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Mechanisms of cancer metastasis

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ABSTRACT

Metastatic cancer is almost always terminal, and more than 90% of cancer deaths result from metastatic disease. Combating cancer metastasis and post-therapeutic recurrence successfully requires understanding each step of metastatic progression. This review describes the current state of knowledge of the etiology and mechanism of cancer progression from primary tumor growth to the formation of new tumors in other parts of the body. Open questions, avenues for future research, and therapeutic approaches with the potential to prevent or inhibit metastasis through personalization to each patient's mutation and/or immune profile are also highlighted.

1. Introduction

Patients with metastatic cancer have considerably lower 5-year survival rates than those with localized cancer [1,2]. More than 90% of cancer-related deaths result from metastatic disease [3]. Metastasis is a dynamic, multifaceted process during which normal cells transform into oncogenic cells that proliferate uncontrollably, evade the immune system, become resistant to programmed cell death, stimulate angiogenesis, acquire invasive potential, survive in the bloodstream, and establish cancerous growths in distant organs (Fig. 1) [4,5]. Patients with localized disease have a range of treatment options, often with lower toxicity than the limited treatment options available to metastatic patients [6,7]. The first line of therapy for metastatic cancer is systemic chemotherapy, which can be effective, but patients usually suffer from severe side effects such as organ failure and high infection rates [8]. Recent advances in cancer therapies have generated a small arsenal of less toxic treatments for metastatic cancer, including immunotherapies (e.g., pembrolizumab and margetuximab-cmkb), epigenome-modifying agents (e.g., azacitidine), and drug conjugates (e.g., sacituzumab and govitecan), and new surgical resection techniques that can extend and provide a higher quality of life to patients. However, these treatments have minimal effects on metastatic dissemination, and disease eventually recurs and progresses [2]. A major hurdle in developing new therapies that effectively target cancer is our lack of understanding of the metastatic process. A more robust understanding of topics such as the epithelial-mesenchymal transition (EMT), epithelial-mesenchymal plasticity (EMP), and immune system modulation during cancer progression may usher in a new age of more effective cancer treatment. This review describes our current understanding of cancer metastasis, detailing what is known about each step of the metastatic process. Key future directions are presented, and the need for innovative therapeutic approaches that combine therapies personalized to each patient's mutation and/or immune profile are also discussed.

2. The primary tumor

Cancer is caused by various factors including chemical carcinogenesis, viral infections, epigenetic changes, and somatic mutations [9,10]. Two distinct models inform our current understanding of the transformation of normal cells into cancer cells (Fig. 2). Proponents of the deterministic model hypothesize that the transformation of somatic stem cells through somatic mutations generates a distinct subpopulation

Abbreviations: CSC, cancer stem cells; EMT, Epithelial-Mesenchymal Transition; EMP, Epithelial-Mesenchymal Plasticity; MMP, Matrix Metalloproteinases; MET, Mesenchymal-Epithelial Transition; MDSCs, myeloid derived suppresor cells; Tregs, T regulatory cells; TAMs, Tumor associated macrophages; FOXC2, Forkhead Box C2; ECM, extracellular matrix; MHC, major histocompatability complex; GEMMs, genetically engineered mouse models.

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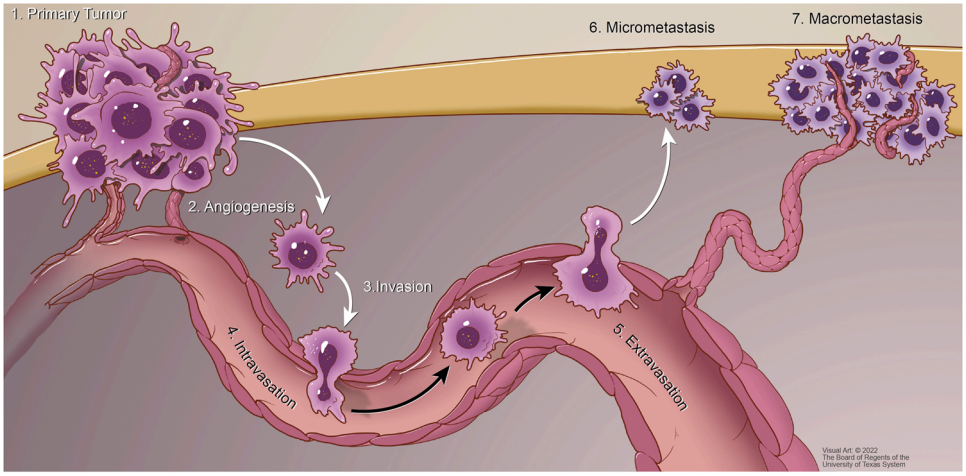

Fig. 1. : Progression of cancer metastasis. Illustration of the stages of progression from primary tumor formation to the establishment of a metastatic tumor.

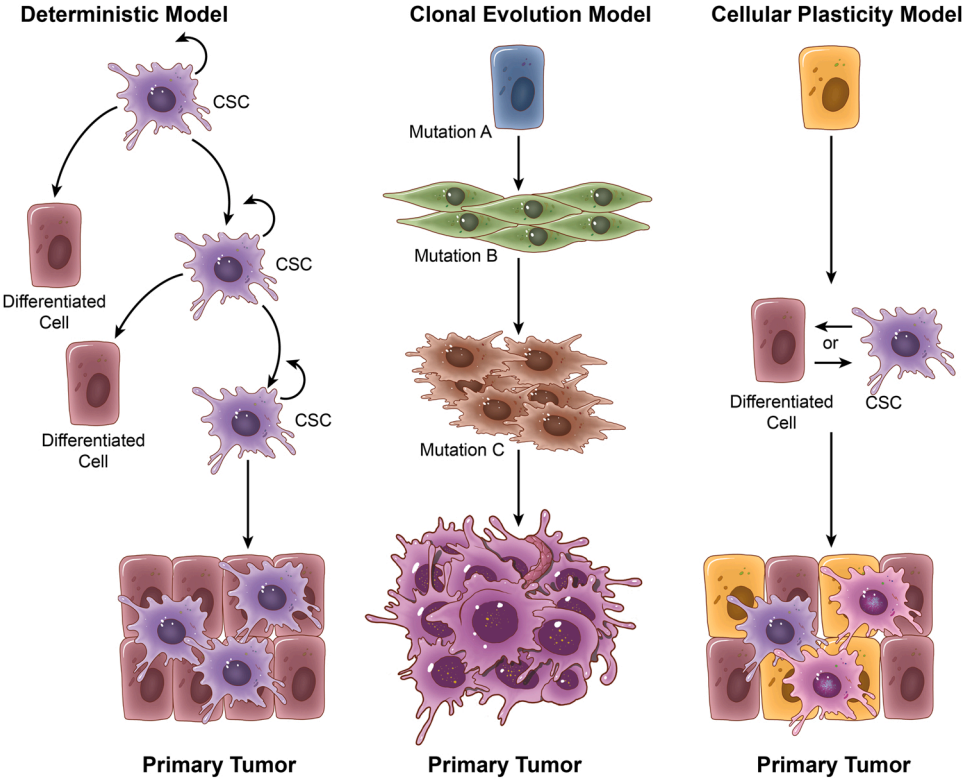

Fig. 2. : Models for tumor initiation. In the deterministic model (left), CSCs are the apex of the hierarchical structure. CSCs, which are capable of self-renewal, are postulated to generate less tumorigenic differentiated cells. In the clonal evolution model (middle), mutations or epigenetic modifications accumulate to provide tumor cells with reproductive advantages over normal cells. The cellular plasticity model (right) assumes that CSCs are not the cells of origin; instead, plasticity is a characteristic of normal cells, which can become either CSCs or differentiated cells resulting in a heterogeneous primary tumor.

of tumor cells with self-renewal capabilities; these cells are known as cancer stem cells (CSCs) [11,12]. CSCs produce daughter cells with limited tumorigenic and metastatic potential that form primary tumors [13,14]. The clonal evolution model of cancer origin (also known as the stochastic model) states that mutations or epigenetic modifications provide a cell with a selective reproductive advantage over normal cells, leading to unregulated growth and a primary tumor [15–17]. In the clonal evolution model, selection at different stages of tumor growth results in increased genetic and epigenetic modifications and decreased tumor-suppressing mechanisms leading to a vulnerability to oncogenesis [18,19]. Neither of these models explains the high degree of heterogeneity in primary tumors. Recently, a hybrid of these two models, the cellular plasticity model, was proposed (Fig. 2). This model postulates that the “cell of origin” is not a CSC. Instead, this model contends that normal cells are inherently plastic and can undergo phenotypic changes when exposed to internal or external stimuli [20]. This innate plasticity enables normal cells to undergo epigenetic and phenotypic changes that allow them to become CSCs. External stimuli can cause the accumulation of many mutations within the cells of a tumor, producing a high degree of heterogeneity in primary tumors [21]. Both types of altered cells result in a heterogenous tumor [21].

Mutations in oncogenes and tumor-suppressor genes lead to uncontrolled proliferation and growth of cells. Mutations in oncogenes need only be heterozygous, with a mutation in one copy of the oncogene sufficient for transformation [22]. In contrast, mutations in tumor-suppressor genes are inactivating and typically need to be homozygous, requiring both copies of the genes to be inactivated for the tumor-suppressor function to be lost [22]. Over 100 oncogenes have been identified, and the best-characterized oncogenes encode RAS, JUN, FOS, ABL, RAF, GSP, SIS, FMS, and BCL2, which are factors involved in

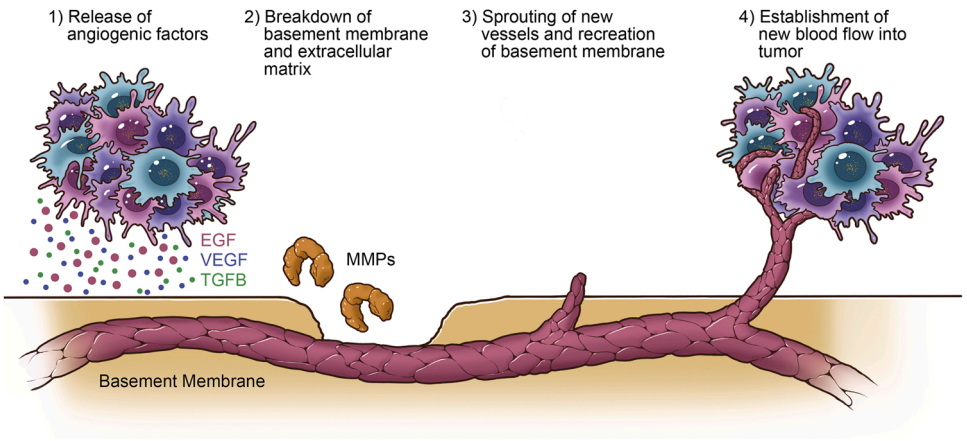

Fig. 3. : The process of angiogenesis. Angiogenesis begins with [1] release of angiogenic factors (e.g., EGF, VEGF, and TGFB), leading to the recruitment of matrix metalloproteinases (MMPs), which [2] break down the extracellular matrix. The breakdown of the extracellular matrix allows [3] sprouting of new blood vessels and [4] establishment of a blood supply to the tumor. The new blood vessels are often malformed and leaky, leading to the dissemination of cancer cells into distant sites.

the regulation of transcription, growth factor signaling, and kinase activity, among others. One of the most well-known oncogenes is *RAS*, with *KRAS* being the isoform most commonly mutated in cancers [23]. Mutations in *KRAS* lead to uncontrolled cell growth and evasion of cell death signals, and induction of chemoresistance [23]. In contrast to the effects of oncogenes, tumor suppressors function to restrain uncontrolled proliferation; the gene encoding p53 is one of the best-characterized tumor suppressor genes [22]. P53 is stabilized upon DNA damage and induces cell-cycle arrest to prevent replication of the damaged DNA. When *p53* is mutated, neither apoptosis nor cell cycle arrest is induced when DNA damage occurs, leading to aberrant cell-cycle progression and uncontrolled proliferation [22].

It remains unclear at what stage of tumor development a primary tumor becomes metastatic [24,25]. It had been assumed that external stress, nutrient deficiencies, and oxygen deficiencies stimulate the migration of cells out of an established primary tumor. However, in $HER2^{+}$ breast cancer, recent evidence indicates that cancer cells disseminate even before the primary tumor is palpable and that circulating cancer cells can seed secondary sites of tumor growth [25]. That there are circulating tumor cells before the clinical detection of a palpable primary tumor suggests that the mechanism of cancer metastasis differs from what is currently accepted. Additional studies into this phenomenon and the role of the EMT in early dissemination are needed.

3. Angiogenesis

A major route of dissemination of cancer cells from the primary tumor to other sites is through angiogenesis (Fig. 3). Newly formed blood vessels in the tumor are malformed, hyperplastic, contain excessive branching, and are highly permeable and leaky, allowing tumor cells to escape from the primary site [26,27]. The malformation of the vasculature in primary tumors results from an imbalance in angiogenesis regulators; for example, *VEGF-A* expression is often elevated in primary tumors [28,29]. In primary tumors, there is suboptimal perfusion of nutrients and oxygen, resulting in hypoxic and acidic regions within the tumor and high interstitial pressure [30–32]. The leaky vasculature also impedes the proper function of immune cells and, in patients receiving systemic chemotherapy, impairs the transport of chemotherapeutic drugs into the tumor [33]. Primary tumors remain small and localized when the angiogenic switch is off. The angiogenic process can be activated months or years after initial tumor formation, and it leads to the renewal of tumor growth, sustained replication of tumor cells, and dissemination of cancer cells from the tumor to secondary sites [34–37]. Thus, cancers that are ostensibly in remission (i.e., that appear to be dormant or present as a micrometastasis) may simply be awaiting the angiogenic switch.

Neovascularization can be halted by treatment with compounds such as the VEGF inhibitor bevacizumab, the mTOR inhibitor everolimus, or the tyrosine kinase inhibitor pazopanib. However, these drugs do not improve overall patient survival [38]. Drugs that inhibit angiogenesis have severe cardiotoxicity and, as with many agents, patients often develop resistance [39–41]. Anti-angiogenic therapies can be used in combination with immune checkpoint inhibitors; the latter help the body recognize and attack cancer cells. The two therapies, in principle, work in concert. The anti-angiogenic treatments provide some immune modulatory effects by upregulating T-cell recruitment and the maturation of dendritic cells, resulting in a higher efficacy of the immune checkpoint inhibitors [42,43]. However, anti-angiogenic treatments are only effective in solid cancers and do not prevent cancer cell dissemination; cancer cells within solid cancers with migratory properties remain capable of invading through the basement membrane and metabolizing through pre-existing blood vessels [44–46].

In addition, cancer cells can disseminate through lymphatic and perineurial routes. In lymphatic dissemination, cancer cells disseminate to lymph nodes and then disseminate to distant organs [47]. Overexpression of *VEGF-C* or *VEGF-D* promotes the growth of tumor-associated lymphatic vessels that meditate cancer cell dissemination [48]. These lymphatic vessels recruit dendritic cells toward the tumor via *CCL21*, found in lymph nodes, creating an immune-suppressive environment [49]. In perineurial metastasis, cancer spreads between neural axons and the surrounding perineural layer. The perineural spread has been described in breast, pancreas, prostate, colorectum, and head and neck cancer [50]. In the perineural spread, a peripheral environment is formed by neural cells, inflammatory cells, extracellular matrix, blood vessels, and immune components to initiate metastasis [51]. Both methods can occur with or without the induction of angiogenesis. That there are multiple routes for dissemination of cancer cells from the primary tumor complicates the targeting of this key step in metastasis.

4. Epithelial-mesenchymal transition

During angiogenesis, not all cancer cells have the capability to enter the vasculature and survive. To successfully metastasize, tumor cells must acquire invasive and stem cell-like properties [5]. Cancer cells accomplish this process by hijacking the developmental program of EMT [52–54]. Activation of EMT causes epithelial cells to lose their apical-basal polarity and cell-cell junctions and gain invasive and migratory capabilities, which are characteristics of mesenchymal cells [55]. Cells in the epithelial state form cell-cell interactions through

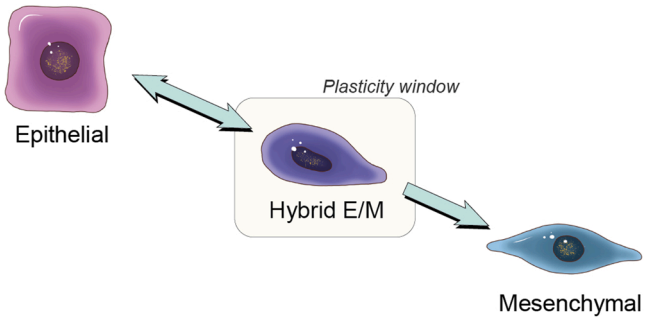

Fig. 4. : EMT and EMP. The epithelial to mesenchymal transition is a spectrum with the two extremes: epithelial and mesenchymal cells. Within EMT exists epithelial-mesenchymal plasticity. This plasticity allows cells to convert between epithelial and mesenchymal states.

tight, adherens, and gap junctions [56,57]. In the progression through EMT, cells acquire stem cell-like properties and become chemoresistant [58,59]. In a population of cells undergoing EMT, cells that simultaneously express both epithelial and mesenchymal markers are detected. These so-called hybrid E/M cells can form cell-cell interactions through E-cadherin- and N-cadherin-mediated adherence, but these interactions are considerably weaker than in the epithelial state [60]. Cells with more mesenchymal properties, which are the primary drivers of metastasis, have cytoskeletons containing actin and vimentin that facilitate migration and invasion [55]. The diversity of cells with hybrid epithelial and mesenchymal states contributes to intratumoral heterogeneity.

EMT is regulated by multiple transcription factors including FOXC2, Twist, Snail, Slug, ZEB1/2, and Goosecoid [52,54,61–63]. These transcription factors activate a variety of signaling pathways, including those mediated by NOTCH, Wnt, GSK3β, and TGFβ, and affect DNA replication, the immune response, and the invasive and migratory capabilities of cancer cells [64–69]. Thus, the EMT program bestows attributes associated with the metastatic process. However, how these transcription factors cooperate during EMT is still not fully understood. For example, transcription factors like Snail and Twist can activate FOXC2, and feedback loops may exist between these factors [54,70]. Additionally, a hierarchy may exist in which factors regulate the function of other factors to promote EMT. Finally, not all carcinoma types rely on the same EMT-regulating transcription factors. For example, Snail activation is frequently associated with EMT in breast cancer, but ZEB-1 is the major driver of EMT in pancreatic cancer [71–73]. EMT-inducing transcription factors such as FOXC2 also regulate stem-like properties [74] including self-renewal abilities and proliferation [75,76].

Recently, it also has been demonstrated that a complete mesenchymal transition is not required for metastatic competence. Rather, it is the hybrid E/M state that promotes cancer metastasis. In other words, EMT is a spectrum of states with different degrees of epithelial and mesenchymal properties, and EMP allows cells to change states within the EMT spectrum (Fig. 4). EMP is defined as the ability of cells to interconvert between epithelial and mesenchymal states [5,77]. This plasticity is critical to cancer metastasis, as shown by the acquisition of a mesenchymal state is important for migration and invasion, but that metastatic seeding requires reversion to an epithelial phenotype in a phenomenon known as the mesenchymal-epithelial transition (MET) [78,79]. It appears that cells with EMP can undergo EMT or MET to adapt to changes in the microenvironment [80–82]. It is also hypothesized that cells with EMP possess higher tumor initiation and metastatic potential than cells on either end of the EMT spectrum [80,83]. Hybrid E/M states are associated with higher metastatic, stemness, and tumor-initiating potentials, therapy resistance, and worse prognostic outcomes [84]. Hybrid E/M states lead to enhanced resistance to therapies such as anti-estrogen therapies and an increase in PD-L1 levels

Table 1
Overview of drugs tested or in clinical testing for treatment of metastatic cancer.

Status	Study Title	Condition	Interventions
Completed	SU5416 and Paclitaxel in Treating Patients With Recurrent, Locally Advanced or Metastatic Cancer of the Head and Neck	Head and Neck Cancer	Drug: paclitaxel Drug: semaxanib
Completed	Gamma Secretase Inhibitor RO4929097 in Previously Treated Metastatic Pancreas Cancer	Adenocarcinoma of the Pancreas Recurrent Pancreatic Cancer Stage IV Pancreatic Cancer	Drug: RO4929097
Completed	A Pilot Study of EZN-2968, an Antisense Oligonucleotide Inhibitor of HIF-1alpha, in Adults With Advanced Solid Tumors With Liver Metastases	Neoplasms Liver Metastases	Drug: EZN-2968
Completed	Gemcitabine, Carboplatin, and Lenalidomide for Treatment of Advanced/ Metastatic Urothelial Cancer and Other Solid Tumors	Urethral Neoplasms Neoplasms, Urethral Cancer of the Urethra	Drug: Gemcitabine Drug: Carboplatin Drug: Lenalidomide
Completed	Radiation Therapy in Treating Patients With Liver Metastases	Metastatic Cancer	Radiation therapy
Completed	Docetaxel With or Without Imatinib Mesylate in Treating Patients With Androgen-Independent Prostate Cancer and Bone Metastases	Metastatic Cancer Prostate Cancer	Drug: Docetaxel Drug: Imatinib Mesylate
Terminated	Interleukin-12 Gene Therapy in Treating Patients With Skin Metastases	Metastatic Cancer Skin Cancer	Biological: interleukin-12 gene Drug: Fludarabine
Terminated	Cell Therapy for Metastatic Melanoma Using CD8 Enriched Tumor Infiltrating Lymphocytes	Metastatic Melanoma	Drug: Cyclophosphamide Biological: IL-12 transduced TIL
Recruiting	A Study to Compare the Administration of Encorafenib + Binimetinib + Nivolumab Versus Ipilimumab + Nivolumab in BRAF-V600 Mutant Melanoma With Brain Metastases	Acral Lentiginous Melanoma Stage IV Cutaneous Melanoma Metastatic Cutaneous Melanoma	Drug: Binimetinib Drug: Encorafenib Biological: Ipilimumab Biological: Nivolumab
Recruiting	Pembrolizumab and Recombinant Interleukin-12 in Treating Patients With Solid Tumors	Metastatic Malignant Solid Neoplasm Unresectable Solid Neoplasm	Biological: Edodekin alfa Biological: Pembrolizumab
Recruiting	Autologous CD8 + SLC45A2-Specific T Lymphocytes With Cyclophosphamide, Aldesleukin, and Ipilimumab in Treating Patients With Metastatic Uveal Melanoma	Metastatic Malignant Neoplasm in the Liver Metastatic Uveal Melanoma	Biological: Aldesleukin Biological: Autologous CD8 + SLC45A2-specific T Lymphocytes Drug: Cyclophosphamide

(*continued on next page*)

Table 1 (*continued*)

Status	Study Title	Condition	Interventions
			Biological: Ipilimumab Drug: Keytruda Injectable Product Drug: Yervoy Injectable Product Drug: GM-CSF Procedure: Non-ablative Cryosurgical freezing
Recruiting	Metastatic Solid Cancer Clinical Trial	Metastatic Cancer	
Recruiting	Autologous Tumor Infiltrating Lymphocytes in Patients With Pretreated Metastatic Triple Negative Breast Cancer	Metastatic Triple Negative Breast Cancer	Drug: Tumor infiltrating lymphocytes (TIL) LN-145 Drug: Palbociclib
Recruiting	Palbociclib With Fulvestrant for Metastatic Breast Cancer After Treatment With Palbociclib and an Aromatase Inhibitor	Metastatic Breast Cancer	Drug: Fulvestrant Biological: Dendritic cell immunotherapy
Active, not recruiting	MIDRIX4-LUNG Dendritic Cell Vaccine in Patients With Metastatic Non-small Cell Lung Cancer	Non-small Cell Lung Cancer Metastatic	Biological: Antigen-specific DTH Biological: Control DTH

[85]. In addition, these hybrid cells create an immunosuppressive environment through the recruitment of myeloid-derived suppressor cells (MDSCs), T regulatory cells (Tregs), and tumor-associated M2 macrophages (TAMs) by releasing cytokines and chemokines such as IL-8 [84]. These immune-modulatory mechanisms are supported by defects in caspase-dependent apoptotic pathways and the down-regulation of antigen-presenting machinery found in hybrid E/M cells [86]. The immunosuppressive environment created by hybrid E/M cells can decrease the efficacy of immune-modulatory therapies. A summary of past and current clinical trials targeting cancer metastasis is given in Table 1. Current clinical trials focus on targeting particularly genomic alterations and immune system modulation rather than use of DNA damaging agents.

Although evidence suggests that EMP is required for metastasis, a host of questions remain. Experimental models do not currently exist that adequately recapitulate the spectrum of EMT, thus limiting our ability to understand how hybrid E/M cells influence chemoresistance, immune modulation, and invasiveness. Furthermore, the context of the carcinoma type is important, as in different carcinomas, EMT is initiated by different pathways [71–73]. Future studies should explore how EMT initiates specific steps of the metastatic cascade and whether alternate programs or dissemination methods require characterized EMT-inducing transcription factors or even EMT at all. Recent literature suggests that a subset of transcription factors such as Nrf2 are responsible for maintaining a hybrid E/M state. Nrf2 functions as a phenotypic stability factor for the hybrid E/M state by inhibiting the completion of EMT [87]. More research is needed to better understand the function of Nrf2 and other EMT-modulating transcription factors.

5. Invasion

Invasion of primary tumor cells into the blood stream can occur through single-cell dissemination or collective migration. Cells involved in each type of invasion have specific morphological features, and the molecular mechanisms of the two types of invasion differ [88]. Both single-cell dissemination and collective migration involve changes in morphology and remodeling of tissue to form migration pathways [89]. Single-cell dissemination involves five steps (Fig. 5). The first is the polarization of the cytoskeleton, which creates a leading protrusion. The second step is the engagement of the leading protrusion with the extracellular matrix (ECM) to form clusters on the surface of the cell to couple extracellular adhesion to intracellular mechano-signaling and

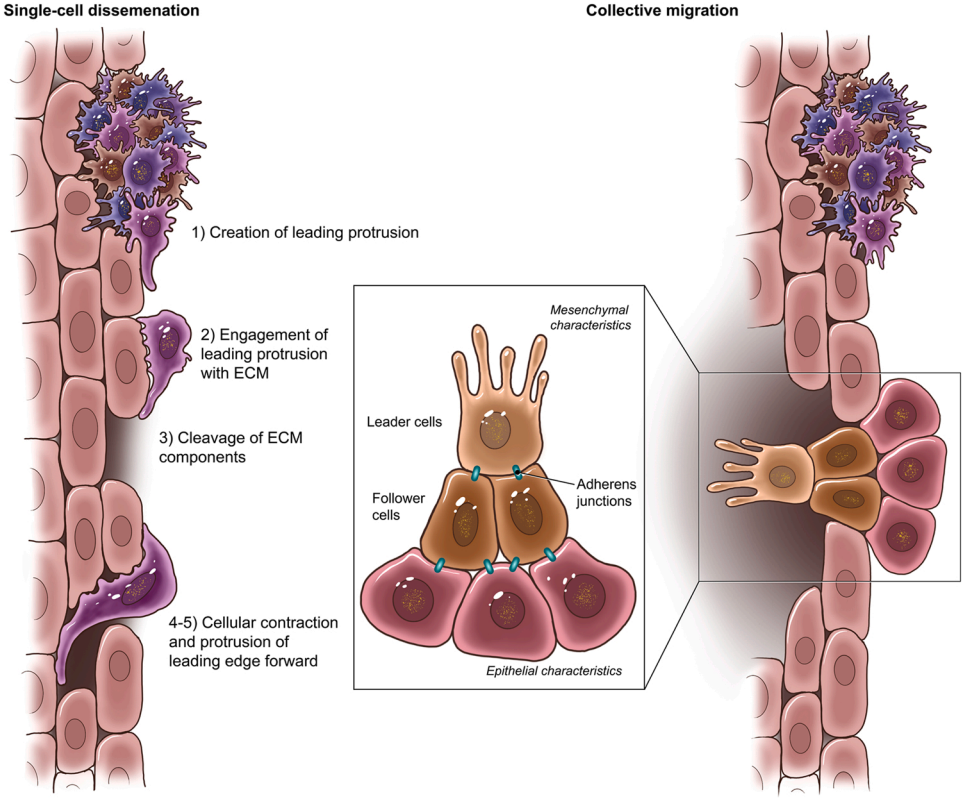

Fig. 5. : Types of invasion during cancer progression. Invasion of cancer cells into blood vessels adjacent to the primary tumor can occur through single-cell dissemination (left) or collective migration (right). Single-cell dissemination involves adaptable single cells that gain mesenchymal traits after protrusion. Collective migration is believed to be the main type of invasion. During collective migration, a cluster of cells invades into a blood vessel. The leading edge contains cells with mesenchymal traits and the follower cells have epithelial characteristics.

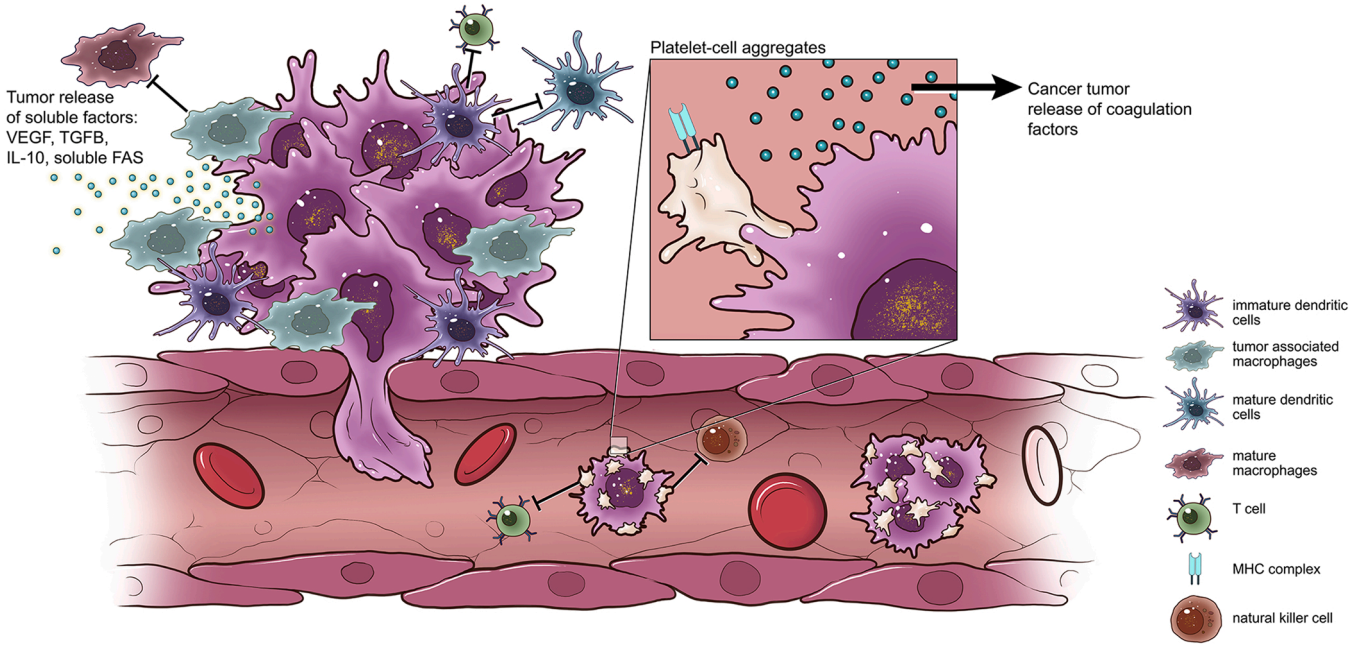

Fig. 6. : Tumor cell-immune system interplay. Cancer cells within a tumor release soluble factors that attract immature dendritic cells and TAMs. The immature dendritic cells inhibit the functions of mature dendritic cells and block T cell activation. TAMs block phagocytosis activation within blood vessels. The aggregation of cancer cells with platelets endows cancer cells with MHC, which prevents recognition by the immune system.

force generation. The third step is the activation of cell-surface proteases at the rear of the leading edge resulting in the cleavage of ECM components. In step four, the tension generated through the actomyosin cytoskeleton results in cellular contraction. Finally, adhesion bonds are detached at the trailing edge, resulting in the forward motion of the cancer cells [90]. Central to single-cell dissemination is a robust cytoskeleton that can cope with migration-related stresses. The mesenchymal intermediate filament vimentin, which is gained during EMT, bolsters a migratory cell's resistance to stress by creating an elastic meshwork that protects against compressive and shear stress [91–93]. The nuclear membrane-associated intermediate filament lamin also provides resilience to mechanical stress. Lamins are expressed in nearly all adult mammalian cells and prevent nuclear fragmentation during invasion [94]. During single-cell dissemination, mechanical stress is distributed across a solitary cell; thus, mechanisms to manage this stress, such as a robust cytoskeletal network, are critical.

In collective migration, the cells that disseminate are interconnected by adhesion molecules such as E-cadherin, N-cadherin, and CD44 [60,95–98]. Collective migration results in higher invasive and metastatic potential than single-cell dissemination [99,100]. Collective migration also appears to protect against chemotherapeutic intervention [89,101]. During collective migration, there is a clear difference in gene expression, morphology, and function between the leader cell and follower cells (Fig. 5) [102]. The leader cells are more mesenchymal, and the follower cells are more epithelial. The leader cells resemble cells involved in single-cell dissemination, except that they retain their adherence to the bulk of the follower cells [89]. The leader cell is linked to the follower cells by adherence junctions; cadherins are the main transmembrane components of these junctions [103]. The follower cells are more epithelial in character than the leader cells and have a tighter organization and uniformity in intracellular contacts [89,103]. The leader cells play a major role in ECM remodeling and are exposed to higher concentrations of external signals (such as soluble factors like VEGF) than the follower cells [104,105]. The leader cells contact the ECM through integrins, such as integrin-β1, which transduce chemical and mechanical signals and aid in cytoskeletal rearrangement, structural reorganization, and morphological polarization of the collective of cells [106]. ECM remodeling is key to tumor cell migration, and the deposition of the matrix, realignment of matrix fibers, and secretion of growth factors by the leader cells cause mechanical stress that aids invasion [101]. Due to differences in position along the EMT spectrum, the leader and follower cells differ with respect to the types of intermediate filaments expressed. The leader cells experience similar invasion-related stresses (e.g., shear stress, cellular compression) to cells that disseminate singly and express cytoskeletal proteins such as vimentin and the basal cytokeratin-14 to mediate this stress [91–93,107–109]. In contrast, follower cells have a more epithelial cytoskeleton composed of cytokeratin intermediate filaments [107]. Cytokeratins distribute mechanical stress across the cluster of follower cells [102,110–112].

Regardless of whether invasion occurs by single-cell dissemination or collective cell migration, invasion results in movement away from the primary tumor, resulting in the entry of cancer cells into circulation. Recent advances in inhibiting invasion involve targeting factors such as vimentin to block assembly of intermediate filaments [113,114]. Disruption of normal vimentin phosphorylation leads to reduced stemness properties [114]. However, further research is needed to fully understand whether the targeting of intermediate filaments can inhibit cancer metastasis.

6. Intravasation and immune modulation

Once tumor cells enter the vasculature by intravasation, they are usually destroyed by shear stress or immune surveillance; less than 0.01 % of cells that leave a primary tumor extravasate successfully upon reaching a secondary site [115–118]. There are two types of intravasation: active and passive [119]. In passive intravasation, most cells die or undergo apoptosis [119]. These cells are believed to be shed because of the dwindling nutrient supply due to the tumor's hypoxic environment and leaky vasculature[119,120]. During active intravasation, cells migrate toward a blood vessel along nutrient and growth factor gradients through the process of chemotaxis [121,122]. These cells digest the ECM and basement membrane and actively intravasate into a blood vessel [119]. In circulation, these cancer cells associate with platelets, allowing the tumor cells to withstand shear force [117,123]. EMT induction within these circulating tumor cells allows for the reorganization of intermediate filaments to withstand this sheer force [124].

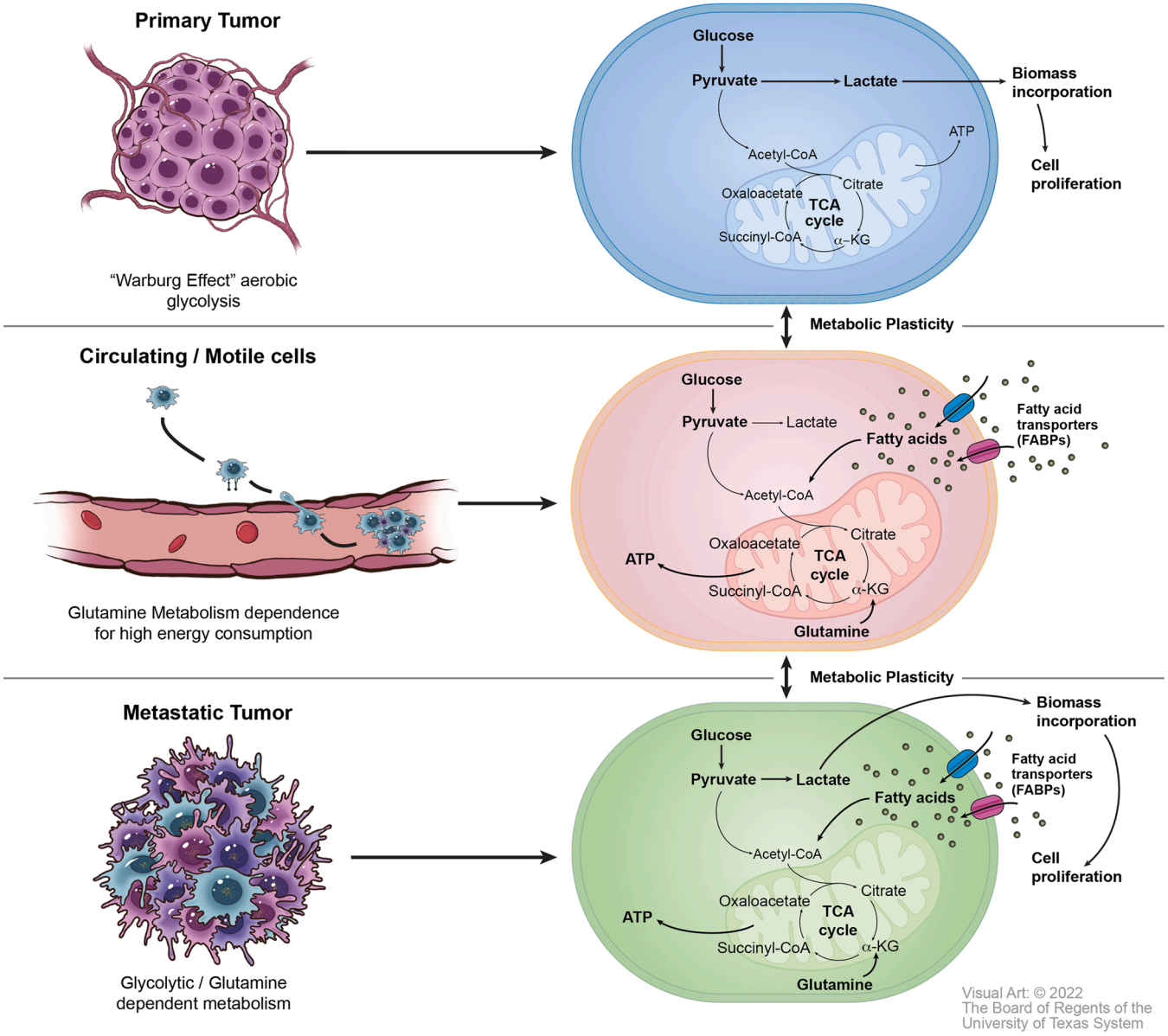

Fig. 7. : Metabolic plasticity. Metabolic dependencies of cancer cells depend upon nutrient availability and energy requirements. During primary tumor growth the main objective is proliferation, thus there is increased conversion of pyruvate into lactate. When a cell enters circulation, there is a switch to glutamine metabolism in order to produce glutathione. Increases in pyruvate and acetyl co-A, lipid accumulation, and fatty acid uptake all help modulate damage to these circulating cells by reactive oxygen species, enhancing cell survival. During macrometastasis formation upon secondary site seeding the cancer cells revert back to an anabolic glycolysis metabolism in which proliferation and growth are the primary objectives. However, metabolism likely differs depending on nutrient availability at the secondary site.

Cancer cells can escape destruction by the immune system through several mechanisms (Fig. 6). Release of soluble factors such as VEGF, IL-10, TGF-β, prostaglandin E, and Fas from tumor cells contributes to the formation of an immunosuppressive environment [125–129]. For example, VEGF secretion leads to the recruitment of immature dendritic cells and macrophages [130,131]. Tumor-associated dendritic cells and tumor-associated macrophages suppress the abilities of mature dendritic cells and macrophages to eliminate tumor cells by blocking T cell activation and phagocytosis [129,132]. In addition, antigen presentation, particularly through presentation by the major histocompatibility complex (MHC), is downregulated on surfaces of tumor cells, allowing them to evade immune surveillance [133]. Furthermore, a platelet cell coating may shield circulating tumor cells from natural killer cells and T cells, and platelets can transfer MHC to tumor cells, fooling the immune system [134]. Immune checkpoint blockade therapies can circumvent immune escape by enhancing T cell-mediated killing of cancer cells [135].

7. Metabolic reprogramming

As cancer cells journey to metastatic sites, their metabolism adapts [136]. Most cancer cells convert glucose to lactate under aerobic conditions; a phenomenon termed the Warburg effect [137]. This mechanism leads to the reduction of ATP and produces an abundance of subunits required for cellular growth through the convergence of pyruvate to building blocks for nucleotide synthesis, meeting the proliferative requirements of primary tumor cells [137,138]. Cancer cells are metabolically plastic, and as cells switch from a proliferative to migratory phenotype necessary for metastasis, there is an increase in pyruvate carboxylate, and a higher percentage of pyruvate reaches the TCA cycle to be converted to lactate [139]. However, the high energy demands of these motile cells mean that the production of ATP through the TCA

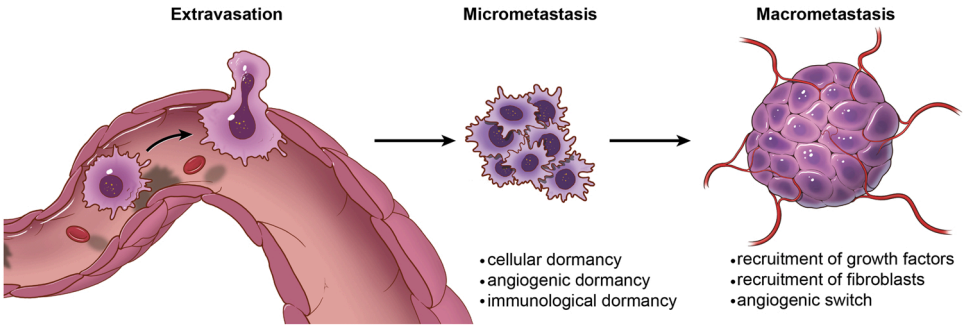

Fig. 8. : Extravasation to micro- and macrometastases. Following extravasation, cancer cells can form micrometastases or macrometastases. In micrometastases, cells are non-proliferative due to cellular dormancy, angiogenic dormancy, or immunological dormancy. A macrometastasis is pathologically detectable, actively recruits growth factors and fibroblasts, and turns on an angiogenic switch to recruit nutrients and oxygen.

cycle is not enough. As glutamine is the most abundant amino acid, it can be utilized to replenish the TCA cycle and produce ATP. In addition to these metabolic switches, an increase in lipid accumulation, fatty acid uptake, and overexpression of fatty acid transporters result from the induction of an invasive and migratory phenotype. These processes are also linked to poor prognosis in patients [136]. In summary, primary tumor cells' main objective is proliferation, whereas energy production is a higher priority in circulating cells. In addition, antioxidant defense mechanisms are upregulated in circulating cells to avoid anoikis [136]. This is accomplished through the upregulation of pyruvate and lactate levels compared to cells in the primary tumor [136]. Pyruvate acts as an antioxidant via a non-enzymatic reaction with hydrogen peroxide, whereas the lactate-driven pentose phosphate pathway and fatty acid oxidation generate NADPH [136]. NADPH is required for the scavenging of reactive oxygen species. The mechanisms of metabolic reprogramming that occur as cancer cells move into circulation are understudied.

Metabolic plasticity is also important as cancer cells adapt to their new environment and create a pre-metastatic niche [136]. Cancer cells at secondary sites rely on increased pyruvate production, glutamine metabolism, and increased fatty acid uptake [136]. Metabolism of cancer cells in pre-metastatic niches appears to be context-dependent depending on nutrient availability at the secondary site. It is clear that cancer cells have the plasticity to switch from catabolic to anabolic metabolism as necessary to adapt to their environment and nutrient availability (Fig. 7). This metabolic plasticity could be targeted by therapeutics that block mechanisms that ensure that energy requirements are met at each key step in the metabolic cascade, taking advantage of differences in metabolic signaling between cancerous and normal cells.

8. Extravasation

To establish metastatic lesions, cancer cells must undergo the demanding process of extravasation, which involves adhesion to endothelial cells at the secondary site, modulation of the endothelial barrier, and trans-endothelial migration into the underlying tissue[132]. The predominant form of extravasation is paracellular migration, in which tumor cells migrate between two endothelial cells [132]. During this process, many ligands and receptors, including selectins, cadherins, and integrins, contribute to the adhesion between the tumor cell and the endothelial cells [140,141]. In addition, extravasation relies on the interaction between tumor cells and blood cells, including platelets, MDSCs, and TAMs [132]. Platelets induce an invasive mesenchymal phenotype by releasing TGFβ1 and granule-derived ATP, modulating endothelial junctions, and promoting tumor cell trans-endothelial migration [132,142,143]. Myeloid cells induce upregulation of VCAM1 and VAP1 on TAMs, which also release VEGF to increase vascular permeability [144]. Cancer cells also form invadopodia on their basal surfaces upon gaining mesenchymal properties; these structures are protrusive and adhesive and release matrix metalloproteinases such as MMP-9 and MMP-2 to break down the endothelial barrier [145,146].

9. Dormancy in micrometastases

The vast majority of cancer cells that successfully extravasate do not result in metastases [147]. Most cancer cells that extravasate remain as single cells or form small clusters of cells called micrometastases and become dormant (Fig. 8) [147,148]. Metastatic dormancy is the process through which single cancer cells or micrometastases become non-proliferative [149]. Dormancy results from the absence of growth factor signaling and the actions of metastatic suppressor genes (cellular dormancy), the absence of an activated angiogenic switch at the secondary site (angiogenic dormancy), and the presence of immunological factors (immunologic dormancy) [149–152]. Some of the characteristics of cellular dormancy are the absence of proliferative and apoptotic markers, a low ratio of ERK and MAPK to p38 MAPK, an inactive JNK pathway, low levels of PI3K/AKT signaling, and activated AXL/Gas6 signaling [151,153–155]. Downregulation or inactivation of MAPK, JNK, and PI3K pathways reduces cellular proliferation, whereas activated AXL/Gas6 signaling inhibits TGFβ1 signaling by activating TGFβ2-mediated growth suppression [151,155]. During angiogenic dormancy, tumor cells are unable to induce angiogenesis, resulting in a lack of nutrient and oxygen influx and blocking metastatic expansion [36,152]. In immune-mediated dormancy, immune defenses suppress the outgrowth of micrometastases [156]. Immune dormancy can result from T cell-mediated release of IFN-γ and from TNF-mediated regulation of cell-cycle progression [156]. In addition, the release of anti-angiogenic chemokines (CXCL9 and CXCL10) by $CD4^+$ T cells contributes to the anti-antiangiogenic phenomenon [156].

The absence of proliferation can be a double-edged sword as it can cause common therapeutic interventions, which target highly proliferative cells, to fail. Cells in dormant micrometastases may remain in a dormant state or may return to an actively proliferating state. Signaling pathways such as AXL/Gas6 can be targeted to induce dormancy, but the durability of this cell suppression is unclear. Inducing dormancy and preventing macrometastasis formation are delaying tactics, not cures for cancer. There is currently controversy about whether the goal of treatment should be to maintain cancer cells in the dormant cell state or to awaken them and target them with chemotherapeutics.

10. Pre-metastatic niche and macrometastasis

When micrometastases escape from dormancy, growth or immune inhibition, the cancer cells can form macrometastases, defined as tumors greater than 2 mm in diameter [157,158]. Re-initiation of growth at a secondary site results from interactions of tumor cells with the microenvironment that establish a pre-metastatic niche [157]. Stephan Paget proposed that each cancer cell must be viewed as an organism capable of

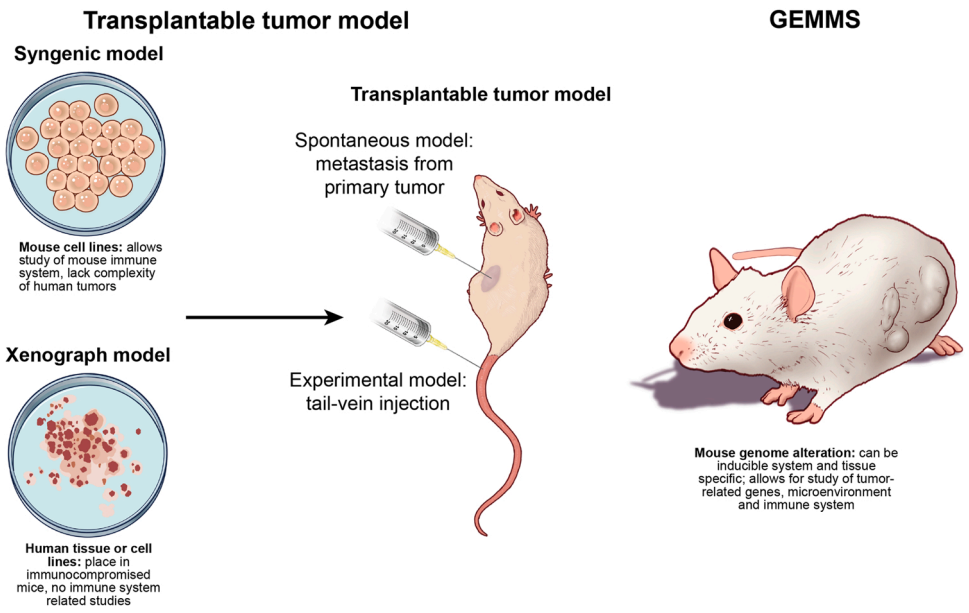

Fig. 9. : Cancer mouse models. Transplantable models of metastasis are either spontaneous or experimental; neither is optimal for investigation of immune system involvement in tumor growth. GEMMS are engineered with constitutive or inducible germline mutations that can result in cell type- or tissue-specific metastasis. GEMMs more realistically mimic patient cancer progression than do transplantable models and allow immune system investigation.

developing entire tumor, and, as when a plant sheds seed and seeds grow only in acceptable soil, cancer cells are spread in all directions, with growth occurring only in tissues with suitable characteristics; this is known as the seed and soil hypothesis [159]. This heterogeneity helps to explain why cancer cells that originate from distinct primary tumor sites metastasize to different secondary sites [160,161]. At a secondary site, tumor cells work in conjunction with macrophages, and stromal cells to initiate, polarize, and establish a pre-metastatic niche [162]. Primary tumors release cytokines and chemokines to recruit TAMs, Tregs, and MDSCs to create an immune inhibitory and tumor proliferative environment [162]. In particular, MDSCs secrete soluble factors such as VEGF, TGF-β, and TNF-α, resulting in proliferation of the cancer cells and inflammation [158,163].

Exosomes, which are extracellular vesicles that contain genetic material, proteins, and lipids are also key to establishment of the pre-metastatic niche [164]. Exosomes derived from the primary tumor have a repertoire of integrins on their outer surfaces that drive exosome adhesion to specific cell types; this can allow tumor cells to bind the ECM or to transfer their contents into recipient cells to dictate organ tropism [160,162]. Exosomes can also carry PD-L1 from the primary tumor site to other sites within the body to suppress the immune response in the pre-metastatic niche [165].

In a suitable pre-metastatic niche, cancer cells must undergo an angiogenic switch to recruit various cells that modify the local tissue, producing an environment that facilitates the growth and expansion of the metastases [166,167]. Although organ tropism is not well understood, research into exosomes and the formation of pre-metastatic niches are expanding our knowledge in this area. It is possible that targeting of exosomes or other factors important for pre-metastatic niche formation could prevent metastatic cancer seeding, but we are far from fully understanding pre-metastatic niche formation.

11. Cancer models

Two general types of animal models are used to simulate cancer metastasis in humans: transplantable tumor models and genetically engineered mouse models (GEMMs) (Fig. 9) [168]. Transplantable models are either syngeneic models or xenograft models [169]. In syngeneic transplant models, the growth of tumors from cell lines derived from mice are studied; thus, the microenvironment is from the same species as the tumor cells [169]. These models lack the genetic complexity of human tumors, and mouse versus human differences can result in observations that differ from those made in cancer patients [169]. Xenograft models are based on the transplantation of human cell lines or human tumor tissues into immunocompromised mice. The absence of a functional immune system limits the utility of these models [169]. The recent development of mice with humanized immune systems has enabled the study of human carcinoma progression in the context of an intact immune system. These mouse models also have limitations, however, such as species specificity of MHC antigens and suboptimal lymphoid architecture, and the multiple model types lead to variabilities [170]. Transplantable models of metastasis are further classified as either spontaneous or experimental (Fig. 7). In spontaneous models, the cancer cells spread from a primary tumor to a secondary site. In experimental metastasis models, cancer cells are injected intravascularly, bypassing primary tumor formation. Transplantable models allow examination of the ability of cancer cells to survive in circulation, extravasate, and colonize the secondary site but are not a physiological representation of metastasis from primary tumor growth to pre-metastatic niche formation [171,172].

Transplantable models do not allow the study of the natural progression of tumors from initial mutations to secondary metastasis, so researchers rely on GEMMs. In GEMMs, the mouse genome is altered to promote tumor formation using a tissue- or cell-specific genetic technique that is constitutive or inducible [171]. GEMMs can be engineered to express oncogenes, such as *PyMT*, *Wnt1,* or *Ras*, under the control of a tumor virus or promoter such as *MMTV* [173]. GEMMs allow study of tumor-related genes, the microenvironment, and the immune system [173,174]. Unfortunately, metastasis development in GEMMs may require months, so these studies are usually time-consuming and labor-intensive. Furthermore, primary tumors may grow rapidly in GEMMs and may be difficult to surgically resect to allow the study of metastatic burden [175]. Despite these limitations, the use of mouse models has considerably furthered our understanding of metastasis.

12. Future directions

Although progress has been made in understanding the mechanisms

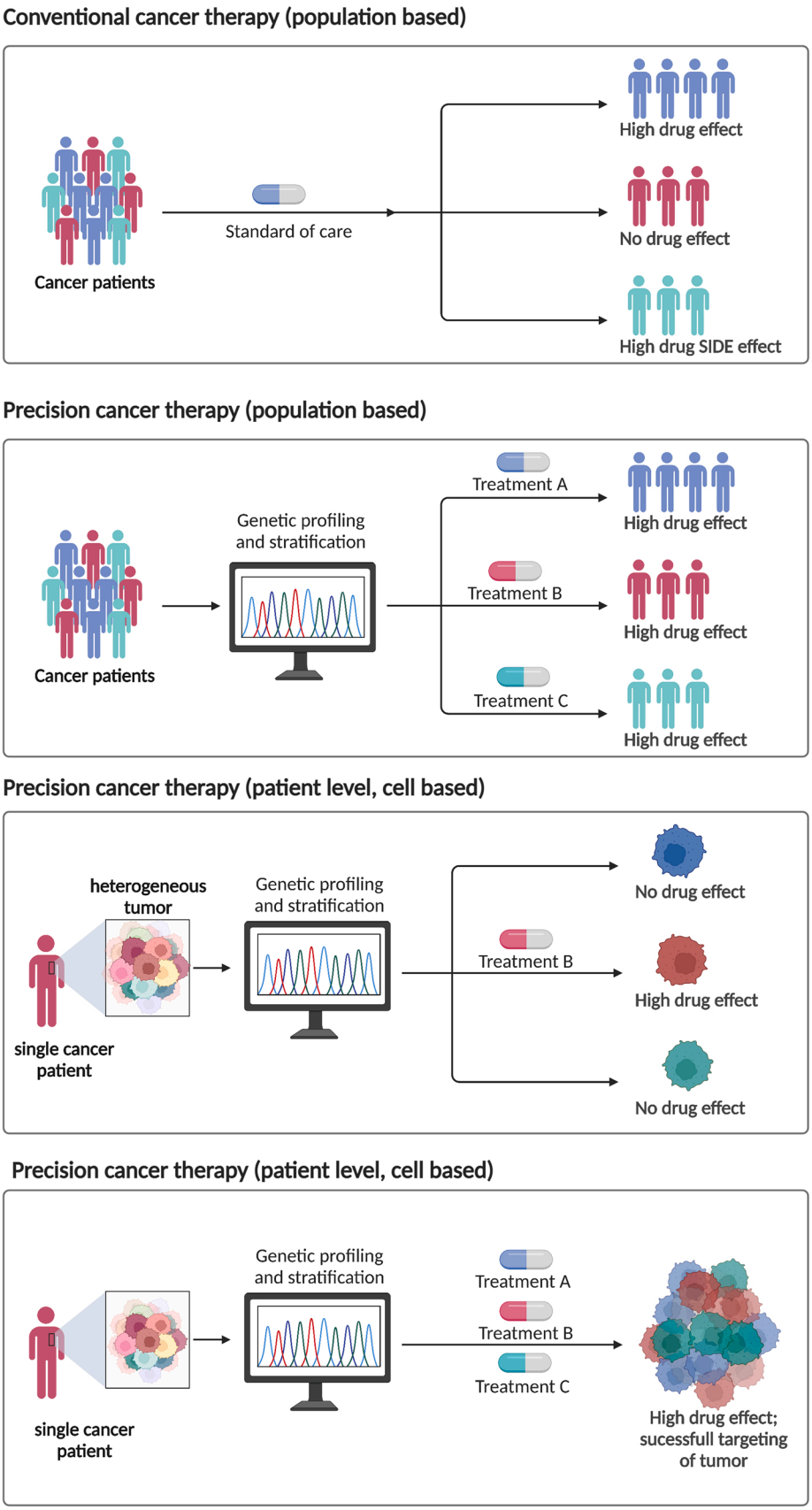

Fig. 10. : Precision cancer therapy. Precision therapy is envisioned as a targeted approach unique for each individual within a population. The use of genetic profiling or other types of analysis will identify targetable pathways. The reality of prevision therapy is that each individual tumor found in each patient contains multitude of genetic abnormalities, thus, use of a single treatment might lead to selective advantages of certain mutations over others.

that result in the growth and spread of cancer, the complexity of the metastasis process has made blocking the process difficult [176]. Immunotherapy interventions have been very successful in treating certain cancers, as celebrated by the award of the 2018 Nobel Prize in Medicine to James Allison and Tasuku Honjo for their discoveries of immune checkpoint proteins CTLA-4 and PD-1 [177]. Immunotherapies activate the patient's own immune system, enabling it to kill cancer cells. Although no immunotherapy tested to date has successfully inhibited metastatic disease [177], clinical trials are underway exploring the use of immunomodulatory therapies (e.g., ipilimumab, Keytruda, and many more) in combination with chemotherapeutic agents (Table 1).

Drugs focused on targeting proteins that trigger steps in metastasis, including metabolic inhibitors, angiogenic inhibitors, and EMT inhibitors,(Table 1) have not been successful clinically for various reasons. Metabolic inhibitors have higher toxicity to normal tissues than tumor cells [40,178–180]. Angiogenic inhibitors lead to hypoxic environments and the resulting selection of cancer cells with higher metastatic competence [181]. EMT inhibitors have been proven difficult to translate into the clinic as they have the potential to increase cancer stemness and host organ homing [182–184]. The limited efficacy of metabolic, angiogenic, and EMT inhibitors are likely due to the dissemination of cancer cells prior to primary tumor detection and to the adaptability of tumor cells. In the case of angiogenic inhibition, cancer cells can induce vascular formation independent of angiogenesis, and pathway switching also occurs during metabolic inhibition of EMT [184].

Prevention or treatment of metastatic cancer may require combination therapy such as immunotherapy plus a metabolic inhibitor [185–187], or it may prove impossible to prevent metastasis requiring that the consequences of the metastatic cascade be targeted. For example, the treatment could focus on circulating tumor cells, metastatic lesions, or dormant metastatic cells. Nanomaterials and antibody-drug conjugates could target drugs specifically to tumors to limit the side effects of chemotherapies [188]. Nanomaterials may enable drug delivery to micrometastases, and a combination of targeted chemotherapy and immunotherapy may make checkpoint inhibitors useful against metastatic cancer [185,188,189]. Immunotherapy and metabolic inhibitors, which target two aspects of metastasis, might also prove efficacious [186].

Personalized therapies, in which treatment options are adapted to a patient's cancer specific mutation status or in which components of the patient's immune system, such as T cells, are exploited as treatment options [120,190]. However, using personalized or targeted therapies to treat cancer metastasis will be challenging. While personalized medicine is advocated as the cure for cancer, the lack of targetable mutations, logistics in genomic screening, and tumor heterogeneity remain problematic (Fig. 10) [191]. The idea of a personalized treatment to cure cancer metastasis based on genetic profiling does not consider the genetic and epigenetic heterogeneity of cancer cells found in each patient. As such, a precision medicine approach that attacks the dominant mutation in a patient's tumor may slow cancer progression but will not be a cure as cancer cell clones with different mutations may remain and relapse. Thus, development of personalized treatments must ensure that all cancer cells are eradicated.

Developing more effective therapies for treating patients with metastatic cancer will necessitate a better understanding of cancer metastasis. A deeper exploration into hybrid E/M cells, EMP, metabolic rewiring, immune reprogramming, and the intertwining of these programs will be required. The future of cancer metastasis treatment will depend on our comprehension of the processes that initiate metastasis, how EMP is gained in certain cells, what causes organ-specific metastatic homing, and how the immune system is exploited by cancer cells. No single agent or treatment will be the cure, but rather a multi-targeted approach will be key in combating cancer metastasis.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data Availability

No data was used for the research described in the article.

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