and/or IL-10) is

responsible for B cell-mediated immune suppression [32, 33]. In addition, complement C5a in a TME recruits MDSCs, thus suppressing CTL function and promoting tumor growth [34].

Beyond the immune cells, there is also an abundance of cancer-associated fibroblasts (CAFs) [35, 36], and their gene expression predicts clinical outcomes [36, 37]. CAFs produce chemokines and cytokines such as stromal-derived factor 1 (SDF-1 or CXCL12) [38] and platelet-derived growth factor C [39], and promote tumor progression and resistance to therapy such as anti-VEGF (vascular endothelial growth factor) [39]. TGFβ is a critical mediator in the CAF tumor-promoting function [40–42].

Tumor infiltrating immune cells crosstalk with each other and with tumor cells. For example, tumor cells and macrophages produce PDL-1 (ligand for PD-1) that activates PD1-(programmed cell death protein 1) mediated inhibitory immune checkpoint in T cells; immune therapies blocking immune-inhibitory checkpoints (PDL1/PD1 and CTLA-4) lead to T cell activation and tumor regression [25, 26]. IL-4-expressing CD4+ T lymphocytes regulate phenotype and function of CD11b+F4/80+ macrophages, which in turn enhance epithelial growth factor receptor (EGFR) signaling in mammary epithelial cells and promote tumor invasion and metastasis [43]. Recently studies show TAM also express PD1, which impairs their phagocytic activity [44]. In addition, TAMs capture the PD-1 mAbs on the T cell surface thus compromise the effectiveness of immune therapeutics [45]. These insights should be helpful in addressing relapse and resistance in immune checkpoint blockade. TGFβ-mediated inflammatory response is critical in the crosstalk between myeloid cells and metastatic breast cancer cells [9, 46, 47]. In addition, epithelial cells and CAFs influence one another and enhance tumor progression [40–42]. Some studies have shown genetic alterations/somatic mutations in stromal fibroblasts and support a tumor-stroma coevolution [48–52]; however, such findings remain highly controversial [53, 54]. Nevertheless, it is clear that host-derived stromal cells collectively create an environment that favors tumor progression by providing growth factors, pro-angiogenic factors, proteases, and adhesion molecules that facilitate tumor cell proliferation, angiogenesis, invasion, and metastasis as well as therapeutic resistance [55, 56]. This very dynamic TME likely serves as a selective pressure for tumor cell variants through genomic instability, genomic heterogeneity, and epigenetic alterations [57, 58].

3. Mechanisms of inflammation

Chronic inflammation is a hallmark of cancer [59]. Different from acute inflammation that can clear infection, heal wounds, and maintain tissue homeostasis, tumor-associated inflammation is often low in grade and chronic. Many factors can trigger inflammatory response in tumors, including infection, tissue damage, activation of oncogenes, and loss of tumor suppressors (TS) (Fig. 2). Oncogenes like those encoding protein tyrosine kinases (RTKs) are often persistently activated in a ligand-independent manner [60, 61]. Emerging literature supports a role of RTKs in inflammation induction. **RET** (REarranged during Transfection), an RTK with cadherin-like domains in its extracellular region, is altered in cancers in the forms of fusion (thyroid and non-small cell lung cancer), overexpression (breast, prostate, pancreatic cancers, and several more), and point mutations (in multiple

endocrine neoplasia type 2A and familial medullary thyroid carcinoma) [62, 63]. Activation of RET, either by oncogenic mutations or binding of ligands and co-receptors, stimulates pro-inflammatory gene expression and increases tumor-associated inflammation [63–66]. This biological property may explain higher malignancy and resistance to endocrine therapies in patients with breast cancer exhibiting increased RET expression [63]. **EGFR** signaling activates NF-κB through MALT1, a scaffold protein, via recruiting E3 ligase TRAF6 to IκB kinase β (IKKβ) complex [67]. Overexpression of **Neu or Her2** driven by the MMTV promoter induced inflammatory response through Stat3-dependent overexpression of C-terminal tensin-like (Cten) focal adhesion protein, which disrupts cell-cell junctions and enhances tumor cell metastatic ability [68].

In addition to RTKs, publications also support a role of **oncogenic Ras** in inflammation induction. **Ras** activation exacerbated pro-malignant paracrine activities through IL-6 and IL-8 [69]. High Ras activity leads to acinar cell senescence and increased inflammation and fibrosis, subsequently resulting in the formation of pancreatic intraepithelial neoplasia and progression to both cystic papillary carcinoma and metastatic pancreatic ductal adenocarcinoma [70]. In the presence of oncogenic Ras, inflammatory stimuli trigger an NF-κB-mediated positive feedback mechanism involving cyclooxygenase 2 that amplifies Ras activity [71]. In skin carcinogenesis, Ras activation induces inflammation through IL-1R-MyD88 signaling [72]. Activation of oncogenic Ras enhances the expression of squamous cell carcinoma antigens and IL-6 via NF-κB inflammatory pathway [73]. In pancreatic cancer, oncogenic KRAS (G12D) accelerates inflammation-induced carcinogenesis through NFATc1-STAT3 cooperativity in gene transcription [74]. Lastly, **p53 mutant**, with gain-of-oncogenic activity, such as p53R273H, is capable of activating NF-κB, which leads to chronic inflammation, tissue damage, and accelerated tumor development [75, 76]. Together these studies suggest that oncogene activation promotes inflammation and tumor progression through production of cytokines, chemokines, growth factors, prostaglandins, reactive oxygen, and nitrogen species, as well as recruitment of inflammatory cells in tumor tissues (Fig. 2).

Tumor suppressors are powerful transcriptional and signaling regulators counteracting the growth-promoting activity of oncogenes [77]. Loss or silencing of TS promotes neoplastic transformation and malignant progression. To date, most work on TS has focused on cell-autonomous effects. We recently proposed an important non-cell-autonomous role for TS in the control of tumor-associated inflammation [78]. These TSs include p53, APC (adenomatous polyposis coli), and TGFβ. **p53** mutations are documented in over 50% of human cancers. Loss of normal p53 function is frequently associated with an increased susceptibility to inflammation-driven cancers such as ulcerative colitis-associated colorectal cancer [79–81]. A strong association of p53 mutation and NF-κB activity has been observed in patients with head and neck squamous cell carcinoma (HNSCC) [82]. In mouse models, p53 mutations induced inflammation in several tumor models [83–85]. Thus, the ability of WT p53 to inhibit tumor-associated inflammation is critical in its tumor suppressive activities [86–88]. **APC** germ line allelic loss is a hallmark of human familial adenomatous polyposis syndrome as well as sporadic colorectal cancer. Studies suggest a strong correlation between colon cancer progression and tumor-associated inflammation [11, 89].

In mouse models, mono-allelic deletion of *Apc* in intestinal epithelial cells ($Apc^{+/\Delta}$) results in tumor development upon inactivation of the wt *Apc* allele due to a loss of heterozygocity (LOH) [90–92]. When $Apc^{+/\Delta}$ mice are crossed with transgenic mice expressing constitutively active IKKβ in intestinal epithelial cells (IECs), the compound mice exhibit more β-catenin positive (+) early lesions and small intestinal and colonic tumors relative to the parental $Apc^{+/\Delta}$ line [93]. Targeted deletion of *Apc* allelic by Cdx2-Cre transgene in mice leads to colorectal tumors with upregulation of several pro-inflammatory cytokines, including IL-23 and IL-17A [94]. Interestingly, loss of APC in this model (which is also due to LOH) results in rapid epithelial barrier deterioration and microbial invasion, thus providing a major impetus for tumor-elicited inflammation [94]. **TGFβ** possesses potent anti-inflammatory activity; loss or down-regulation of TGFβ signaling in epithelial cells induces inflammation in mouse models of mammary, pancreatic, intestinal, colon, and head-and-neck squamous cell carcinomas, and accelerates malignant progression and metastasis through effects on inflammation and the TME [9, 78, 95, 96]. In addition, inactivation of TGFβ signaling in CAFs also results in expression of pro-inflammatory genes whose products promote tumor development [40, 42]. Deletion of SMAD4 in T lymphocytes increases the expression of IL-5, IL-6, and IL-13, and results in tumor infiltration with inflammatory cells, thereby promoting the development of gastrointestinal carcinomas [97]. However, it should be noted that opposite results have also been observed [97–99], reflecting the context-dependent nature of TGFβ signaling in cancer and its complexity.

The major mechanisms underlying loss of TS function and activation of inflammatory responses involve NF-κB and STAT3 [76, 85, 100], key regulators of inflammation [89, 101, 102]. The inflammatory mediators include nitric oxide synthase, cytokines, and chemokines such as SDF-1, CXCL1/5, and CXCR4, as well as prostaglandin E2 [55, 78, 103]. These studies together lead to a new paradigm regarding the non-cell-autonomous function of TS as a negative regulator of inflammation, thus modulating the interaction between the malignant cells and their microenvironment (Fig. 2).

4. Angiogenesis and challenges in anti-angiogenesis therapies

Tumor vascular networks provide a route for metastasis spread [5, 104, 105]. It is well-documented that tumor vascular density positively correlates with metastatic potential [106–109]. Accordingly, targeting tumor vasculatures offers potential for halting tumor invasion and metastasis [5, 110]. During tumor progression, a subset of tumor cells at the primary tumor site acquire aggressive properties due to genetic/epigenetic modifications or stimulation by TME components. They invade surrounding tissues and neighboring organs, or intravasate into blood and lymphatic vessels, travel through circulation, and extravasate out the vessels, then disseminate into distant organs [111].

Tumor vasculature is disorganized, tortuous, and leaky, which is associated with altered endothelial cell adherens junctions and tight junctions that are critical in maintaining vascular barrier functions [112]. A tumor-derived factor such as VEGF is a potent vascular permeability factor that is capable of disruption of the endothelial barrier. VEGF activates FAK, which in turn facilitates dissociation of VE-cadherin–β-catenin and breakdown of endothelium adherens junctions [113, 114]. Another tumor-derived factor, SPARC, drives

vascular permeability and extravasation through endothelial VCAM1 signaling [115]. Interestingly, microRNA105 from tumor cells delivered to the endothelium through exosomes, increases vascular permeability, thereby promoting tumor metastasis [116]. Additionally, tumor cells induce programed necrosis (necroptosis) of endothelial cells, thus increasing vascular leakiness and tumor cell extravasation and metastasis [117]. Consistently, inhibition of vascular permeability using angiopoietin-like 4 prevents tumor metastasis [118]. These findings clearly establish the importance of vascular properties, particularly vascular leakiness in tumor metastasis.

Oxygen availability decreases as the distance from the nearest blood vessel increases. When tumor tissue growth outpaces the growth of blood vessels, it often results in hypoxia, a low oxygen status. Naturally, hypoxia is a common feature of solid tumors. It is well-established that hypoxia positively correlates with and controls tumor metastasis [119, 120]. Mechanistically, hypoxia induces abnormal levels of molecular mediators in the TME and leads to defective structure and function of the vasculature. In addition, hypoxia activates signaling pathways in tumor cells, and alters extracellular matrix deposition, remodeling, degradation, and potentiate cancer metastasis [119]. Hypoxia targets multiple cell types in the TME including MDSCs or neutrophils by regulating the expression of multiple angiogenic genes to promote tumor angiogenesis [8, 121]. Hypoxia also promotes Treg recruitment through CCL28 induction, which in turn promotes immune tolerance and angiogenesis [121], two programs closely connected for sustained tumor progression [121]. Indeed, we originally reported that MDSCs, a potent immune suppressive cell population present in tumor conditions, also promote tumor vascular formation and metastasis [8, 9].

Hypoxia regulates gene expression in multiple cell types through a transcription factor, hypoxia inducible factor (HIF). HIF is a heterodimeric complex composed of an alpha and beta subunit. The alpha subunit of HIF is hydroxylated at conserved proline residues by HIF prolyl-hydroxylases, allowing their recognition and ubiquitination by von Hippel–Lindau (VHL) E3 ubiquitin ligase and subsequent proteasome-mediated degradation. Hypoxic condition inhibits HIF prolyl-hydroxylase, which results in an accumulation of the alpha subunit, activation of HIF, and its targeted gene expression to restore homeostasis [122]. Similar to the close association of hypoxia with metastasis, overexpression of HIF is frequently associated with metastasis and poor clinical outcomes [123–125]. HIF upregulates the expression of proteases such as MMP1 and MMP2 and simultaneously downregulates the expression of MMP inhibitors, which collectively promotes tumor vascular permeability and intravasation [126–128]. Moreover, HIF-1 induces the recruitment of myeloid cells, as well as endothelial and pericyte progenitor cells via induction of HIF target genes to regulate tumor angiogenesis and glioblastoma invasion [129].

Accordingly, targeting tumor vasculature offers a unique opportunity for cancer therapy. FDA has approved several antiangiogenic drugs, such as bevacizumab, a monoclonal antibody against VEGF; sorafenib and sunitinib, neutralizing antibodies against VEGF receptors; pazopanib, an RTK inhibitor; and everolimus, an mTOR inhibitor [130]. In some cancers, angiogenesis inhibitors are most effective when combined with additional therapies. It has been proposed that angiogenic inhibitors help normalize the blood vessels that supply a tumor, thus facilitating the delivery of other anticancer agents [131]. Despite the critical

role of vasculature in tumor growth and dissemination, most anti-angiogenic cancer therapies have had limited success. Furthermore, some tumors including prostate cancer, pancreatic adenocarcinoma, and melanoma are resistant to anti-angiogenic therapy [130]. To improve therapeutic efficacy, it is important to identify specific biomarkers for patient selection to determine who most likely will respond to therapy, balancing efficacy and toxicity as well as developing novel combination strategies with other types of drugs. For example, immunotherapy has achieved unprecedented success recently, a combination of ipilimumab, an immune checkpoint blockade, and bevacizumab yielded roughly twice expectations compared to that from ipilimumab alone in metastatic melanoma in a phase I study [132]. A better understanding of the immunomodulatory functions and other mode of actions of anti-angiogenic drugs will likely lead to better treatment strategies for cancer patients.

5. Tumor heterogeneity, clonal evolution, and the influence of the tumor microenvironment

Resistance to therapy is the foremost challenge in cancer treatment. The emergence of therapy-resistant escape variants is fueled by intra-tumor heterogeneity [133]. It is proposed that cancers emerge from an ongoing Darwinian evolutionary process, often leading to multiple competing subclones within a single primary tumor and eventually culminate in metastatic phenotypes [134–137]. Scientific evidence suggests three possible mode of actions: lineage progression, parallel progression, or a hybrid model between lineage and parallel progression [137]. Lineage progression implies that metastatic competence can arise from preexisting genetic variations in parental populations with high levels of concordance between primary tumors and metastasis, which is supported by comparative genetic studies of primary tumors and metastases of colorectal, breast, pancreatic, renal, and prostate [137]. The preexisting genetic alterations include TP53 [138–140], MYC [141, 142], KRAS(G13D), BRAF(G464V) [143], and PI3K [144]. Reflected in its name, parallel progression suggests that metastatic clones and subclones in primary tumor continue to evolve in parallel, resulting in substantial genetic divergence between primary and metastatic lesions [145, 146], including mutations in Hras and Kras during skin carcinogenesis [147].

Mechanisms responsible for tumor heterogeneity are emerging (Fig. 3). Genetic divergence is a well-known driver for heterogeneity. However, non-genetic factors such as epigenetic regulation and the TME also add significant variability [148]. First, alterations in histone markers, DNA methylation, and noncoding RNA contribute to tumor heterogeneity [149–152]. Second, splice variants drive tumor heterogeneity such as androgen receptor (AR) variants in prostate cancer [153, 154]. Third, interactions of CAFs with tumor cells could lead to co-evolution of tumor cells and their microenvironment [38, 48, 52]. Finally, host-derived CAFs, myeloid cells, and Treg cells produce various soluble factors. These TME or non-cell-autonomous factors could drive clonal heterogeneity, enable inter-clonal interactions, and lead to new phenotypic traits. In a mouse xenograft model, non-cell-autonomous factors IL11 and VEGF-D were found to drive tumor clonal interference and sub-clonal stabilization [155]. Paracrine signaling via FGF-2 and MAPK among SCLC subclones is a critical determinant in early steps of the metastatic process, which drives

metastatic dissemination of the neuroendocrine tumor subclones [156]. Invading tumor cells programed with epithelial-mesenchymal transition (EMT) are one of the noticeable heterogeneity [111, 157, 158], which is controlled by transcription factors including Snai1, Twist, Zeb1, and SIP1 [159–161]. Signal transducers supplied from TME such as TGFβ activates EMT-related transcription factors [161, 162]. The cellular plasticity displayed by EMT/MET (mesenchymal-epithelial transition) could generate multiple, distinct cellular subpopulations leading to intratumoral heterogeneity [163, 164]. Another example is cancer stem cell (CSC) [158]. Solid tumors are hierarchically organized and sustained by CSCs. Emerging evidence suggests that CSCs contribute to therapeutic refractoriness and dormant behavior [165].

Adding another layer of complexity in tumor heterogeneity is that the circulating tumor cells, which obtain additional genetic or epigenetic alterations, return to the primary tumor site through self-seeding [166]. In addition, the existence of polyclonal seeding in human malignancy has been observed [167], and metastasis-to-metastasis spread was also found to be common [167]. The collective action of tumor cells traveling together in clusters also gives rise to metastases heterogeneity [168–170], which is different from the conventional view that metastases are seeded by single cells from primary tumors [171].

Single cell sequencing technology is powerful in revealing the distinct genotypic and phenotypic states of tumors. It has led to a series of unanticipated discoveries, such as high heterogeneity and stochastic changes in cancer-cell populations, new driver mutations, and molecular mechanisms of clonal evolution [172]. RNAseq from 4645 single cells isolated from 19 patients with melanoma showed transcriptional heterogeneity in tumor cells, as well as distinct TME patterns, including exhaustion of tumor-infiltrating T cells [173]. Single cell sequencing of patients with glioblastoma revealed tumor heterogeneity in oncogenic signaling, proliferation, complement/immune responses, hypoxia, and molecular program for stemness [174]. In prostate cancer, single cell sequencing of 77 circulating tumor cells isolated from 13 patients showed considerable heterogeneity, which includes expression levels of androgen receptor (AR), gene mutations, and splicing variants, as well as activation of noncanonical Wnt signaling responsible for resistance to AR inhibitor treatment [175]. These publications are just snapshots from many ongoing studies that reveal previously unappreciated heterogeneity and diverse regulatory programs central to tumor biology, prognosis, and therapy. Understanding how cancer genetics synergize with emerging epigenetic and TME factors will be key for the development of therapies capable of tackling tumor escape, and thereby improve cancer patient survival.

6. Targeting opportunities

Strategies focusing on the cancer genome have limitations [176, 177]. One major hurdle is limited sampling of tumor tissues (e.g., by a single biopsy), which prevents from understanding clonal structure and heterogeneity of tumors. For example, the spatial and temporal diversity in genomic instability defines lung cancer evolution [178]. The cooperative behavior of subclones influences disease progression and adds complexity to therapeutic interventions [179]. Cytotoxic cancer therapies impose intense evolutionary selection pressures on the surviving cells and thus increase the evolutionary rate [176].

Additionally, the influence of tumor micro-environment heterogeneity on therapeutic response is also noteworthy [56]. In fact, the significance of the TME in tumor progression has been utilized to monitor the biological phenotype. The subtype, density, and location of immune cells within human colorectal tumors predict clinical outcome. While $CD4^+$ Th1 cells and $CD8^+$ CTL constitute a positive prognostic sign in human colorectal cancer [180], Th17 (T-helper cell producing interleukin 17) cells negatively correlates with patient survival [181]. Gene expression signatures of tumor stroma can predict clinical outcome in breast cancer [37].

Tumor invasion at the primary tumor site is the first and a crucial step in the metastatic process. Patients who are initially diagnosed with regional tumor invasion and visible metastasis have low survival [1]. Moreover, locoregional and metastatic relapse after standard therapies are also mortal for patients; e.g., in HNSCC [182] and triple-negative breast cancer [183]. Once these events happen, there are no good options to control tumor progression. Thus, it is paramount to investigate the mechanisms underlying both tumor invasion and locoregional or metastatic recurrence events. Any interventions aimed at this initiating step in primary tumors should have significant therapeutic outcome. However, in the case of TS loss in cancer, it is difficult or even impossible to design small molecules that directly restore TS function, with the exception of compounds that target Mdm2:p53 interaction [184]. With the ample evidence revealing the association between TS loss and enhanced tumor-associated inflammation, one could explore this targeting option by using anti-inflammatory drugs [78]. Along this line, several targeting opportunities are envisioned (Fig. 4), including IKK inhibitors and Metformin, as well as non-steroidal anti-inflammatory drugs (NSAIDs) [103]. Immunotherapies including those aimed at clonal neoantigens, as well as neutralizing antibodies and antagonists, will likely offer additional options to inhibit the inflammatory TME and enhance anti-tumor immunity [185–187]. In addition, neutralizing antibodies and antagonists targeting tumor angiogenesis have been tried and are under further evaluation [188–191]. Furthermore, cell type specific targeting may also provide alternative options [192, 193], including TAMs [194, 195], MDSCs [7, 46], and B cells [196]. This is demonstrated in the case of TGFβ, in which myeloid specific inactivation of TGFβ signaling diminished cancer metastasis in a number of mouse models [9, 46, 47, 78].

Technology modalities that monitor temporal and spatial response of immune cells and multiple signaling transduction pathways could be utilized to properly assess therapeutic efficacy. Such approaches combining high-content, multiplexed measurements with informatic and computational modeling methods offer better understanding of biological functions at various scales. For example, histo-cytometry allows us to visualize and quantify phenotypically complex cell populations as well as signaling transduction molecules and transcription factors directly in tissue sections [197, 198]. However, due to the heterogeneity and plasticity nature of cancer cells [164], the paramount challenges remain: how to monitor and catch or aim precisely at a moving target? It should be noted that mutation, selection, and adaptation are mainly studied within primary tumor. Cancer cell evolution could occur after leaving primary tumor tissues. The disseminated cells acquire key traits of fully malignant cells, which points out new challenges and opportunities for diagnosis and adjuvant therapies [145, 146].

Concluding remarks

It is clear that the tumor microenvironment is an indispensable participant in metastatic progression. There are a variety of cellular and molecular players at the primary tumor site where metastatic cascade initiates. Understanding the regulation of tumor-associated inflammation, and its impact on tumor cell compartment is critical to address tumor heterogeneity, clonal evolution and therapeutic resistance. It remains challenging to have a thorough knowledge of the dynamic nature and to discover the major mediators of many host-derived factors that cross talk with autonomous tumor suppressors and oncogenic pathways. Concerted effort in understanding the tumor microenvironment together with genomic targeting approaches will likely improve metastasis treatment outcome.

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Abbreviations

APC	adenomatous polyposis coli
AR	androgen receptor
CAFs	cancer-associated fibroblasts
CTL	cytotoxic T cells
EMT	epithelial–mesenchymal transition
EPCs	endothelial progenitor cells
FSP1	fibroblast specific protein 1
HNSCC	head and neck squamous cell carcinoma
IKK	IκB kinase
LOH	loss of heterozygosity
MDSC	Gr-1+CD11b+ myeloid derived suppressor cells
NO	nitric oxide
NSAIDS	non-steroidal anti-inflammatory drugs
PD1/PDL-1	programmed cell death protein 1/PD1 ligand
PTEN	phosphatase and tensin homolog
RTK	receptor tyrosine kinase
SDF-1	stromal derived factor 1
TAM	tumor-associated macrophages

TAN	tumor-associated neutrophils
TGFβ	transforming growth factor beta
Tgfbr2^{fspKO}	mice with *Tgfbr2* deletion in stromal cells expressing fibroblast specific protein 1
TβRII	TGFβ type II receptor
TME	tumor microenvironment
TS	tumor suppressors
VEGF	vascular endothelial growth factor

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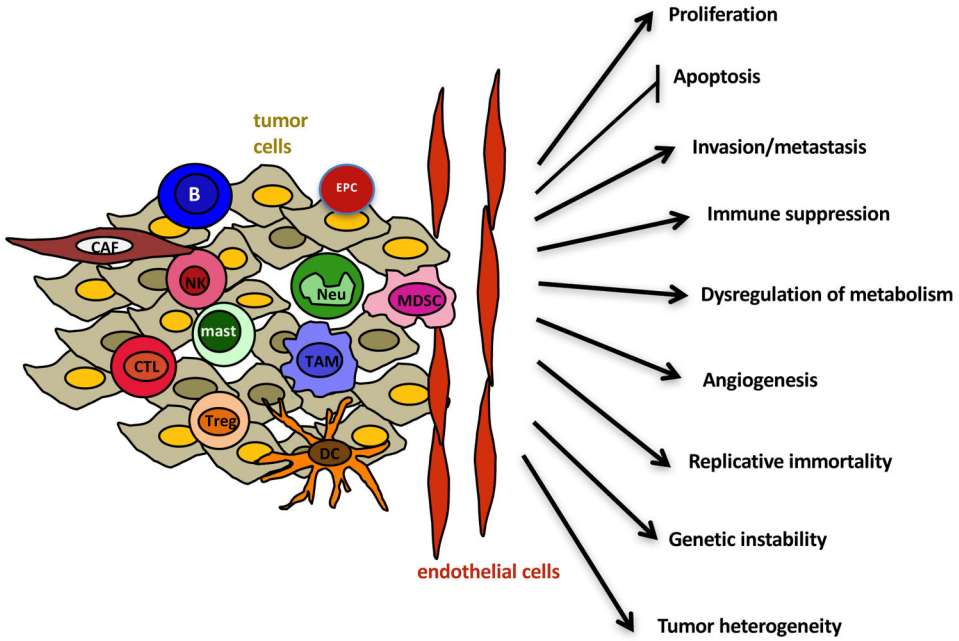

Fig. 1.

Players in the TME and the outcome resulting from tumor-associated inflammation. CAFs: cancer-associated fibroblasts; CTL: cytotoxic T cells; DC: dendritic cells; EPCs: endothelial progenitor cells; MDSC: Gr-1+CD11b+ myeloid-derived suppressor cells; Neu: neutrophiles; NK: natural killer cells; TAM: tumor-associated macrophages; Treg: regulatory T cells.

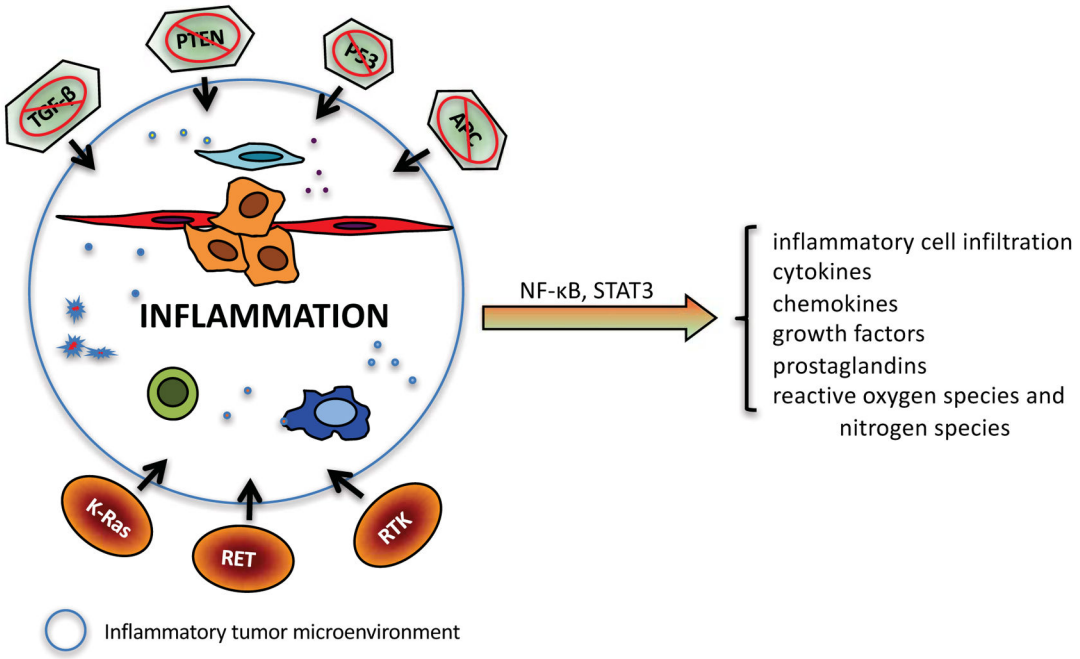

Fig. 2.

Loss of TS and/or activation of oncogenes (*input, black arrows*) induces the inflammatory microenvironment. Loss of p53, TGFβ, APC, and PTEN, and/or activation of K-ras, RET, and RTK induces infiltration of host-derived inflammatory and stromal cells (in the big circle), and increases expression of growth factors, cytokines, chemokines, production of prostaglandins, reactive oxygen, and nitrogen species. The underlying mechanisms involve NF-κB and STAT3 signaling pathways.

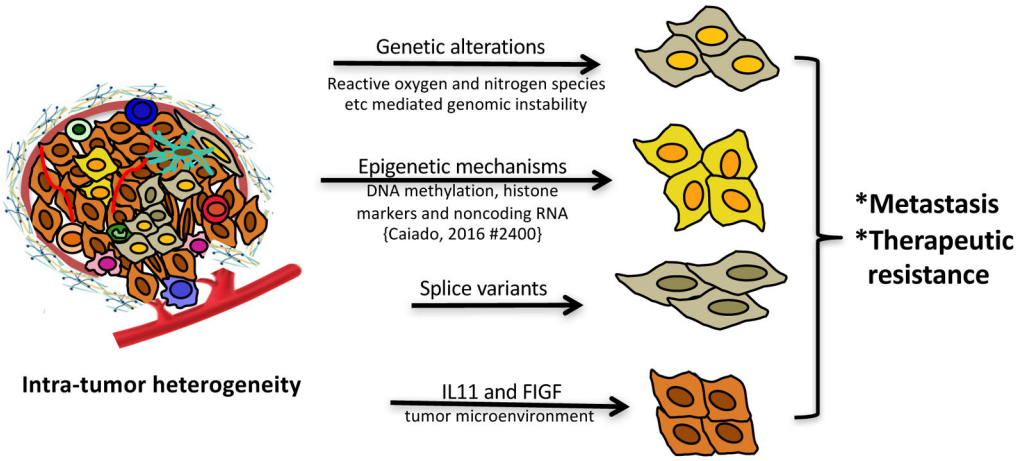

Fig. 3.

TME promotes tumor heterogeneity through genomic instability, epigenetic alterations, splice variants, and soluble factors produced by inflammatory cells, stromal cells, and endothelial cells in the TME.

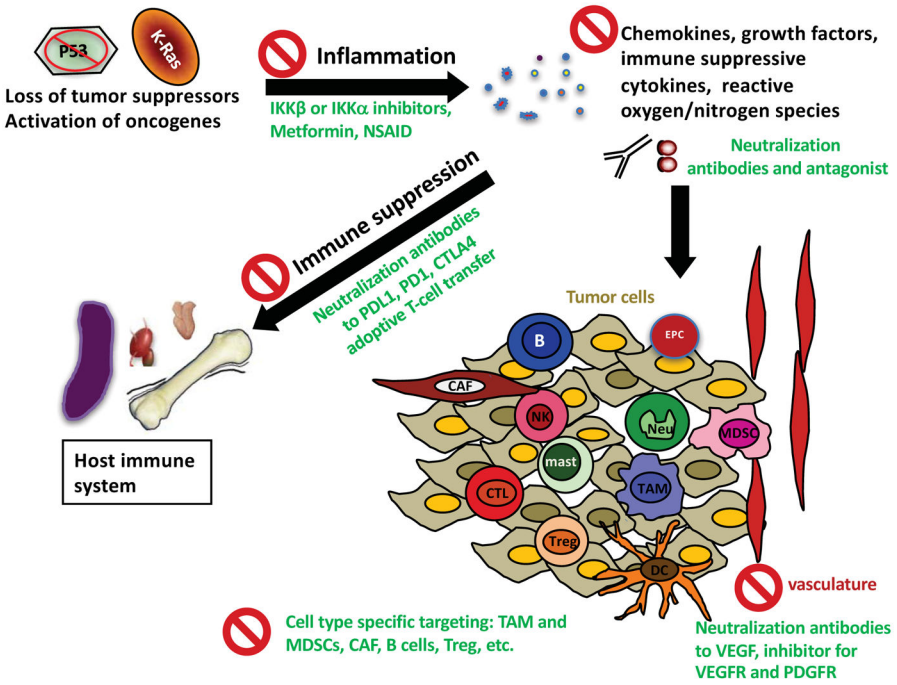

Fig. 4.

Targeting opportunities (**green color**) aimed at inflammation and the TME. IKKβ or IKK**α** inhibitors, Metformin, as well as anti-inflammation drugs can be combined with conventional chemotherapeutic agents, radiation therapies, and targeted therapies. Immunotherapy and neutralization antibodies and antagonists have shown efficacy in targeting soluble factors. Vasculature targeting and cell type specific targeting offer additional options to inhibit the inflammatory TME and enhance host anti-tumor immunity.