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REVIEW ARTICLE

Steps in metastasis: an updated review

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Abstract

Metastasis is the most complex and deadly event. Tumor-stromal interface is a place where invasion of tumor cells in the form of single-cell or collective migration occurs, with the latter being less common but more efficient. Initiation of metastasis relies on the tumor cell cross-talking with stromal cells and taking an epithelial-mesenchymal transition (EMT) in single cells, and a hybrid EMT in collective migratory cells. Stromal cross-talking along with an abnormal leaky vasculature facilitate intravasation of tumor cells, here the cells are called circulating tumor cells (CTCs). Tumor cells isolated from the primary tumor exploit several mechanisms to maintain their survival including rewiring metabolic demands to use sources available within the new environments, avoiding anoikis cell death when cells are detached from extracellular matrix (ECM), adopting flow mechanic by acquiring platelet shielding and immunosuppression by negating the activity of suppressor immune cells, such as natural killer (NK) cells. CTCs will adhere to the interstituim of the secondary organ/s, within which the newly arrived disseminative tumor cells (DTCs) undergo either dormancy or proliferation. Metastatic outgrowth is under the influence of several factors, such as the activity of macrophages, impaired autophagy and secondary site inflammatory events. Metastasis can be targeted by multiple ways, such as repressing the promoters of pre-metastatic niche (PMN) formation, suppressing environmental contributors, such as hypoxia, oxidative and metabolic stressors, and targeting signaling and cell types that take major contribution to the whole process. These strategies can be used in adjuvant with other therapeutics, such as immunotherapy.

Keywords Invasion · Metastasis · Cancer-associated fibroblast (CAF) · Epithelial-mesenchymal transition (EMT) · Circulating tumor cell (CTC) · Survival · Transforming growth factor (TGF) · Tumor microenvironment (TME) · C-X-C chemokine (CXC) · Platelet

Introduction

Metastasis is the deadliest event in tumorigenesis, and distant metastasis is regarded as the end result in tumorigenesis [1]. In prostate cancer, for instance, virtually all deaths occur as a result of metastasis [2]. Previously, metastasis was viewed as the stage of an advanced tumor, namely happening mostly at the time of tumor progression [3]; however, recent investigations approved the occurrence of metastasis often early in tumorigenesis. This infers that metastasis can occur both at early and late tumorigenesis, but the two represent distinct pathogenesis in which cells that elicit early metastasis just carry truncal mutations, whereas late-arising metastatic cells show subclonal mutations [1].

Metastasis is an organ-selective and multi-stepping process that is started by escape of tumor cells from the primary tumor and ended with colonizing secondary tumors in the distant sites [4]. The multi-stepping process of metastasis accounts for the complexity of the whole event, which imposes a huge burden on effective tumor therapy. This is also a reason for failure of developing drugs (so called migrastatics) to combat tumor metastasis with high efficiency. A number of promoters of metastasis has been identified so far (see Table 1). Developing drugs against the whole process of metastasis is not applicable. This is a reason for an ongoing research in the area. Every year more

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Table 1 Metastatic inducers in solid tumors

Factor name	The identity	The process promoted by the factor and the cancer type	Mechanism of action	References
CRMP2	A cytosolic phosphoprotein that regulates cytoskeletal dynamics	CRMP2 reduced expression is associated with lymph node metastasis of breast cancer	CRMP2/RECK interaction blocks WNT and NF-κB signaling	[128]
IMPAD1 and KDELR2	IMPAD1: a Golgi resident protein KDELR2: localizes at ER/Golgi intermediary complex	Invasion and metastasis of NSCLC	IMPAD1 and KDELR2 mediate release of proteases like MMPs from Golgi	[129]
TMPRSS2	A serine protease	Invasion of prostate cancer	TMPRSS2 mediates the effect of androgen on cancer cell invasion	[130]
ENO2	A glycolytic enzyme and a well-established biomarker of tumor	ENO2 activation enhances glycolysis and metastasis of PDAC	ENO2 induces EMT in cancer cells	[131]
NSD2	A histone methyltransferase regulated by EZH2	Prostate cancer	NSD2 targets the H3K36me2 to drive a lethal metastasis	[2]
CDK4/6	CDKs are a family of protein kinases discovered at first for their role in regulation of cell cycle	Breast cancer	CDK4/6 stabilizes ZEB1, known as a major transcription factor in EMT	[132]
ITGBL1	An integrin family	ITGBL1 promotes distant metastasis of CRC	ITGBL1 stimulates NF-κB signaling to activate fibroblasts in distant organ that by turn promote formation of PMNs	[127]
HuR	An RNA-binding protein	HuR promotes invasion of breast cancer cells	HuR interacts with the mRNA of Snail and MMP-9	[133]
REGγ	A proteasome activator	REGγ promotes metastasis of lung cancer in mice	REGγ induces TGF-β/Smad activity	[26]
VCAM-1	An immunoglobulin superfamily of transmembrane proteins	VCAM-1 promotes invasion and metastasis of lung cancer	VCAM-1 activates MAPK and AKT signaling in cancer cells	[134]
tenascin-C	An ECM factor	Tenascin-C reduces cancer cell apoptosis and induces its plasticity in breast cancer in mice	Tenascin-C activates TGF-β	[135]
PRC1	PRC1 consists of multiple subunits implicated in the mono-ubiquitination of the H2A histone	PRC1 promotes stemness and immunosuppression in prostate cancer	PRC1 induces CCL2 expression, implicated in the recruitment of Treg and M2 and promotion of stemness	[115]
ZNF367	A transcriptional factor that contains a zinc finger motif with two types of contiguous Cys2His2 fingers	ZNF367 promotes anoikis resistance, thus increasing the number of breast cancer CTCs	ZNF367 suppresses the Hippo pathway	[62]
BCL11A	A transcription factor that contains a zinc finger that is identified initially by representing a transcription site in B cell leukemia	BCL11A is upregulated preferentially in the TNBC, compared to the other subtypes, and is contributed to the maintenance of stemness	BCL11A represses the MBNL1	[136]
UCHL1	A deubiquitinase that protects proteins from ubiquitination	UCHL1 is highly present in serum of TNBC patients, and it promotes migration and extravasation of cancer cells	UCHL1 facilitates TGF-β activity	[137]
CXCL14	An orphan chemokine	CXCL14 is highly expressed in CAFs of breast cancer, and it promotes EMT and survival of tumor cells	CXCL14 acts in a mechanism mediated by ACKR2	[138]

Table 1 (continued)

Factor name	The identity	The process promoted by the factor and the cancer type	Mechanism of action	References
MBNL2	An RNA-binding proteins	MBNL2 is expressed at lower levels in lung and breast cancer tissues, compared to the normal tissues, and acts as a suppressor of metastasis	MBNL2 regulates pAKT/EMT pathway	[139]

CRMP2 Collapsin response mediator protein 2, *IMPAD1* Inositol monophosphatase domain containing 1, *KDELR2*,KDEL endoplasmic reticulum protein retention receptor 2, *MMP* matrix metalloproteinase, *NSCLC* non-small cell lung cancer, *ENO2* enolase-2, *PDAC* pancreatic ductal adenocarcinoma, *EMT*,epithelial-mesenchymal transition, *NSD2* nuclear receptor binding SET Domain Protein 2, *CDK* cyclin-dependent kinase, *ZEB1*,zinc finger E-box binding homeobox 1, *ITGBL1* integrin beta-like 1, *CRC* colorectal cancer, *PMNs* pre-metastatic niches, HuR, Hu receptor, *TGF* transforming growth factor, *VCAM-1* vascular cell adhesion molecule-1, *MAPK* mitogen-activated protein kinase, *ECM* extracellular matrix, *PRC1* Polycomb Repressor Complex 1, *M2* macrophage type 2, Treg, regulatory T cell, *ZNF* Zinc finger protein, *CTC* circulating tumor cell, *TNBR* triple-negative breast cancer, *BCL11A* B cell leukemia/lymphoma 11A, *MBNL1* muscleblind-like splicing regulator 1, *ACKR* atypical chemokine receptor, *CDK* cyclin-dependent kinase, *H3K36me2* histone H3 di-methyl mark on lysine 36, *MBNL2* Muscleblind-like 2

insights into the complexity of this phenomenon will come to the understanding. Knowing more about the whole process and the major drivers of this key phenomenon is critical for identification of the main targets in tumor metastasis. This may let us to think of factors and cells that take major responsibility in tumor metastasis. Novel approaches for suppressing tumor metastasis or reducing the extent of this process are the current concern in the area. In this review, we are focusing particularly over the key steps in metastasis of solid tumors by interpreting papers published recently in the relevant subject. The steps in metastasis are as followings: escape of cancer cells from primary tumor, intravasation, survival maintenance, extravasation (secondary site seeding) and outgrowth (colonization) [5–9]. The whole process is illustrated in Fig. 1.

Invasion

Invasion takes an early step and a pre-requisite to metastatic dissemination [10] so that cancer cell escape is a known characteristic of most advanced tumors [11]. Cancer cells are required to take a plastic phenotype to attain an invasive state [12]. Harnessing this inherent plasticity through evoking a systemic inflammatory response, which is occurring in certain primary tumors like breast, can impede metastatic establishment [13]. Higher frequency of driver mutations (alterations in the somatic copy numbers and mutational burden) [2, 14], and the presence of abnormal chromosomes (called aneuploidy) [14] can also lead cancer cells to drive a metastatic potential.

Single-cell migration vs. collective invasion

Invasion occurs at the tumor-stromal interface (also called Edge or invasive front) of tumor by either single-cell migration (monoclonal metastasis) or collective invasion (polyclonal metastasis). For the former, tumoral cells to attain an invasive phenotype will modify their shape and attachment profiles. For the latter, invasion occurs as a cohesive multi-cellular strands still maintaining cell-to-cell adhesions. Colon, breast, thyroid, prostate, lung and glioblastoma cancer cells take features of collective invasion [10, 15]. Collective invasion is less common but more efficient for taking an efficient metastatic route, as compared to the single-cell migration [16]. E-cadherin is a marker of epithelialization (cell-to-cell adhesion) that is generally downregulated in single-cell invasion. Padmanaban and colleagues in an invasive ductal breast cancer has come to the finding that E-cadherin expression was negatively related to invasion, while its relation with metastasis was positive. The negative relation can be interpreted by collective invasion in which

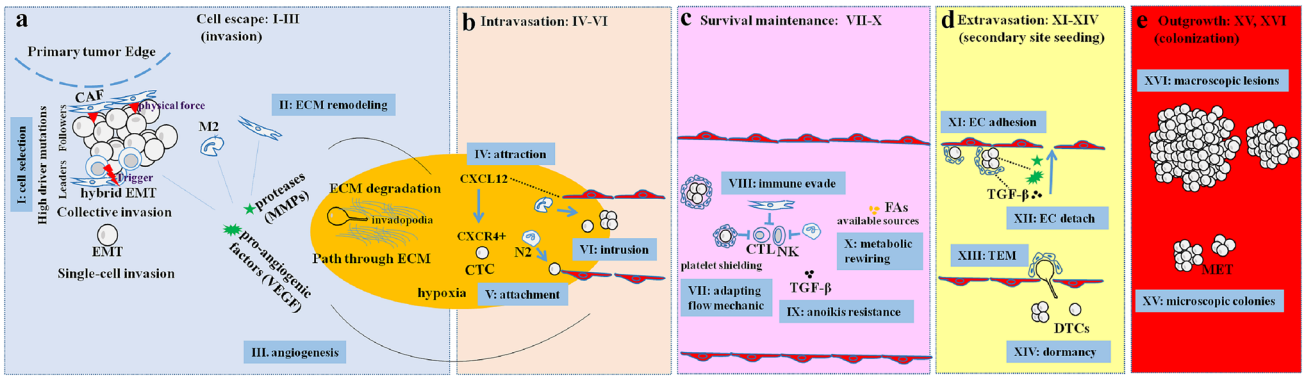

Fig. 1 Sequential steps in metastasis. **a** Cellular escaping. Acquiring an epithelial-mesenchymal transition (EMT) enhances invasive behavior of cancer cells and ECM degradation required for cell escape. Transforming growth factor (TGF)-β released from cancer-associated fibroblasts (CAFs) fosters EMT mediated invasion. Physical forces exerted from CAFs over tumor cells change physical properties of basement membrane (BM) in the interface of tumor and stroma to make it permissive for invasion of tumor cells. Macrophage type 2 (M2) cells and CAFs release ECM degrading factors. M2 cells release pro-angiogenic factors to stimulate angiogenesis and lymphangiogenesis, which are for providing a path for metastasis. M2 cells also support metastatic niche formation. **b** Vascular entrance. Exosomes possibly derived from CAFs increase vascular permeability. Circulating tumor cells (CTCs) with EMT phenotype form invadopodia to pass through ECM (via releasing ECM degrading proteases), dissociate from the Edge front, and to intrude tumor vasculature. Neutrophils facilitate bondage between cancer cells and endothelium, and hypoxia inducible C-X-C chemokine ligand 12 (CXCL12)/C-X-C chemokine receptor type 4 (CXCR4) is a key promoter of cancer cell intravasation. **c** Survival maintenance. CTCs have a potential to avoid anoikis cell death within circulation. EMT is a possible contributor. Flow mechanics also influences cancer cell survival. Rewiring metabolic pathways and reprograming gene expression profile are contributed to cancer cell survival within circulation. Signals of survival are directed by exosomes. Cell-to-cell interactions between cancer cells with CAFs, neutrophils and platelets favors immune escaping. **d** Extravasation (secondary site seeding. Blood platelets through release of TGF-β enable extravasation by disrupting cell-to-cell endothelial junctions. Extravasation is also potentiated by factors released from TME in distant organs into the circulation, such as vascular endothelial growth factor (VEGF). Disseminative tumor cells (DTCs) with EMT phenotype undergo intravascular arrest and evolve cellular protrusions to help trans-endothelial migration (TEM) of cancer cells and their entrance into the ECM of the metastatic site. Exosomes increase vascular permeability (leakiness) and cellular seeding. Physical forces within the circulation would determine the success of distant metastatic seeding. Here, cancer cells may remain dormant for a period prior to colonization, which gives cells the ability to escape immunosurveillance, and to colonize successfully in the distant organ/s. **e** Tumor outgrowth (colonization). DTCs acquire a MET phenotype for proliferation into forming secondary tumors. Exosomes derived from cancer cells restructure the metastatic sites through supporting dynamic interplay with the TME in order for promoting tumor colonization (outgrowth). One of the outcomes of these interactions is reprogramming of glucose metabolism in cancer cells

E-cadherin mediated cell-to-cell adhesion is reported to be critical for reinforcing cancer cell survival during the early step of metastasis [17]. Thus, in the collective invasion two or more tumor cells retain some properties of epithelial cells, such as cell-to-cell adhesion; this enables them to take a collective migration and enter blood circulation as multicellular circulating tumor cell (CTC) clusters. Detection of these cellular clusters within the circulation indicates worse prognosis, compared to the detection of single CTCs by solo [15]. Generally, $^{<}$ 5 tumor cells are dissociated from the invasive (Edge) area of tumor as clusters. Tumor cells dissociated as clusters higher than this amount (5 or higher) without aggregating as glandular structures are called poorly differentiated clusters (PDCs). In colorectal cancer (CRC), PDCs are linked positively with high local and distant metastasis, tumor grade and patient mortality [18]. Components of Wnt/PCP signaling are dysregulated abundantly in solid tumors, and mediate collective cell migration, acting possibly for generation of CTC clusters and their maintenance inside the circulatory system. Targeting Wnt/PCP signaling is thus being a promising for metastatic intervention [19].

Epithelial-mesenchymal transition in tumor cell invasion

Epithelial-mesenchymal transition (EMT) is known as the key mechanism in promotion of cancer metastasis. Generally, a cell type with mesenchymal phenotype is more prone to acquire drug resistance and invasion [20]. The point is that both single cells and collective migratory cells show EMT and stem cell-like characteristics with some differences. Single tumor cells will lose cell-to-cell adhesion and undergo EMT [10]. EMT seemingly drives metastasis initiation [21], and induction of this phenotypic state in tumors cells requires cross-talking with stromal cells, especially with cancer-associated fibroblasts (CAFs) [22], known as the most abundant cell types within the microenvironment

of tumors that their presence within the stroma indicates poor prognosis [23].

Tumor cell clusters in collective migration take a partial (hybrid) EMT [24]. In this type, some cells are served as leaders (in the front-line of migration) and others are followers [10]. The EMT phenotype is more pronounced in leader cells compared to those following them [25]. In an animal model of lung cancer, it has shown that the activity of transforming growth factor (TGF)-β-Smad signaling is important for metastasis. TGF-β acts through diminishing E-cadherin expression [26]. The impedance of EMT by A-Kinase Anchor Protein (AKAP8) is reported to suppress metastasis of breast cancer [20].

There is evidence that tumor cells expressing a mixture of mesenchymal and epithelial phenotypes have more potency to complete metastatic steps [27]. The EMT phenotype in some collective migratory cells infers the heterogeneity of the cellular clusters, and that the pack of cells with such phenotype may suggest the presence of cancer stem cells (CSCs) and/or cancer cells acquiring the EMT phenotype. Compared to the non-EMT cancer cells, cells with EMT phenotype have more developed anti-apoptotic systems and show more resistance to therapy [28, 29].

Mechanical forces in collective migration

Physical forces exerted by CAFs are required for collective migration of tumor cells [30]. Contractile forces exerted by CAFs (independent on matrix metalloproteinases [MMPs]) can pull and stretch the basement membrane segregating cancer cells from the nearby stroma, which finally alter physical properties of this barrier to become permissive for cellular invasion [31].

Intravasation

Cancer cells intruded toward the blood vessels are called CTCs, while their extrusion from the blood and rooted toward the secondary cite/s of metastasis is called disseminative tumor cells (DTCs). Intravasation is a complex process involving numerous intrinsic and extrinsic factors. Tumoral cell intrinsic factors include EMT and production of proteases. Extrinsic factors include the activity of protumor neutrophils (N2 type), fibroblasts (CAFs) and macrophages (M2 type) [32]. Intravasation of cancer cells is facilitated possibly by the abnormal leaky vasculature [33]. Endothelial cells (ECs) secrete C-X-C chemokine ligand 12 (CXCL12), which attracts C-X-C chemokine receptor type 4 (CXCR4)-expressing cancer cells toward the CXCL12 gradient, thus facilitating their further intravasation. The point is that both ligand and receptor in the CXCL12/CXCR4 axis are hypoxia inducible, which indicates a key role taken by hypoxia in intravasation [34] of tumor cell clusters [35]. Perivascular tumor-associated macrophages (TAMs) promote cancer cell intravasation through upregulating EGF [36] and MMP-9 [37] expressions. The stiffened extracellular matrix (ECM) induces invadopodia formation in cancer cells [38]; cancer cells then required to path through ECM in order for intruding the blood vessels. Invadopodia are actin-rich special protrusions from cancer cell membrane effective for taking a pass through ECM by releasing proteases, such as MMPs at the tip of these unique structures. Here, TAMs and CAFs are acting together to cleave the ECM through releasing MMPs (2 and 9) [39–42]. The activity of CAFs in reorganizing the ECM and drilling the holes is important in paving the paths smoother for invading tumor cells [43, 44]. ECM degradation is required not only for creating tumoral cell tracks (paths) [9, 45] but also for liberation of growth factors implicated in promotion of angiogenesis [46] and lymphangiogenesis [47], as well as promoting extravasation of cancer cells [45]. These are indicative that how both ECM stiffness and degradation are functional for promoting tumor metastasis.

Survival maintenance in tumor cells isolated from the primary tumor

CTCs have a half-life of about 2.4 h in human subjects. CTCs although are under exposure to the nutrient rich bloodstream, the cells still exhibit the same invasive phenotype undertaken upon sequestration from the nutrient-low, invasive (Edge) area of tumor. This is possibly due to the short time lived within the circulation, which is not sufficient to make a change in the phenotype of CTCs in spite of accessing to the high amounts of nutrients within the blood [48]. This infers the importance of timing in determining cellular plasticity. The cells rooted toward circulation or when firstly reside within the secondary site/s of metastasis are under continuous exposure to the destructing signals. Within the circulation, CTCs confront several environmental stressors including oxidative stress, shear forces and an assault imposed by immune system [15], so many of the CTCs are condemned to die within circulation. Only a few number of cells remain alive and take the next step, namely secondary site seeding, among these secondary site seeding cells there are also a considerable rate of cell death, so the fraction of cells achieving the final metastatic fate is too low. However, these low fractions of DTCs are highly competent to take an effective metastatic fate. Effective metastasis relies heavily on developing mechanisms of survival, highly efficient to keep the cells 'hale and hearty' from the harm conditions encountered by. There are four potential mechanisms

to maintain survival of cancer cells within circulation (i.e. CTCs) and in the metastatic site/s (i.e. DTCs) as followings:

Rewiring metabolic demands

Metabolic rewiring leads cancer cell survival within circulation and their adaptation to the foreign metastatic milieu [49]. The differences in the metabolic predilection between cancer cells from primary tumor with micrometastatic cells in the secondary organ are important therapeutically. Reduced capacity for glucose uptake and further blockade of glycolysis in CTCs can potentially cause anoikis cell death [16, 50, 51]. CTCs either exploit mechanisms to retrieve their glucose uptake systems [50] or to use other sources of energy available within circulation, such as fatty acids [52].

Higher OXPHOS/glycolysis in micrometastatic breast cancer cells deposited into the lung, and the reversed ratio for primary breast cancer cells reported by Davis et al. highlight the potential of targeting OXPHOS to preclude metastasis of breast cancer [53]. How about primary tumors? Are they more responsive to glycolysis targeting? The response to this question is somewhat challenging, which may be covered by another question. Is there a strategy to enhance glucose content in the TME (for potentiating immune activation), while simultaneously reducing glycolysis (for avoiding cancer cell growth and resistance)? Although TME factors involved in the promotion of cancer cell glycolysis are known to an extent, such as hyaluronan [54], we leave this question open for forthcoming research and see how it can be translated into clinic.

Avoiding anoikis cell death

Anoikis resistance is known as a cornerstone step for a cell attaining a metastatic feature [10]. The capacity to avoid anoikis cell death when cells are detached from ECM is a distinctive characteristic of most epithelial tumors, isolating them from normal epithelial cells [55], and enabling cancer cells to maintain their survival within circulation [10]. The activity of TGF-β signaling is contributed to the anoikis resistance [56], possibly by maintaining an EMT state in CTCs [50]. TGF-β signaling increases the activity of extracellular-signal-regulated kinase (ERK), the activity of which promotes Slug upregulation and resulting inhibition of E-cadherin [57].

CTCs highly express hypoxia inducible factor (HIF)-1α activated in a mechanism independent on hypoxia (CTCs have access to the O_2 within the blood). HIF-1α protects CTCs from anoikis cell death possibly through promotion of metabolic reprogramming (increasing cellular uptake of glucose) [51]. Due to the key roles taken by HIF-1 [58] and TGF-β [29] in maintaining EMT, it is fair to postulate that HIF-1α are activated in CTCs possibly under the influence of TGF-β. It has found that patients with HIF-1α^+ tumors have lower overall survival (OS) and 5-year survival rates [59]. Topotecan is an inhibitor of HIF-1 that its application in tumors like cervical [60] and endometrial [61] cancers, and the results were promising to an extent.

It is presumable that anoikis resistance might has negative relation with Hippo pathway. Wu and colleagues have found an increase in the rate of metastasis in breast cancer tissues upregulating Zinc finger protein 367 (ZNF367). ZNF367 is a transcriptional factor that its upregulation can promote metastasis via suppression of Hippo pathway. Anoikis resistance can be an outcome of the inhibition in the Hippo pathway [62]. The question here is that whether Hippo reactivation can be used as an approach for reducing the number of CTCs and the subsequent reduction of the chance of tumor metastasis? The answer to this question is yes. From what discussed above, and adhering the results of clinical trials in the relevant context activation of the Hippo pathway can be a good prognostic value and an effective approach for retarding tumor invasion. Generally, malignant mesothelial cells inactivate the Hippo pathway, the result of which is the YAP activation [63]. Maille and colleagues carried out a study in patients with malignant pleural mesothelioma, and they found a link between MST1/hippo kinase inactivation with the poor prognosis. Loss of expression for the MST1/hippo pathway resulted in the nuclear accumulation of YAP, silencing of which reduced invasion in MST1-depleted cells [64]. Hippo reactivation by statin has found to be effective for reducing the proliferative activity of hepatocellular carcinoma cells, thus improving the prognosis in the treated patients [65].

Adapting flow mechanics

Flow mechanic and co-option between lymphatic and blood circulatory systems influences the effective transit of cancer cells from a primary tumor, their survival within circulation, as well as extravasation and seeding in the metastatic site/s. Vascular size, flow rates and shear stress can potentially influence survival of cancer cells within circulation and their organotropic seeding. High flow velocity and shear forces (as in arterial vessels) can cause mechanical stress and cancer cell death, while moderate velocity and shear stress (as in venous vessels) favor intravascular arrest of CTCs and their extravasation [66]. CTCs must endure the pressures imposed by blood vessels to maintain their survival [27]. Platelets within the circulation form aggregates around the CTCs in response to stimulatory signals received from cancer cells; these aggregates provide a shield to protect the CTCs from shear stress and immune responses [16, 67, 68].

Immune suppression

To avoid destruction by immune system upon taking a circulatory route, CTCs promote interactions with CAFs, neutrophils and platelets. It seems that the physical impact of CTC clusters can augment the recruitment of these immunosuppressive cells, protecting them from being attacked by anti-tumor immune cells, such as NK cells [15]. A recent study by Owen and colleagues has shown that prostatic cancer cells seeded within the bone will lose their intrinsic interferon 1 (IFN1) signaling; this allows them to repress tumor immunogenicity and to dampen tumor responses to immunotherapy, which indicates a reason for failure of therapy in metastatic solid tumors [69]. The activity of natural killer (NK) is important for suppression of tumor metastasis. Higher expression of epithelial genes and cell-to-cell adhesion markers in CTC clusters is related negatively with the expression of ligands responsible for activation of NK cells. This along with an increase in the rate of monocolonal versus polyclonal metastasis upon depletion of NK cells infers the lower sensitivity of CTC clusters than single-cell tumor cells to NK cell-mediated metastasis blockade [15].

Prognostic value of CTCs

Liquid biopsies can be obtained from cancer patients to track CTCs. Counting the total CTCs and the fraction of mesenchymal-type (M^+) CTCs can be exploited for monitoring therapeutic resistance and for predicting the prognosis in patients with advanced cancers, as for breast cancer [70]. In renal cell carcinoma (RCC) it was found that the initial CTC count (one day prior to the operation) was not correlated with the cancer relapse or metastasis, attested by no considerable difference between metastasis-free patients with metastasis cases. However, counting the number of epithelial (E^+) M+ (mixed) CTCs at 12 months after surgery showed a significant increase, as compared to that performed at 6 months' post-operation or one day pre-operation [71]. Cai and colleagues in a study analyzed the peripheral blood from 91 CRC cancer patients, and they noticed a noticeable relevance between the number of M^+ CTCs with the distance metastasis. The authors collected M^+ CTCs from 73 patients for assessing the expression of cyclooxygenase (COX)-2. 38 patients showed COX-2 expression in the CTCs, and its expression in M^+ CTCs showed higher rates in the metastatic patients, compared to that for non-metastatic cases [72]. Overexpression of COX-2 is linked positively with the invasive behavior of CRC cells [73]. These data indicate that evaluation of CTCs can be used as diagnostic marker and a marker for predicting the efficacy of therapy.

Extravasation

Release of cytokines into the systemic circulation facilitates extravasation of cancer cells within the distant organs. The activity of platelets is important for extravasation of tumor cells. Platelet shielding of cancer cells can cause an imbalance in the homeostatic control over coagulation, thus promoting blood clotting varied from micro-thrombi to pulmonary emboli [8]. The cells promote coagulation and weaken endothelial barrier [68] possibly through releasing TGF-β [67]. Coagulation abnormalities is experienced in more than 50% of all cancer cases and in 90% of patients with metastatic tumors [74]. In non-small cell lung cancer (NSCLC) patients with cancer emboli and the subsequent cerebral infarction the risk of brain metastasis will be increased considerably, as documented in a recent study by Kim and coworkers [75].

TME in distant organs also send signals (such as vascular endothelial growth factor (VEGF) to promote cancer cell extravasation [33]. N2 cells [76] and M2 cells [77] are active in such process. When cells leaving the primary tumor and metastasize to an organ including lung, it is important to be survived for an extended period of time. This means that the metastatic cells to survive for longer times are needed to activate pro-survival signals in the organ of target. Interactions between fibronectin fibrils (promoted by metastatic breast cancer cells) with integrin (in pneumocyte type 1 cells) is an example in this context [78].

Extravasation relies on interactions between circulating tumor cells with endothelium and the secondary site

A key event in metastasis is the interaction between CTCs with endothelium (adhesion between CTCs and ECs is a required step for the subsequent extravasation), and then with the secondary site (the requirement of which is the shedding of endothelial glycocalyx) [79, 80]. Cell adhesion molecules (CAMs) are expressed on both CTCs and ECs [79], and these molecules mediate endothelial interactions, either directly or indirectly, within the specific tissues [81], inferring their role in organ tropism. Barbazán and colleagues in mouse colon cancer model liver metastasis reported the presence of fibronectin deposits in the luminal liver vasculature as a site for attachment of talin1$^+$ CTCs, and the depletion of this focal adhesive component on CTCs has found to impair endothelial adhesion and cellular migration through the endothelium, and the further reduction of liver metastasis [82]. CTCs exploit low-energy adhesion (integrin β3 and CD44 involvement) and stronger adhesions (integrin β1 involvement) for the respective initiation of

transient vascular arrest, and the stable bonds to the endothelium and extravasation [83, 84]. The point is that a combination of biological (EC interactions) and physical (flow dynamics) factors are involved in the vascular colonization of CTCs, namely CTC intravascular arrest of CTCs [83, 85]. Blood flow may cause shear stress in arrest CTCs arrested within the vessels; to oppose the stable bond between CTCs and endothelium is evolved [84].

Besides the adhesion between CTCs with endothelium, CTC-ECM interaction is also important for the final cellular seeding within the secondary site/s. Adhesion between ECM with tumor cells is mediated through integrin family [86]. Tumor cells form protrusions called invadopodia at endothelial junctions to extend between ECs [87]. Williams and colleagues in breast cancer brain metastasis mouse model have found the chemosensing activity of these cellular protrusion. In this model, they notified the presence of chemokine receptors EGFR and GABA receptor in the invadopodia protrusions, which are responding to their respective ligand available within the brain environment. This infers a positive relation between invadopodia protrusions with organotropism (here is brain) of metastatic tumor cells. PAK1 mediates this chemotaxis potential by controlling responses from invadopodia to the ligands in the brain environment, as its suppression renders tumor cells irresponsive to the stromal chemotactic stimuli. Therefore, the 'soil fertility' (brain in this study) is important for extravasation of tumor cells toward the microenvironment of distant metastatic site [88]. Structurally, invadopodia are in fact protrusions of actin filaments, which also include assembly of the intermediate filaments and the cytoskeletal linking proteins. Yoneyamaa and colleagues in a study have found that the assembly of the intermediate filament vimentin and cytoskeletal linker plectin was necessary for generation and stabilization of invadopodia of highly metastatic bladder cancer cells, and that the disruption of the link between vimentin, plectin and actin filaments has found to reduce the capacity of the cells for migrating toward the endothelium and their further intrusion into the site of metastasis [89]. From these results, it could be understood that targeting invadopodia by either addressing their interactions with the stroma of the secondary site or disruption of these protrusions structurally can be effective approaches to repress extravasation and secondary site seeding of tumoral cells.

Outgrowth (colonization)

From the whole cancer cells routed toward circulation a vast majority are condemned to die, and among them only a subset (0.01%) stays alive and form secondary tumors [22, 90]; the survived cells take either one of the two routes: dormancy or proliferation. Generally, DTCs upon entering the secondary site/s undergo dormancy for a while. The proliferative cancer cells are able to grow macrometastatic tumors [14, 91, 92]. Acquisition of either route is determined by multiple intracellular and extracellular signals, as well as by factors rendered from the bone marrow niche [27]. Actin assembly has found to play a critical role for promotion of a dormancy-to-proliferation switch in DTCs. Gau and colleagues in a study attested the positive role for the actin cytoskeleton regulator myocardin-related transcription factor (MRTF) in survival and outgrowth of DTCs from breast cancer. MRTF loss of function has found to reduce the activity of Profilin-1 (Pfn1). Pfn1 activity is essential for regulation of the actin dynamics [93]. 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (Pfkfb3) is an essential mediator of glycolysis that its elevated expression has found to be positively related to the metastatic outgrowth of breast cancer. This is mediated through the positive impact of Pfkfb3 on emergence of CSCs from the dormant metastatic state toward reactivating programs related to the cellular proliferation and outgrowth [94].

Evaluation of p38/ERK ratio can determine whether the cells take the either phenotypes, with the higher p38-to-ERK ratio causing cellular reawaking, while the higher ERK-to-p38 ratio causing cellular dormancy [27]. It has found that activation of p38 can suppress metastasis of breast cancer. Guereño and colleagues exploited the efficacy of Glypican-3 (GPC3) on this process, and they found a switch from mesenchymal-to-epithelial in breast cancer cells mediated by activation of p38, which further impairs metastasis and induce dormancy in tumor cells [92]. This infers how switching from one phenotype to another will define the metastatic potency of a cell type.

The point here is that DTCs chose specific target organs where they can promote metastatic colonization. The term 'organ tropism' is used in this context. Rodrigues and colleagues in a study found that tumor-derived exosomes containing cell migration-inducing and hyaluronan-binding protein (CEMIP) are more prone to take a metastatic predilection toward the brain than the other organs including bone and lung. They noticed that tumoral cell depletion of CEMIP impaired metastasis into the brain [95]. This infers how the microenvironment in the secondary organ is preferentially influenced by signals from primary tumor organ to direct organ tropism.

Promoters and suppressors of metastatic outgrowth

The activity of macrophages

A number of factors are related to the colonization or outgrowth of DTCs (see Table 2). Macrophages play important

Table 2 Tumor cell colonization: Promoters and repressors

Factor's name	Identity and function on colonization	Mechanism of action	Tumor type-species	References
NBR1	An autophagic cargo receptor; promoter	NBR1 is accumulated when autophagy is impaired. NBR1 binds to the specific proteins and organelles within the cytosol for degradation of autophagy	Breast cancer model-mice	[101]
CCR2 & IL-4	CCR2 is a chemokine implicated in the recruitment of monocytes	CCR2 and IL-4 promote the activity of macrophages	Breast cancer bone metastasis-mice	[96]
Calcineurin	An endothelial stabilizing factor, repressor	Calcineurin suppresses production of BMP2	Retinal tumor model, lung metastasis- mice	[140]
Rab11b	A small GTPase implicated in the recycling of surface proteome, promoter	Rab11b mediates ingrain β1 recycling from the cell surface	Breast cancer brain metastasis-human	[141]
IMPACT	A GCN1 inhibitor, repressor	IMPACT inhibits the activity of GCN1-ATF4 and mTORC1	Pancreatic cancer liver metastasis-mice	[142]
TAZ-AXL-ABL2	A feed-forward loop of signaling, promoter	TAZ-AXL-ABL2 acts for colonizing metastatic cells within the brain	Lung cancer brain metastasis	[143]
LIGHT-VTP	A cytokine-based therapy for normalizing the leaky tumor vasculature, repressor	Increasing the presence of effector T cells, reducing the deposition of ECM contents, reducing the hyper permeability in the tumor vasculature	Melanoma lung metastasis-mice, human	[116]
ERK1/2	A member in the MAPK signaling, promoter	ERK1/2 upregulates the activity of CXCR4 & and ANGPT2	CRC liver metastasis	[144]
CEMIP	CEMIP is a WNT related protein that promotes brain metastasis through generation of a pro-metastatic milieu	CEMIP uptake by microglial and ECs induces inflammation in PVNs and EC branching (vascular remodeling) in brain	Breast & lung cancer brain metastasis	[95]
IL1β	A cytokine protein that its elevation is related with the poor prognosis in many solid tumors, promoter	IL1β is produced by bone marrow cells, tumor cells and osteoblasts, and it acts by expanding the metastatic bone niche for further proliferation of dormant tumor cells seeded within the bone	Breast cancer bone metastasis	[145]
CXCR3	A chemokine receptor that is expressed particularly in CSCs, promoter	CXCR3 in tumor cells binds to the CXCL9/10 in CAFs to confer an inflammatory CAF phenotype in lung fibroblasts for lung colonization	Breast cancer lung metastasis	[112]
PCK1/DHODH	PCK1 is a gluconeogenic enzyme, DHODH is an enzyme that acts in pyrimidine biosynthesis, promoter	PCK1 promotes initial outgrowth of metastatic tumor cells, mediated through pyrimidine synthesis under the effect of hypoxia	CRC liver metastasis	[146]
Abl kinases	Abl family of kinases including ABL1 and ABL2 are non-receptor tyrosine kinases that act for transduction of extracellular signals implicated in cellular proliferation and survival	ABL1 and ABL2 promote proliferation of metastatic tumor cells within the brain	Breast cancer ($HER2^+$) brain metastasis	[147]
EGR1	EGR1 is a transcription factorthatis involved in wound healing, growth, outgrowth, apoptosis and differentiation, promoter	EGR1 promotes angiogenesis and osteoclastogenesis in prostate cancer bone metastasis	Prostate cancer bone metastasis	[148]

NBR1 Neighbor to BRCA1, *CCR2* CC chemokine receptor 2, *BMP2* bone morphogenetic protein 2, *GCN1* general control of amino-acid synthesis 1-like 1, *VTP* vascular targeting peptide, *CRC* colorectal cancer, *MAPK* mitogen-activated protein kinase, *CEMIP* cell migration-inducing and hyaluronan-binding protein, *PVN* perivascular niche, *EC* endothelial cell, *IL1β* interleukin 1 beta, *CRC* colorectal cancer, *PCK1*, phosphoenolpyruvate carboxykinase 1, *DHODH* dihydroorotate dehydrogenase, *Abl* Abelson, $HER2^+$ epidermal growth factor receptor 2-positive, *EGR1* early growth response-1

roles for metastatic colonization within the bone [96]. Macrophages have two phenotypes: anti-tumor M1 (so called classically activated cells) and pro-tumor M2 cells (so called alternatively activated cells) [97]. M1 macrophages are implicated for improving the recruitment of T cells, supporting normalization of tumor vessels, and suppressing the activity of M2 cells, whereas M2 cells are responsible for promotion of cancer cell proliferation, angiogenesis, immune escape, invasion and metastasis [98]. A study by Ma and colleagues documented that macrophages expressing CC chemokine receptor 2 (CCR2) and IL4R are more prone to promote metastatic colonization of breast cancer cells within the bone. They noticed high amount of macrophages in the bone of breast cancer in both human and mice, and that ablation of CCR2 or IL4R suppressed metastatic outgrowth within the bone [96]. CCR2 is a chemokine that its interaction with CCL2 (the CCL2/CCR2 axis) acts for recruitment of immunosuppressive M2 cells into the TME, and its blockade can be an approach for restoring anti-tumor immunity [99]. The two clinical trials are performed in this context: in the first study, the immunosuppressive (M2) cells were targeted by CSF-1 inhibition (by emactuzumab) in advanced solid tumors. Roca and colleagues in this study found the attenuation of the immunosuppressive TAMs, but its administration either alone or in combination with paclitaxel did not show anti-tumor activity from the clinical standpoint [100]. In another study, the CCR2 inhibitor PF-04136309 was used in combination with FOLFIRINOX for targeting pancreatic ductal adenocarcinoma (PDAC), and the combination therapy considerably improved the local tumor control (in 32/33 patients) [99].

A suggested strategy is to polarize macrophages from a suppressive into immune activating M1 phenotype. M1 polarization can normalize tumor vasculature, through which more infiltration of $CD8^+$ T cells into the tumor area is possible; the higher presence of $CD8^+$ tumor infiltrating lymphocytes (TILs) will be effective for hampering immune escape capacity of tumor cells, thus reducing the chance of tumor metastasis. This is applicable by targeting factors responsible for polarization of macrophages into M2 cell phenotype, such as VEGF and ILs 4, 10 & 13 [98].

Autophagy

The ideas behind the roles for contribution of autophagy in metastatic systems are somewhat controversial, which can be interpreted differently from what reported in experimental studies with that published for clinical trials. Outcomes of the two experimental studies in the relevant concept are favoring the use of enforced autophagic systems as a potential approach for preventing metastatic outgrowth: Marsh and colleagues reported that the autophagy system acts differently in primary and advanced tumors in which in an autophagic competent tumor, induction of autophagy promotes the growth of primary tumor. A deficient autophagy system has found to cause accumulation of Neighbor to BRCA1 (NBR1), an autophagic cargo receptor, accumulation of which mediates the impact of autophagy suppression on metastasis, as authors noticed that the degradation of NBR1 will restrict the outgrowth of breast tumor cells, and suggest targeting this receptor as a potential strategy to combat outgrowth of tumor cells in the metastatic sites. The authors noticed that the enforced autophagy took a preventive role on colonization of DTCs. This presumably infers the role for autophagy for promoting a dormant state in the DTCs. The authors also evaluated the outcomes on the OS in human breast cancers, and they noticed the positive relation between decreased autophagy with the reduced survival in these patients [101]. In line, Flynn and colleagues in an experimental model of dormant breast cancer have found a diverse relation between autophagy with emergence of breast CSCs from dormant metastatic state. The authors declared that the autophagic machinery can be activated in order for extending the OS by maintaining disseminative CSCs in perpetual dormancy, inhibition of which drives the escape of tumoral cells from metastatic dormancy thus rendering them competent to promote metastatic colonization [94].

Hydroxychloroquine (HCQ) is an inhibitor of autophagy used in clinic. Jyoti and colleagues exploited the role for HCQ when is used in combination with chemotherapy (paclitaxel, carboplatin with or without bevacizumab) in metastatic NSCLC. The authors noticed that the combination therapy improved objective response rate (ORR) and progression-free survival (PFS), $KRAS^+$ tumors in particular, and they declared that HCQ addition to the standard chemotherapy regimen may be an effective approach for overcoming tumor resistance to chemotherapy [102]. Improvement in ORR by addition of HCQ to the chemotherapy regimen (gemcitabine plus nab-paclitaxel) has also been approved in advanced pancreatic cancer by Karasic and coworkers. However, the authors found no improvement in the OS upon addition of this autophagy inhibitor to the chemotherapy [103]. The results of the two clinical trials are mostly objective, so it is impossible to compare the results with that found in the experimental studies, discussed above. This indicates the requirement for more studies both on human cancers and animal models to extract more knowledge about the impact of autophagy machinery on tumors at both lower and higher (or metastatic) stages.

Inflammation

Inflammation plays a key role in tumorigenesis, representing a tight link with the incidence of over half of human cancers [104]. Inflammation plays a key role in all aspects of

tumorigenesis (development and progression of tumor) [105] including its inducible effect on metastatic colonization. The study by Rodrigues and colleagues showed that CEMIP inducible effect on microglial cells and the resulting inflammation is associated with the brain colonization of metastatic breast cancer cells [95]. The link between inflammation with metastatic colonization is also depicted in the study by Du and colleagues. They noticed the positive link between activation of nuclear factor kappa B (NF-κB) in local fibroblasts with the intra-pulmonary colonization of lung cancer cells [106]. NF-κB is a master regulator of inflammation that shows constitutive activity in several advanced-stage cancers [107, 108]. Activation of NF-κB leads to the generation of pro-inflammatory cytokines [109]. Granulocyte colony-stimulating factor (G-CSF) is a pro-inflammatory cytokine that its upregulation in brain tissue has found to be contributed to the recruitment of immunosuppressive neutrophils, which act for driving metastatic colonization [110]. The study performed by So and colleagues showed that colonization of breast cancer cells at distant sites of metastasis requires epigenetic reprogramming. They noticed a positive relation between the activity of inflammatory mediators IL-6 and prostaglandin E_2 (PGE2) with altering pathways required for proliferation, survival and colonization of tumor cells at distant sites, mediated through induction of DNA methyltransferase 3B (DNMT3B) [111]. Pein and colleagues documented that breast cancer lung colonization requires orchestration of an inflammatory phenotype in lung CAFs in a mechanism mediated by NF-κB [112].

Surgical resection of primary tumor may sometimes deteriorate the condition of patients by predisposing them to overt metastasis. Evidence of which is in the study by Miarka and coworkers who found a positive relation between surgical resection of primary pancreatic ductal adenocarcinoma (PDAC) with the outgrowth of micrometastatic lesions seeded within the liver. They notified that abdominal surgery can induce inflammation within the liver, which is a trigger for hepatic stellate cell (HSC) activation into myofibroblasts, enabling the metastatic cells to escape growth arrest [113].

How to combat cancer metastasis?

The complexity of tumor metastasis is a major concern. Despite huge efforts, there is no drug or agent approved specifically to suppress tumor metastasis until now. Some inducers of metastasis in solid tumors is presented in Table 1**,** and the clinical trials carried out to target mediators of metastasis is presented in Table 3. Over viewing of the whole process is required to seek for more appropriate modality to target this devastating condition. The point here is that tumor cells taking an invasive phase reside more within the Edge of tumor, and the cells are more resistant to therapy than the ones not taking the invasive step. The current strategies to combat cancer metastasis are focusing on combating tumor relevant signaling pathways, as well as addressing tumor promoting cell types, in brief targeting signaling and cell types that take major contribution to the whole process. CAFs and platelets are examples of cells important for metastasis, and among the pathways TGF-β, MMPs and signaling related to the EMT are the key contributors, so they can be targeted using appropriate regimen. For example, SMAD4 can be targeted in the TGF-β/SMAD4 pathway using SIRT7, and the results are promising for retarding breast cancer lung metastasis [114]. Due to the important contribution taken by tumor-mediated immunosuppression in metastasis, it is reasonable to think of exploiting adjuvant immunotherapy with the target signaling of metastasis, for example TGF-β inhibition plus PD-L1 blockade, as it has been under the current focus. PRC1 is known to promote metastasis of prostatic cancer cells by inducing stemness and recruiting the immunosuppressive M2 and Treg cells, thus being an appropriate choice to be used in combination with immune checkpoint inhibitor (ICI), such as PD-L1 blockade therapy for suppression of tumor metastasis [115]. As discussed, the architecture of tumor vessels is abnormal. The leaky vasculature in the pre-metastatic niche facilitates tumor outgrowth in the metastatic site. A study by He and colleagues showed that a repair in the leaky vasculature through a cytokine-based therapy will preclude the possibility of metastatic outgrowth and sensitizes established metastatic tumors to ICI [116].

Environmental events such as hypoxic, oxidative and metabolic stresses are the key contributors to the tumor progression and metastasis, so they can be a target [107, 117–119]. Relationship between mitochondrial oxidative stress and immunosuppression within the TME is positive [117], and application of oxidative modulators, such as melatonin [120–123], resveratrol [124], metformin [125] and curcumin [126] has shown promising results.

Tumor metabolism can be targeted to avoid further metastasis, and in tumors forming PMNs, strategies can be expanded to target the main promoters of PMN formation. CAFs take the key role in PMN formation. In fact, primary tumor cells send signals to either recruit or activate fibroblasts in the secondary sites to form PMNs for their subsequent metastasis [127]. For tumors initiating the process without any evidence of detectable tumoral cells in the target secondary organs, strategies can be switched to target signaling implicated in early metastatic cascade, such as EMT. Both single-cell invasion and collective migration use EMT at the early metastatic event, so EMT inhibitors can be used in combination with PD-1/PD-L1 blockade therapy with the aim of retarding the whole events. However, failure of response in the long-term to such therapies is expectable, so knowledge in the area must be expanded to seek for strategies that are more effective, more durable and less invasive.

Table 3 Clinical trials for targeting various events in tumor metastasis

Agent's name	Cancer type	Mechanism of action	Outcome	References
Targeting epithelial-mesenchymal transition (EMT) (effective for retarding tumor invasion)				
Ascorbate	Pancreas	Ascorbate preferentially suppresses NAD in cancer cells which resulted in the ATP depletion and augmented α-tubulin acetylation. Depletion of ATP led to the cancer cell death, and over acetylation of tubulin suppressed mitosis and motility	Suppression of EMT, inhibition of metastasis, and prolonging patient survival	[149]
Galunisertib	Advanced cancers, majorly glioma	Galunisertib is an inhibitor of TGF-β receptor I. TGF-β is a key factor for induction of EMT. Galunisertib reduced pSMAD2 (SMADs are placed downstream of TGF-β signaling)	15% of glioma patients showed a durable SD	[150]
Eribulin	Metastatic breast cancer	Eribulin is a microtubule dynamic suppressor. Eribulin treatment increased EMT conversion (enhanced E-cadherin expression in cancer cells) and improve vascular normality (decreased CA9 expression in tumor vasculature)	Patients showed higher rate and durable responses, explained partially by decreasing the evolving of new metastatic foci in cancer patients	[151]
Targeting vascular abnormality (Vascular abnormality accounts for tumoral cell intravasation and reducing drug delivery)				
AuNPs	Melanoma	Gold NPs (AuNPs) facilitated vascular normalization, enhanced blood perfusion and reduced hypoxia, and reversed EMT	Patients receiving AuNPs represented lower rates of lung metastasis	[152]
Cediranib	GBM	Cediranib is a pan-VEGF receptor inhibitor. Cediranib caused a durable increase in blood perfusion	Patients receiving showed prolonged survival	[153]
Targeting CXCL12/CXCR4 axis (The activity in this axis is responsible for tumor cell intravasation)				
Plerixafor	GBM	Plerixafor is a CXCR4 inhibitor. Post-radiation administration of plerixafor inhibited revascularization of tumor vasculature within the field of irradiation.	Improvement of local control over cancer relapse	[154]
Targeting MMP (MMPs are involved in the intravasation of tumor cells)				
Andecaliximab	Advanced gastric cancer	Andecaliximab is a selective inhibitor of MMP-9. Andecaliximab was used in combination with mFOLFOX6*	The combination therapy represented encouraging clinical benefits (ORR, 48%; and PFS, 7.8 months)	[155]
Targeting hypoxia mediators (HIF-1 protects CTCs from anoikis cell death)				
Topotecan	Metastatic cervical and endometrial cancers	Topotecan is an inhibitor of HIF-1.	27.7% of cervical cancer patients showed stable disease The ORR and OS in endometrial cancer patients were 20% and 6.5 months.	[60, 61]
Hippo reactivation (Hippo inhibition promotes anoikis resistance and invasion of tumor cells)				
Statin	MM	Statins are cholesterol lowering drugs. Statin administration reactivated Hippo pathway and inactivated YAP and its downstream CD44	Statin administration seemingly reduced invasion of tumor cells by decreasing the number of $CD44^+$ CSC-like cells	[63]
Targeting TGF-β (TGF-β promotes tumor invasion, anoikis resistance and ItalicItalicextravasation)				
Galunisertib	Advanced HCC	Galunisertib is a selective inhibitor of TGF-β receptor type I. The drug changed the plasma levels of related proteins	Median OS was improved in the treated patients	[156]

Table 3 (continued)

Agent's name	Cancer type	Mechanism of action	Outcome	References
Galunisertib	Advanced HCC	Galunisertib was used in combination with sorafenib	The combination therapy prolonged OS	[157]
Galunisertib	Pancreatic cancer	Galunisertib was used in combination with gemcitabine. The drug changed circulating proteins	The combination therapy improved OS	[158]
Targeting ERK1/2 (The activity of ERK1/2 promotes EMT and invasion)				
Ulixertinib	Advanced solid tumors	Ulixertinib is an ERK1/2 inhibitor. In this dose escalation trial, ulixertinib showed acceptable pharmacokinetics and safety profile	Ulixertinib showed clinical activity in MAPK mutant patients	[159]
Targeting autophagy (Impaired autophagy is a promoter of tumor outgrowth)				
HCQ	Metastatic NSCLC	HCQ is an inhibitor of autophagy. HCQ combination with chemotherapy** improved ORR and PFS more in $KRAS^{+}$ tumors	Addition of HCQ may overcome resistance to chemotherapy	[102]
HCQ	Advanced pancreatic cancer	HCQ combination with chemotherapy*** improved the ORR	Addition of HCQ did not improve OS	[103]
Targeting chronic inflammation (Chronic inflammation is a key promoter of metastatic outgrowth)				
Chiauranib	Advanced solid tumors	Chiauranib is a multi-target kinase inhibitor. Chiauranib suppresses simultaneously suppresses kinases related to angiogenesis, mitosis and chronic inflammation	A majority of patients (66.7%) receiving chiauranib showed SD	[160]
Targeting macrophage type 2 (M2) cells (M2 cells promote metastatic outgrowth)				
Emactuzumab	Advanced solid tumors	Emactuzumab is a mcA against CSF-1 receptor specific for targeting M2 cells. Emactuzumab used either alone or in combination with paclitaxel	No anti-tumor activity for mono or combination therapy	[100]
PF-04136309	PDAC	PF-04136309 is a CCR2 inhibitor. CCR2 acts for recruitments of M2 cells. PF-04136309 used in combination with FOLFIRINOX****	The combination therapy improved considerably the local control over tumor (97% of patients)	[99]

*oxaliplatin, fluorouracil & leucovorin

**paclitaxel, carboplatin with/without bevacizumab

***nab-paclitaxel & gemcitabine

****irinotecan & oxaliplatin plus fluorouracil & leucovorin

TGF transforming growth factor, *SD* stable disease, *CA9* carbonic anhydrase 9, *CXCL12* C-X-C chemokine ligand 12, *CXCR4* C-X-C chemokine receptor type 4, *MMP* matrix metalloproteinase, *GBM* glioblastoma, *CTC* circulating tumor cell, *HIF* hypoxia inducible factor, *MM* malignant mesothelioma, *CSC* cancer stem cell, *HCC* hepatocellular carcinoma, *OS* overall survival, *ERK* extracellular-signal-regulated kinase, *HCQ* hydroxychloroquine, *NSCLC* non-small cell lung cancer, *ORR* objective response rate, *PFS* progression-free survival, *SD* stable disease, *mcA* monoclonal antibody, *CSF-1*colony-stimulating factor-1, *PDAC* pancreatic ductal adenocarcinoma

Conclusion

Metastasis is a complicated event which may occur early at tumorigenesis or upon tumor taking a progressive step. Metastasis is viewed as a systemic disease, being different from a local primary tumor which is treatable by surgery or chemo/radiation therapy [81]. Metastasis is started by selection of some cancer cells taking an invasive phase at the Edge area of tumor, and is finished by colonization and outgrowth in the secondary area. Despite the real progresses in the field, which resulted in the identification of key drivers of the metastatic steps, research in the area is still continuing, which are to shed more lights in regard with the cross-communications occurring between contributing factors in each step with another.

Compliance with ethical standards

Conflict of interest The authors of the paper have no potential conflict to interests, and authors have read and approve the final version.

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