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Metastasis

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SUMMARY

Most cancer-associated deaths occur due to metastasis, yet our understanding of metastasis as an evolving, heterogeneous, systemic disease and of how to effectively treat it is still emerging. Metastasis requires the acquisition of a succession of traits to disseminate, variably enter and exit dormancy, and colonize distant organs. The success of these events is driven by clonal selection, the potential of metastatic cells to dynamically transition into distinct states, and their ability to co-opt the immune environment. Here, we review the main principles of metastasis and highlight emerging opportunities to develop more effective therapies for metastatic cancer.

INTRODUCTION

Metastasis, the growth of cancer cells in organs distant from the one in which they originated, is the ultimate and most lethal manifestation of cancer. The vast majority of cancer patients die as a consequence of their metastatic disease and not due to primary tumors. Metastasis encompasses a series of biological events in which cells from a primary tumor progressively acquire the capacity to invade through the mucosa into deeper tissues; disseminate through the blood, lymphatics, or through direct infiltration of neighboring structures; seed distant organs; and eventually resume proliferation at distant sites to colonize these organs.[1–3] Each of these events is driven by the ability of tumor cells to adopt different phenotypic cell states and co-opt their surrounding immune and stromal cells in the tumor environment to support their growth and evade the immune system.[4] Unlike primary tumors, which often can be cured with local therapies such as surgery and radiation, metastatic cancer is a systemic disease that affects multiple organs, either by directly colonizing organs and compromising their function or by altering their metabolism through altered secretomes, eventually leading to death.[1,5] Even response to systemic treatment can be drastically different in primary versus metastatic disease in the same patient. Clinically evident metastasis remains largely incurable with few exceptions, due to acquired resistance of metastatic tumors to existing therapies.

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DECLARATION OF INTERESTS

K.G. is an inventor on patents related to targeting metastatic cancer.

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Technological advances in the form of next-generation sequencing approaches have been transformative for both basic cancer science and clinical oncology. They have enabled the accumulation of tumor genomic data characterizing disease-specific expression patterns and tumor microenvironments, tracking disease progression and resistance patterns in response to therapies through sequencing circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs), and illuminated the heterogeneity and clonality of primary and metastatic tumors. Together, these efforts have generated unprecedented development of new biomarkers and drug targets and rapidly advanced our understanding of the underlying biology of how metastatic cells hijack their host environments to ensure their survival. Here, we review the established paradigms of metastasis and emerging principles essential for dissemination, survival, and outgrowth of metastatic cells and also highlight recent discoveries and conceptual advances as well as therapeutic implications for the treatment of metastatic cancer.

PHASES OF METASTASIS

Metastasis can be divided into three phases that can overlap in time—dissemination, dormancy, and colonization—during which cancer cells undergo a succession of steps to invade tissues, survive in transit, and colonize organs, collectively termed the metastatic cascade[1,2] (Figure 1). During dissemination, tumor cells harboring oncogenic driver mutations invade through the basement membrane into deeper tissue layers, acquiring competence to survive in the absence of niche-specific growth factors. This is followed by intravasation into proximal blood vessels or lymphatics and ultimately extravasation into distant organs through transendothelial migration and capillary disruption, migration along neurons, or direct local spread into adjacent spaces such as the peritoneal or pleural cavities.[1,2,6] In circulation, CTCs suffer extensive attrition due to physical, redox, and immune stressors, demonstrated in mouse models and inferred from the low number of CTCs in the blood hours after removal of the primary tumor[7–9] (Figure 1). CTCs circulate as single cells or in microclusters enriched with stem-like cancer cells, coated with platelets, neutrophils, or tumor-derived stromal cells, which can protect them from immune surveillance and endow CTC clusters with greater metastatic potential than would single cells.[7] Reaching distant organs, disseminated tumor cells (DTCs) are further eliminated by high oxidative stress, lack of supportive growth factors or nutrients, and active hostile immune defenses in form of tissue-specific macrophages, natural killer (NK) cells, infiltrating T cells, and other immune surveillance mechanisms.[4,6] Surviving DTCs can enter a variable period of dormancy (Figure 1), during which they either exit the cell cycle or enter a dynamic equilibrium with bursts of proliferation countered by immune elimination or other stromal containment of proliferative clones by the tumor microenvironment (TME), such that there is little net metastatic outgrowth.[1,10] Dissemination and dormancy are considered micrometastatic disease because DTCs are undetectable by clinical imaging and patients are unaware of subclinical disease. Clinically apparent macrometastases are derived from successful metastasis-initiating cells (MICs) that have adapted and co-opted their TME to ultimately enable outgrowth and organ colonization by co-opting regenerative, angiogenic, and immune-suppressive programs. The metastatic cascade represents an evolutionary continuum of ongoing cellular and microenvironmental

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reprogramming and clonal selection of cancer cell subpopulations capable of withstanding selective microenvironmental pressures.[1] This results in unfettered tumor growth, leading to organ dysfunction, collapse of systemic organismal function, and ultimately death. This continuum of changes encompasses multiple domains that can be understood as principles of metastasis (Figure 2).

PRINCIPLES OF METASTASIS

Metastatic cells require a large number of traits to undergo each step of the metastatic cascade. Some of these traits originate in the primary tumor and are the result of genetic mutations that activate oncogenes and disrupt tumor suppressor genes, enabling uncontrolled survival and proliferation, self-renewal, migration, and invasion.[11] But even with these oncogenic traits, the vast majority of cancer cells leaving the primary tumor fail to survive and form distant metastasis.[1,2,8] Metastasis thus poses a major evolutionary bottleneck. Metastasis-specific traits emerge either (1) by selection of these clones from the genetic heterogeneity present in the primary cancer cell population or (2) by non-genetic dynamic adaptation of cells leaving primary tumors to the demands of the distinct phases of metastasis (Figure 2).

GENETIC SELECTION

Modes of tumor evolution

Phylogenetic analysis of lineage relationships among matched primary tumors and metastases have suggested two broad modes of tumor evolution during metastasis[12–15]: in the linear model, metastatic cells disseminate late from the primary tumor, whereas in the parallel evolution model, cancer cells disseminate early and share few mutations with and evolve separately from the primary tumor. Different modes of seeding (polyclonal, monoclonal, and metastatic reseeding) further increase cellular heterogeneity of metastatic lesions.[13,16] Genomic clonal evolution studies demonstrate both early and late dissemination in different tumor types and individuals.[12,14,17] Recent multiplexed parallel lineage-tracing efforts combining CRISPR-Cas9 editing with single-cell RNA sequencing, creating an evolvable barcoding system, showed that linear and parallel evolution can also coexist.[18]

Spatial influence of the TME

Spatial genomics have yielded further insights into intratumoral heterogeneity, subclonal variation, and the role of the local TME.[19] The TME comprises diverse immune and stromal cells that interact and co-evolve with cancer cells and can have both pro- and anti-tumor effects.[4] Spatial analysis of the TME in breast cancer patients revealed that distinct cancer subclones were associated with varying degrees of local immune infiltration.[20] An *in vivo* spatial CRISPR screen in murine lung adenocarcinoma demonstrated that specific gene knockouts in tumor cells lead to characteristic changes in the immune environment and T cell infiltration.[21] This suggests that tumor cells experience localized variations in selective pressure and, conversely, that subclonal genetics might instruct the establishment of local microenvironmental niches.[22,23] Loss of heterozygosity of human leukocyte antigen class I alleles was enriched in tumor subclones selected for metastasis

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in multiple cancer types,[23] whereas strongly immunogenic neoantigens were preferentially lost in recurrent pancreatic cancer.[24] Pre-existing germline genetics can also influence metastatic propensity; recent studies have demonstrated that germline variants of the APOE gene alter anti-tumor responses of immune cells and lead to different TME compositions and outcomes in melanoma mouse models.[25] Increasing application of spatial and lineage-tracing technologies in both clinical samples and preclinical experimental models promises to reveal further mechanistic insights into the influence of the TME on MIC selection and how MICs co-opt TME constituents to support their survival.

Copy number alteration

Although mutations are critical for tumor initiation, large-scale genomic studies have revealed that paired metastatic and primary tumors exhibit similar somatic mutational landscapes, and metastasis-specific somatic mutations cannot be readily identified.[26–28] Where novel somatic mutations are identified later in tumor progression, their functional significance is typically linked to therapy resistance.[28–30] However, metastases exhibit increased copy number amplifications and chromosomal abnormalities compared with primary tumors in some cancers but not in all.[26,31] Providing evidence for a functional role for aneuploidy in driving metastasis, Myc amplification was shown to promote metastasis by recruiting more tumor-associated macrophage (TAMs), leading to greater bloodstream invasion.[32] Chromosomal instability could further promote metastasis by increasing cytosolic DNA, leading to activation of the cGAS-STING cytosolic DNA sensing pathway and downstream NFkB signaling.[33] However, the extent to which aneuploidy plays a role in causally driving tumor progression in most cancers remains unresolved. Overall, genetic changes could be permissive for metastasis but in most cases are insufficient to explain why some cancer cells metastasize while others do not.

PLASTICITY

Single-cell profiling of clinical samples and sophisticated animal and patient-derived tumor models have revealed tremendous intra- and inter-tumor transcriptional heterogeneity in advanced cancer, not explained by acquired genomic alterations.[34,35] Phenotypic plasticity, the ability to undergo dynamic non-genetic adaptations to the distinct stresses of the metastatic cascade and respond to changes in the TME, is thus emerging as an overarching hallmark of metastasis.[1,36] Plasticity allows MICs to enter and exit stem-like states, trans-differentiate, and dynamically adjust to metabolic, redox, and immune stressors[37] (Figure 3A). Recent studies in metastatic mouse models demonstrated that cancer cells undergo multiple phenotypic transitions during progression from primary tumor to MICs and organ-specific macrometastasis.[37–40] *In vivo* lineage tracing and single-cell transcriptomic profiling in murine lung adenocarcinoma models revealed that select primary tumor subclones exhibiting high plasticity were highly enriched in matched metastases, suggesting that plasticity at the primary site was a prerequisite for metastasis.[17] An important emerging theme is that the same molecules and pathways could have distinct roles in different contexts during tumor progression, notably during primary tumor proliferation versus tumor dissemination and dormancy. Thus, the historical paradigm of designating cancer-associated genes as oncogenes, to which tumor cells can become addicted, or tumor suppressors, whose

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loss is always deleterious, frequently does not hold in the context of dynamic metastatic plasticity.

Stress-responsive regulation of gene expression

A large body of research has led to the discovery of gene expression programs specific to discrete steps of the metastatic cascade.[3,6,11] Epigenetic mechanisms, including DNA methylation, histone modifications, 3D chromatin organization, and noncoding RNAs, affect gene expression without changing the underlying DNA sequence, offering a variety of possibilities for dynamic responses to microenvironmental inputs.[36,41] Activation of key transcription factors, including SOX10 and RUNX, have been shown to mediate cellular plasticity along an epigenetic continuum toward metastasis.[42,43] Dynamic changes in histone modifications can alter chromatin accessibility and structure, and a high-fat diet containing palmitic acid has been shown to drive metastasis through deposition of histone H3 lysine 4 trimethylation in oral squamous cell carcinoma and melanoma models.[44,45] MICs employ diverse stressresponse mechanisms to rapidly adapt their cell states through posttranscriptional gene regulation, autophagy,[46] and the unfolded protein response, an emerging integrator of stress signals and determinant of metabolic and immune evasive plasticity across solid tumors.[47,48] RNA-binding proteins orchestrate cellular reprogramming and TME interactions during metastasis, regulating RNA modifications, mRNA splicing, localization, translation, stability, and degradation.[49–53] Overexpression of the RNA N6-methyladenosine (m^6A) reader YTHDF3 promotes cancer cell interactions with brain endothelial cells and astrocytes and metastasis by increasing translation of m^6A-enriched transcripts.[54] Cancer cells with low levels of mitochondrial RNA cytosine-5 methylation (m5C) have reduced translation of mitochondrially encoded oxidative phosphorylation complex members, blocking the switch from glycolysis to oxidative phosphorylation required to power metastasis of oral squamous cell cancer.[55] Dynamic expression of specific tRNAs further contribute to plasticity by modulating the translation dynamics of genes with differential codon usage.[56,57] Together, these studies show how cancer cells dynamically toggle their phenotypic states by adapting their epigenetic, transcriptional, and post-transcriptional landscapes in response to signaling cues from the evolving organ-specific microenvironment.

Metabolic adaptation

Although the metabolic shift of tumors to aerobic glycolysis (the Warburg effect) is a well-established hallmark of cancer, there is growing appreciation that flux through metabolic pathways can be dynamically altered during cancer progression.[58] DTCs can adapt their metabolism to environmental stresses including oxidative stress or nutrient availability to survive in circulation and upon seeding distant organs.[59–62] Metastatic cancer cells from diverse tumors have been shown to increase uptake, synthesis, and utilization of lipids as a fuel source.[63–65] Micrometastatic tumors can employ autophagy and macropinocytosis to deal with nutrition or growth-factor scarcity in alien organ environments.[66,67] Overall, cancer cells alter their metabolism from an anabolic to a catabolic state during circulation and seeding and back to an anabolic state to support metastatic outgrowth.[58] Reactive oxygen species (ROS) can induce cell death through ferroptosis. However, cancer cells can switch to a ferroptosis-resistant state *in vivo* by

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decreasing synthesis of polyunsaturated ether phospholipids[68] or through exposure to oleic acid in lymph, in turn increasing metastasis.[69] In murine pancreatic ductal adenocarcinoma, ROS limitation initially supports cancer initiation but later becomes a metabolic liability in metastasizing cells.[70] Reversible effects of ROS thus enable reciprocal switching from a proliferative to an invasive phenotype. Demonstrating similar contextual functions, activity of phosphoglycerate dehydrogenase (PHGDH), the first rate-limiting enzyme in glucose-derived serine synthesis, drives cancer proliferation, while loss of PHDGH-dependent sialic acid synthesis and integrin glycosylation increases metastatic dissemination.[71] These studies underscore the importance of metabolic plasticity in cancer progression, with the same molecules and pathways serving different roles at discrete steps during proliferation and metastatic dissemination.

CO-OPTION OF DEVELOPMENTAL AND REGENERATIVE PROGRAMS

While previous studies have identified diverse individual genes and pathways associated with metastasis, modern systems-biology approaches for unbiased profiling of the entire transcriptomes, epigenomes, and proteomes of metastatic states are unveiling convergent phenotypes. An emerging principle is that many cancers appear to simultaneously capture many of the traits needed for metastasis by activating pre-existing co-regulated modules of functionally related genes. Such gene networks are frequently those required for development or regeneration of the tissues from which the cancers are derived, although novel gene programs unique to cancer might also make important contributions (Figure 3).

Development, regeneration, and metastasis

During embryonic development, the progeny of the totipotent fertilized egg gradually becomes restricted in plasticity and fate as organs mature. Most adult tissues contain subpopulations of tissue-resident stem cells whose progeny can differentiate into various cell types of that tissue but not of other tissues. Acquisition of oncogenic driver mutations in such homeostatic tissue stem cells can lead to cancer, termed “cancer stem cells.”[72] However, recent work has revealed that tissue stem cells need not be a lineage-restricted population but can instead be metastable phenotypic states that many cells can enter and exit through plasticity, especially during tissue regeneration after injury[73] (Figure 3). In the lung, inflammatory damage induces entry of basal cells into a hybrid damage-associated transient progenitor (DATP) state with mixed expression of genes from multiple normal lung epithelial lineages.[74,75] DATPs in turn undergo plasticity into tissue-resident stem-like alveolar type II cells and subsequently differentiate into diverse lung cell types to restore epithelial function and architecture after wounding. In mouse lung adenocarcinoma, a high-plasticity cell state with multilineage differentiation potential was identified as a precursor to metastasis,[76] and reacquisition of more developmentally primitive transcriptional gene programs has been shown in mouse and human lung adenocarcinoma metastasis.[77] Analogously, colorectal primary tumors are initiated by $Lgr5^+$ cancer stem cells,[78] but cells disseminating from the tumor at the invasion front lose Lgr5 expression[79,80] and instead gain expression of novel markers, including L1CAM[79] and EMP1.[81] L1CAM is not expressed by healthy intestinal epithelial cells but is expressed by regenerative progenitors after injury and is required for wound healing, metastasis initiation, and tumor regeneration after therapy.[79]

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Established murine colorectal metastases revert from an $Lgr5^{low}$ metastasis-initiating state to an $Lgr5^{+}$ state during metastatic outgrowth,[80–82] although it remains unclear the extent to which such elasticity is retained in advanced human cancers.

Epithelial-mesenchymal transition (EMT) is the best-studied example of a developmental plasticity program co-opted in metastasis.[83,84] This program encompasses multiple dynamic changes in cellular organization, including the loss of cell polarity and downregulation of epithelial cell adhesion molecules, resulting in the increased ability to migrate and invade adjacent tissues. This is driven by the coordinated and dynamically regulated functions of SNAIL and ZEB transcriptional repressors of epithelial genes.[84] EMT first occurs during embryonic gastrulation and is co-opted at the primary tumor invasion front as DTCs acquire migratory phenotypes. Whether EMT is a prerequisite for metastasis has been debated because it is difficult to demonstrate in clinical samples, with some studies demonstrating that EMT was not required for metastasis but contributed to chemoresistance.[85–87] Recent data suggest that cancer cells most often undergo incomplete or "partial" EMT associated with invasion, metastasis, and chemoresistance.[83,84] Hybrid EMT states with co-expression of epithelial and mesenchymal markers in the same cells were recently shown to drive metastasis in multiple cancer types, challenging the traditional view of EMT as a binary switch.[39,40,88,89] As MICs acquire metastatic niche-specific growth competence in distant sites, they regenerate tumors that can demonstrate (1) elasticity, in phenol-copying the lineage hierarchies of the primary tumors from which they originated, e.g., via mesenchymal-epithelial transition (MET)[84]; (2) deformability, in remaining trapped in an MIC-like state; or (3) transdifferentiation, in undergoing lineage plasticity to enter new cell states distinct from the cognate primary tumor (Figure 3C). The specific contribution of these three metastatic regenerative modes is likely to vary across individuals, tumor genotypes, and originating tissues and remains to be defined in clinical metastasis.

PROGRESSIVE IMMUNE EVASION

The evolving tumor microenvironment

DTCs must evade attack by tissue-resident and systemic immunity in order to successfully colonize distant organs. Striking evidence for the importance of immune surveillance of metastasis comes from case reports of organ transplantation, where immunosuppressed recipients of kidney transplants developed widespread metastasis from micrometastases present in the kidneys of donors who were long thought to be cured of early stage melanoma.[90] Accordingly, increased tumor-infiltrating lymphocytes in primary tumors is a favorable prognostic biomarker for relapse-free survival in patients with colorectal cancer, whereas depletion of T cells and NK cells increases metastasis in experimental models.[9,91] Immune checkpoint inhibitors (ICIs) that enhance anti-tumor immunity have revolutionized clinical practice in many metastatic cancers. Antibodies that block the cancer cell-T cell receptor-ligand interactions of PD-1, CTLA-4, and LAG3 can clinically induce long-term durable responses—unlike chemotherapy and targeted therapy—and are now standard of care across many solid tumors.[9,91] Despite these successes, several tumor types do not respond to ICIs and are found to lack tumor T cell infiltration, described as "cold tumors," due to lack of tumor antigens, defects in antigen presentation, defective T cell

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activation, and T cell exclusion through an immunosuppressive TME.[92,93] Even for tumors that do initially respond to ICIs, resistance can occur due to T cell exhaustion, driven by chronic T cell stimulation leading to hypofunctionality.[92] Crucially, ICIs appear to be much more effective against primary tumors and micrometastases than against established macrometastasis.[9] While addition of the PD-1 ICI pembrolizumab to chemotherapy shows no overall survival benefit in metastatic triple-negative breast cancer with low expression of the PD-L1 ligand on tumor cells,[94] adding pembrolizumab in early stage breast cancers led to significantly longer event-free survival regardless of PD-L1 status.[95] Similar data in melanoma and lung cancer suggest that ICIs are more effective in the adjuvant/neoadjuvant setting than in advanced metastasis.[9] These clinical observations highlight the difference in the underlying biology of primary tumors and metastases and the progressive co-evolution of the tumor with an immunosuppressive niche during cancer progression (Figure 4). Understanding the mechanistic basis of progressive immunosuppression in metastasis is therefore vital to turning immunologically “cold” tumors “hot” and improving the efficacy of immunotherapies in metastatic cancer.

DTCs extravasating into distant organs first encounter tissue-resident macrophages and NK cells, whereas the latter further recruit local and circulating immune cells to combat newly seeded metastasis.[1,93] In response, cancer cells develop adaptive mechanisms to evade or suppress the immune response, restricting antigen recognition, increasing expression of receptors that block the adaptive immune system, and secreting immunomodulatory cytokines, extracellular vesicles, and growth factors.[1] The TME includes T and B cells; NK cells; myeloid cells including TAMs, myeloid-derived suppressor cells, dendritic cells, and neutrophils; stromal cells including cancer-associated fibroblasts, pericytes, and endothelial cells; and extracellular matrix (ECM) components, which all coordinate such adaptations (Figure 4). The TME can have both tumor-promoting and tumor-suppressing roles, but its role becomes increasingly tumor promoting with time[93,96] (Figure 4). In turn, therapeutic targeting of the TME is increasingly being explored in clinical trials either as single agents or in combination with ICIs.[93] Local and systemic immunosuppression can occur prior to metastasis. Extracellular vesicles shed by primary tumors into the circulation can release cytokines to induce recruitment and immunosuppressive reprogramming of bone-marrow-derived immune cells to pre-metastatic organ sites, where they form, together with resident cells, “pre-metastatic niches” conducive to metastatic colonization.[97] Signaling circuits coordinating the function of tissue-resident cells, recruited myeloid cells, and infiltrating tumor cells can further reinforce the emerging immunosuppressive milieu during metastatic colonization.[98]

Lymph node metastasis

Metastatic immunosuppression can also be orchestrated by cancer cells in transit. Macroscopic lymph-node involvement of tumors serves as a robust prognostic biomarker for future metastatic disease, reflected in clinical staging criteria.[99] However, molecular reconstruction of clonal phylogenies reveals that lymph node and distant metastases largely arise from independent subclones in the primary tumor.[100,101] Recent work suggests that the lymph node is not a passive staging post during metastasis but is a critical site for inducing systemic immunosuppression.[102] In an elegant model of lymph-node metastases,

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exposure of DTCs to IFN-γ in lymph nodes induced an interferon-stimulated gene program upregulating PD-L1 and MHCI expression, thereby promoting NK evasion and T cell suppression.[103] Crucially, lymph node colonization altered the systemic immune response by inducing tumor-specific T regulatory cells, increasing PD-L1 expression on macrophages, and shifting dendritic cells from migratory to resident subtypes. Tumor-transplanted mice injected with leukocytes from donors with lymph node tumors were more susceptible to lung metastases, demonstrating that lymph node metastases promoted metastasis by inducing tumor-specific immune tolerance. Further studies of lymph node-dependent immune re-education could yield improved biomarkers and therapeutic targets to perturb immunosuppressive circuits in metastatic cancer.

DORMANCY

During dormancy, DTCs do not form detectable macroscopic lesions, and patients show no clinical evidence of disease until it relapses months or years later.[10] Mouse models of early stage cancers demonstrate that DTCs can be found across every organ[104]; however, DTCs eventually grow only in specific tissues in a cancer-specific manner.[6,10,105] Some cancers such as estrogen receptor-positive breast cancer can show distant relapse after surgery as late as 20 years from their original diagnosis,[106] whereas others, including small-cell lung cancer, show aggressive spread at the time of diagnosis without a measurable dormancy period. These observations suggest variable biology of dormancy entry and reawakening in different tumor types.

Clinical dormancy reflects the dynamic equilibrium of cellular dormancy, where DTCs enter quiescence through regulatory programs that reversibly control growth arrest, and tumor mass dormancy, recurrent stochastic attempts at proliferation and colonization that are aborted by immunological, physical, and metabolic barriers.[1,10] Cellular plasticity is a key feature of maintaining dormancy.[107] In breast cancer models, early DTCs activated mesenchymal-like programs linked to pluripotency-like plasticity that coordinated dissemination and enabled long-lived dormancy, controlled by the transcription factor ZFP281.[108] Dormant DTCs maintain their state through epigenetic regulation, such as histone modifications and enhanced DNA methylation, leading to a more repressed chromatin state, transcriptional/post-transcriptional gene regulation, as well as activation of cellular stress responses and autophagy.[10] Cancer cell proliferation is further controlled by growth inhibitory signals released by DTCs or TME constituents including TGFβ, BMPs, and Wnt antagonists and altered ECM components such as collagen and laminin isoforms.[1,10,109–111] Metabolic adaptations to the TME further promote slow-cycling cell states as a consequence of tumor hypoxia or nutrient limitation.[107] Entering cellular quiescence has been shown to be functionally coupled with immune evasion through activation of the unfolded protein response,[47] downregulation of NK ligands,[112] or upregulation of MacroH2A histone variants that couple cell cycle arrest and senescence-associated inflammatory cytokine secretion.[113] Such coupling of quiescence with immunosuppression is an intrinsic property of some adult tissue stem cells[114] and could be important in calibrating immunity during tissue regeneration, but it also serves to coordinate local immune-evasive niches surrounding quiescent cells in heterogeneous tumors.[115]

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To initiate macrometastatic outgrowth, dormant cancer cells must re-enter the cell cycle or escape immune surveillance. Direct inflammatory stressors to the local host environment, such as surgery, tobacco smoke, or exposure to bacterial lipopolysaccharides, can trigger reactivation and outgrowth of dormant DTCs.[10,116] In murine metastasis models, cancer cells were shown to induce neutrophils that produced metastasis-supporting neutrophil extracellular traps, stimulating breast cancer invasion, migration, and lung metastasis.[117] Reawakening from dormancy is an intrinsic feature of the aging host.[118] In murine models, the frequency of dormant DTCs decreases in aged bone marrow, and cancer cells become more proliferative, associated with increased pro-proliferative inflammatory cytokines and downregulated dormancy-promoting factors.[119] Age-related remodeling of the ECM was shown to stimulate melanoma metastasis,[120] senescent osteoblasts promote bone metastasis,[121] and aged lung fibroblasts can reactivate dormant melanoma cells through increased secretion of the soluble WNT antagonist sFRP1.[122]

ORGAN-SPECIFIC MICROENVIRONMENTAL ADAPTATION

DTCs can spread to virtually all organs; however, different cancer types tend to preferentially relapse in certain organs, a phenomenon termed metastatic organ tropism.[6] Determinants of organ-specific metastasis have been extensively reviewed elsewhere[1,6,97,105,123]; here, we focus on emerging principles. Metastatic tropism is determined by the combination of (1) cancer cells physically reaching an organ and (2) organ-specific environments that favor seeding and colonization by DTCs. The predominant first site of metastasis in colorectal cancer is the liver, since cancer cells hematogenously disseminating from the intestines travel through the mesenteric capillaries into the hepatic portal vein, encountering the hepatic sinusoids as their first capillary beds. However, the liver is also the metastatic site in 90% of patients with uveal melanoma,[124] which is less anatomically obvious given the primary tumor location in the eye. Thus, DTCs become “seeds” that favor specific organ niches with fertile “soil” in order to grow into metastases.[125] Pre-existing transcriptional and metabolic heterogeneity among DTCs enable selection of clones capable of outgrowth in specific organs, while plasticity mechanisms enable dynamic inducible adaptation to novel tissue niches. Thus, intracardiac injection of the triple-negative breast cancer cell line MDA-MB-231 results in metastatic spread of subclones with distinct gene expression signatures with preferential colonization to the brain, bone, and lung.[126–128] Extracellular metabolites present in the local niche further shape metastatic outgrowth and intrinsic cell metabolism, e.g., brain metastases adapt their metabolism toward acetate, glutamine, and branched chain amino acids when glucose sources become limiting.[123] Colorectal liver xenografts enhance secretion of the creatine kinase brain-type enzyme, converting hepatocyte-released extracellular creatine to phosphocreatine, which is then taken up by the solute transporter SLC6A8 into metastatic cancer cells to fuel ATP production.[129]

Niche-adaptive plasticity of cancer cells with novel paracrine signaling can reciprocally induce plasticity of surrounding cells in the target organ, powering co-evolution of the tumor with its new host and formation of the metastatic TME.[130] Initial metastatic niches can facilitate cancer cell reprogramming to increase fitness for widespread secondary metastases, which causes greater clinical morbidity and mortality. *In vivo* lineage tracing

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of breast and prostate cancer cells demonstrated that the bone microenvironment not only provided a compatible niche for initial metastasis but also induced EZH2-mediated epigenetic plasticity, accelerating secondary metastasis to visceral organs at faster kinetics with a more severe tumor burden.[131,132]

Recent studies have comprehensively characterized the TME of primary and metastatic brain tumors by using transcriptomic, proteomic, flow cytometry, and spatial approaches.[133–136] Although the composition of stromal cells was comparable,[136] disease-specific differences in the immune cell composition and their expression characteristics were found among different brain malignancies. Primary brain tumors had fewer lymphocytic and neutrophil infiltrates compared to brain metastases, whereas different metastatic tumors showed distinct enrichment of different immune cell types, implying that primary brain tumors shape their TME differently than do extracranial tumors metastasizing to the brain.[134] These studies give us a better understanding of the different cell compositions of the local TME in different disease types in the same anatomic location and shed light on the limitations of current one-size-fits-all therapeutic approaches attempting to modulate the TME. Importantly, they also demonstrate that “soil” is neither static nor uniform, and although the homeostatic niche of the organ might be initially similar, infiltrating cancer cells sculpt co-evolution of the local TME, in turn recruiting immune cells in a disease- and cell-type-specific manner.

REPROGRAMMING THE SYSTEMIC MACROENVIRONMENT

Metastatic cancer is a systemic disease affecting all organ systems. As such, systemic factors affecting the nutritional, metabolic, neurohormonal, and inflammatory state of the body can influence all three phases of metastasis.[4] These networks of inter-organ system communication during tumor progression are only beginning to be explored for therapeutic benefit. Factors secreted by primary tumors can reprogram myeloid cells and the ECM, creating pre-metastatic niches favorable for metastatic seeding and outgrowth.[137] Inflammatory states, including obesity[138] and aging[118] can promote metastasis, whereas exercise[139] and prudent diet[140] might decrease metastatic risk. Tumor-resident and gut microbiota are emerging as key determinants of metastatic outcome via direct cellular effects of microbial metabolites or through reprogramming of the TME.[141] Intriguingly, bacteria have been found to selectively colonize a wide range of cancer types, including those derived from non-barrier epithelia, although it remains to be uncovered whether such associations are correlative or causal.[142] Intratumoral fusobacterium colonization has been shown to drive metastasis,[143] whereas intratumoral bacteria in murine breast cancer CTCs promoted resistance to fluid shear stress via actin cytoskeletal remodeling and hence promoted metastasis.[144] The emerging field of cancer neuroscience is unveiling roles for electrical activity and neural-immune-cancer interactions in cancer progression beyond the long-recognized role of perineural invasion.[145] Recent studies have shown that circadian rhythms can control both the timing of tumor dissemination[146] and anti-tumor immunity via rhythmic trafficking of dendritic cells to tumor-draining lymph nodes.[147] These observations suggest opportunities to target metastasis by disrupting circadian signaling circuits or synchronizing therapy with circadian rhythms to maximize antitumor efficacy. The most profound manifestation of systemic reprogramming induced by advanced cancer

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is cachexia, a catabolic state defined by loss of muscle mass and function, associated with anorexia, insulin resistance, and loss of adipose tissue.[148] The incidence and severity of cachexia increases with metastasis,[149] limits tolerance of therapy, can induce immune suppression, and is associated with early mortality.[150] Delineating how progressive tumor and microenvironmental remodeling during metastasis can induce cachexia could yield more promising therapeutic approaches for combating this lethal manifestation of metastatic cancer.

THERAPY RESISTANCE

Metastasis selects for cancer cells endowed with the ability to dynamically reprogram themselves to adapt to diverse stresses, evade immune surveillance, and subvert host tissue biology to support tumor regeneration. The same adaptive stress-resistant tumor-regenerative properties can be deployed by cancer cells to resist and regrow tumors after therapy (Figure 2).[9,107,151] The close relationship between metastasis and therapy resistance is evident in the fact that macrometastatic or clinical stage IV disease remains largely incurable, with 5-year survival between 5% and 30%.[152] Therapy applies further selective pressures on metastatic cancer cells, driving the selection of tumor subclones harboring resistance mutations[153] and inducing inflammatory signaling that can drive lineage plasticity.[154,155] Metastatic disease is the target of almost all systemic cancer therapy, including chemotherapy, targeted therapy, and immunotherapy.[9] Cancer patients are administered systemic therapy in two contexts: (1) patients with surgically resectable primary tumors with no clinical evidence of metastatic disease (stages I–III) receive systemic therapy administered before (neoadjuvant) or after (adjuvant) surgery with the primary goal of eliminating micrometastatic disease, i.e., to cure the patient of cancer and (2) in stage IV cancer patients with widespread macrometastatic disease, therapy typically shifts from cure to palliation and prolongation of life (Figure 5). While chemotherapy remains the backbone of medical therapy, breakthroughs over the last two decades with the development of ICIs, targeted therapies (e.g., kinase inhibitors), and antibody-drug conjugates (e.g., in $Her2^+$ breast cancer) have significantly improved overall survival.[9,91,152] The plethora of novel biological insights into the principles and mechanistic mediators of metastasis offer a number of opportunities for further improving therapeutic outcomes for micro- and macrometastatic cancer (Figure 5).

Targeting dormant micrometastasis

To date, drugs targeting mediators of metastatic dissemination, e.g., matrix metalloproteinase inhibitors, have not been successful in clinical trials,[156] consistent with clinical and preclinical evidence that metastasis begins early and can be distinct from tumor initiation (discussed below). Frequently, by the time a primary tumor is detected, DTCs have already seeded distant organs, rendering approaches to block dissemination futile. Perhaps the greatest opportunity to improve cancer outcomes is to expand the portfolio of clinical trials focused on dormant micrometastasis[10] (Figure 5). With growing biological understanding of the dormant state, its plasticity and molecular underpinnings, it is becoming apparent that the markers, signaling pathways, and immune evasive strategies of micrometastases are distinct from those of macrometastasis. However, clinical drug development typically proceeds by first testing novel drugs in the advanced macrometastatic

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setting in patients whose tumors have become resistant to most standard treatments. If successful here, trials advance to previously untreated metastasis and only then move to the adjuvant setting to treat patients without clinical metastasis but who are at high risk of metastatic relapse, likely harboring micrometastasis. As a result, many drugs that might effectively treat micrometastasis but not the more biologically aggressive macrometastasis fail to reach patients.[9]

A critical requirement to rapidly and cost-effectively advance trials targeting micrometastasis is to establish actionable biomarkers of dormant micrometastatic disease that identify patients at highest risk for macrometastatic relapse most likely to benefit from adjuvant therapy. In that regard, “liquid biopsy” ctDNA detection in the blood is a promising biomarker of micrometastasis, but further improvements are needed in the sensitivity of such assays and in the clinical actionability of their results.[157] Another potential approach proposed to identify patients with microscopic disease has been the use of bonemarrow biopsies to identify DTCs in early stage breast cancer patients.[158,159] Future development of tissue-based protein or RNA assays informed by single-cell analysis of metastasis, detection of epigenetic modifications of ctDNA, or assaying CTCs, exosomes, immune cells, and cytokines could provide avenues for predictive real-time biomarker development. These approaches will help stratifying patients who would benefit from continued therapy to eradicate micrometastatic disease versus opting for observation.

Targeting oligometastatic disease

Metastasis can show tumor-type growth-specific patterns, metastasizing slowly or to a single organ site. Oligometastatic disease is postulated to present an intermediate state of metastatic spread, where local ablative therapies can provide meaningful clinical benefit and prolonged survival.[160] In these cases, surgical resection or radiation is often considered to decrease tumor burden, extend life, and potentially cure patients. Combination approaches to oligometastatic disease in form of surgery, radiation, and chemotherapy are standard of care treatment in soft-tissue sarcomas where systemic therapies alone show limited efficacy.[161] Surgical resection of colorectal liver metastases can be curative in 20% of patients[149] and prolongs life in metastatic cutaneous/uveal melanoma and neuroendocrine tumors[162,163] (Figure 5). Liver-directed therapies including hepatic artery infusion chemotherapy, embolization, and radiofrequency ablation have shown survival benefit.[164] Recent randomized controlled trials involving local consolidative radiation therapy for oligometastatic disease demonstrated prolonged disease-free and overall survival in patients with breast, prostate, lung, and other cancers.[160,165,166] The alpha emitter radium-233, which selectively binds to areas of increased bone turnover, shows overall survival benefit in advanced metastatic prostate cancer with bone metastasis, suggesting that specific targeting of the bone niche can influence subsequent widespread metastasis, consistent with observations in mouse models.[131,167] Together, these studies suggest that widespread metastatic and oligometastatic disease are clinically distinct, and thus the historical notion that local therapies do not generally improve survival in stage IV cancer patients needs to be carefully reconsidered. In patients who present with oligometastatic disease, there are currently few clinical tools that can distinguish between disease that is likely to be organ limited versus fast spreading with subclinical micrometastases that will

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soon become clinically evident within a short period of time. These two scenarios require distinct treatment approaches and have disparate outcomes, and there is an unmet need to understand the underlying biology and define biomarkers.

Targeting macrometastasis

The plasticity of advanced metastatic disease poses a great challenge to cancer therapy since in theory, the ability to dynamically reprogram cell states could confer resistance to almost any drug.[9,151] While single-cell profiling of tumors is yielding tremendous insight into tumor heterogeneity, more needs to be done to determine the extent to which adaptive programs are shared across patients with diverse tumor and host genetics or lifestyle factors. In principle, delineating metastasis mediators that result from co-option of conserved developmental or regenerative programs could offer broadly applicable therapeutic targets, as opposed to patient-specific or widely heterogeneous subclonal mechanisms. However, efforts targeting developmental signaling pathways such as Wnt, TGFb, Notch, and Hedgehog implicated in cancer have been largely unsuccessful to date due to substantial off-tumor on-target toxicities, context-dependent pleiotropic roles, and feedback loops of these pathways.[151,156] Other approaches have focused on targeting metastatic cancer metabolism such as inhibition of SLC6A8 phosphocreatine transporter in colorectal liver metastases[168] and delineating metastasis-specific TMEs to overcome metastasis-specific immunosuppressive environments.[93,134,136,169,170] More preclinical studies are needed that (1) focus on delineating the components of developmental and regenerative programs unique to cancer and (2) identify targets that selectively modulate such pathways in metastasis phase-specific contexts in both cancer cells and their evolving TME. This work could offer two paths to combat metastasis. First, delineating plasticity endpoint states could enable anticipatory targeting of those states. Second, inhibiting molecular mediators of such plasticity could constrain the evolutionary space available to metastatic cells and render them more vulnerable to therapy (Figure 5).

EMERGING PERSPECTIVES

Metastasis and tumorigenesis as separable properties?

Metastasis has historically been considered the very last stage of cancer progression; i.e., oncogenic driver mutations transform normal epithelia into hyperproliferative primary tumors, with a subset of these tumor cells strictly and sequentially acquiring the ability to invade, disseminate, and colonize distant organs.[2,11] In this view, normal cells cannot metastasize without first becoming a tumor and then an invasive cancer, aligning with the observation that the likelihood of developing metastatic disease is strongly correlated with the size of the primary tumor, reflected in clinical TNM staging (T, size of tumor; N, extent of spread to regional lymph nodes; M, presence of metastasis) used to select patients for adjuvant therapy.[99] Several lines of evidence are converging to challenge this dogma. Precancerous cells from ductal carcinoma *in situ* and pancreatic intraepithelial neoplasia can be found in circulation, bone marrow, and distant organs of patients without a clinical diagnosis of cancer.[171,172] Early disseminating cells have been shown to be the principal source of later metastasis in some cancers.[172–175] Intriguingly, studies in mouse models suggest that oncogenic transformation might not even be necessary for

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metastasis. Untransformed mouse mammary epithelial cells could seed morphologically normal microcolonies in the lung, and, upon inducible activation of oncogenes, grow into lesions morphologically indistinguishable from spontaneous metastasis from primary mammary tumors.[176] Expanding on this concept, mice harboring conditional deletion of the sodium leak channel NALCN demonstrated widespread dissemination of morphologically normal cells harboring no oncogenic mutations.[177] NALCN-deficient untransformed cells formed morphologically normal, complex structures such as glomeruli in the kidneys of recipient mice. Further, metastases hijack properties such as L1CAM expression that are normally employed during tissue regeneration, distinct from the requirement of unrestricted proliferation in an intact niche that is characteristic of primary tumors.[79] More studies are needed to demonstrate the extent to which normal cells disseminate to distant organs; however, together these observations suggest that metastasis and tumor initiation can be distinct and mutually co-operative properties, which need not be acquired in a strictly hierarchical sequence. Therapeutically, important implications of this changing temporal paradigm are (1) targeting oncogenic driver mutations required for tumor initiation might not suffice to target metastatic cells and (2) mediators that endow cells with metastatic competence should be targeted early during tumor progression, ideally in the context of cancer prevention in high-risk individuals.

Emerging approaches to comprehend and combat metastasis

We are amid a boom in single-cell and spatial technologies and computational systems-biology innovations that enable definition of the evolving TME and clonal dynamics at unprecedented resolution and scale. A growing suite of technologies is enabling analysis of epigenetic marks, proteins, and metabolites at single-cell and spatial resolution and adapting existing transcriptomic technologies to the current clinical standard, formalin-fixed, paraffin-embedded tissues.[19,178] Emerging DNA-editing-based high-fidelity inducible lineage recorder systems allow precise temporal ordering of cell state transitions.[17,88,179] In clinical samples, mitochondrial mutation analysis and liquid biopsy-based real-time clonal tracking are adding to existing approaches to delineate the ordering of subclone emergence during tumor evolution.[157,180] With these technical advances, the growing challenges of the single-cell era of cancer biology are the need for (1) robust, reproducible, and transparent algorithms and computationally trained investigators to analyze, interpret, and share the growing volume of “big data” and (2) experimental approaches that rigorously validate the hypotheses emerging from tumor profiling, define molecular mechanisms, and translate these into novel therapies. Multiplexed engineering of sophisticated organoid and mouse models to simultaneously induce multiple mutations will be needed to accurately model the heterogeneity of genetic and epigenetic cell states within and across tumors and identify shared regulatory pathways.[181,182] Emerging technologies to more accurately represent patient, disease-stage, and site-specific TMEs, including patient-derived organoid:immune co-cultures, tumor explant cultures and humanized mice are yielding novel mechanistic insights and enabling rapid drug-response evaluation.[183,184] Prospective clinical trials employing *ex vivo* models may guide their potential use as pre-treatment “avatars” of patient-specific therapy response to guide clinical decision-making.[181] Finally, artificial intelligence is poised to transform clinical trial design to accelerate biomarker discovery and drug development.[185]

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Conclusions

In summary, metastasis relies on a variety of mechanisms to engage epigenetically encoded programs that enable dynamic adaptation to changing conditions, cellular stress, survival outside the tissue niche, dissemination, immune evasion, and TME co-option and end organ colonization. With emerging insights from new technologies such as single-cell profiling, lineage tracing, and sophisticated preclinical and *ex vivo* models, the challenge now is to define metastasis dependencies that can be safely targeted across heterogeneous patients or within biomarker-defined cohorts. Together, major advances in the landscape of metastasis research and clinical drug development have the potential to improve clinical outcomes for patients with metastatic cancer.

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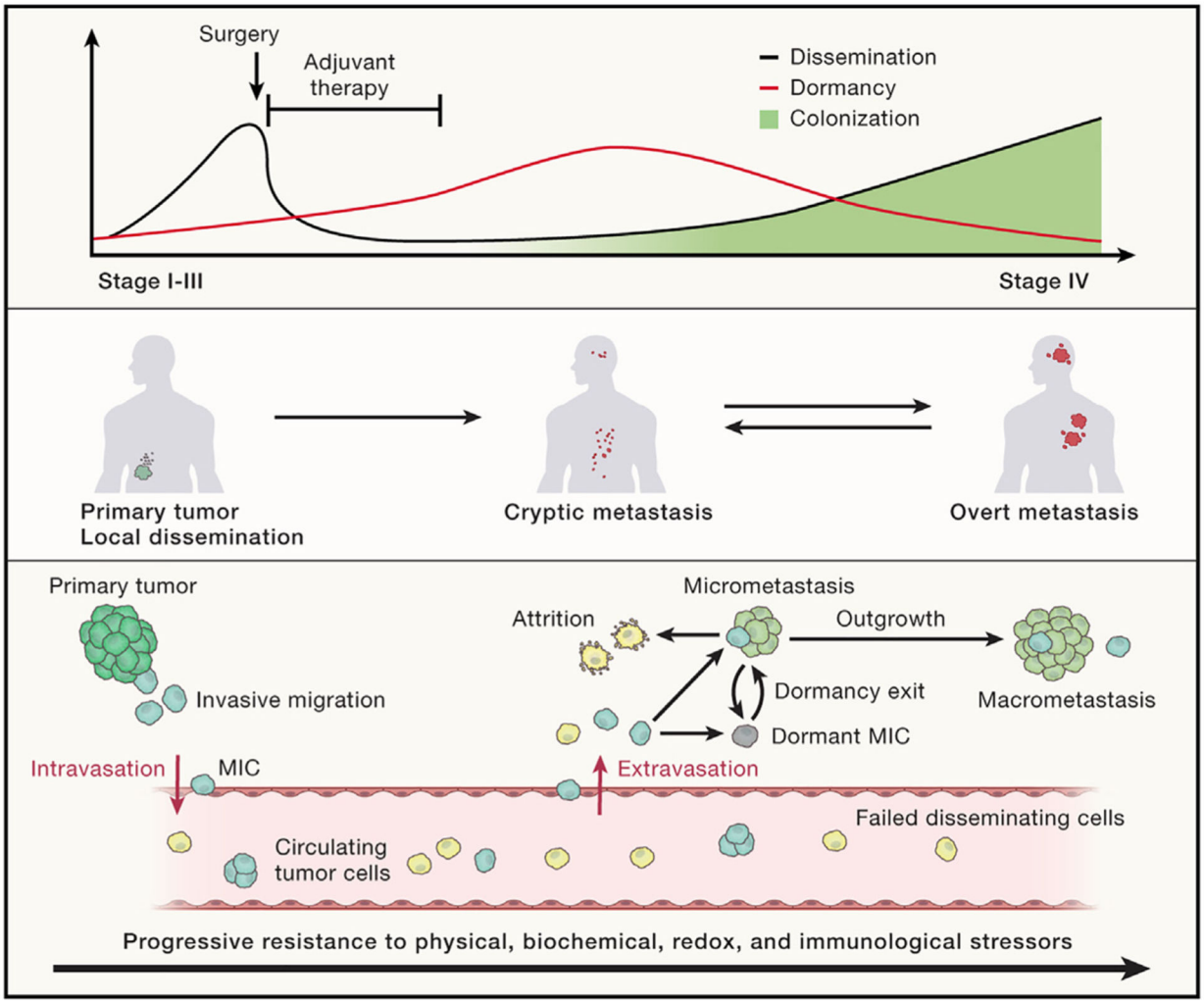

Figure 1. Stages of metastasis

Metastasis comprises three stages: dissemination, dormancy, and colonization, which can coexist and overlap in time. MICs arise from primary tumors and have acquired the ability to undergo invasive migration and then singly or collectively migrate and disseminate via the blood or lymphatics as CTCs. Most CTCs are cleared due to physical, biochemical, and immunological stressors. Trapped in capillary beds of distant organs, CTCs extravasate and migrate into organ parenchyma as DTCs to seed nascent metastasis. DTCs seed in organ-specific, perivascular niches. The majority are cleared by niche-specific or systemic immune defenses, but few MICs survive, entering reversible growth arrest and immune-evasive quiescence, acquire organ-specific growth adaptations, and co-opt their TME to evade immune surveillance. Environmental triggers lead MICs to exit dormancy and form clinically detectable macrometastases.

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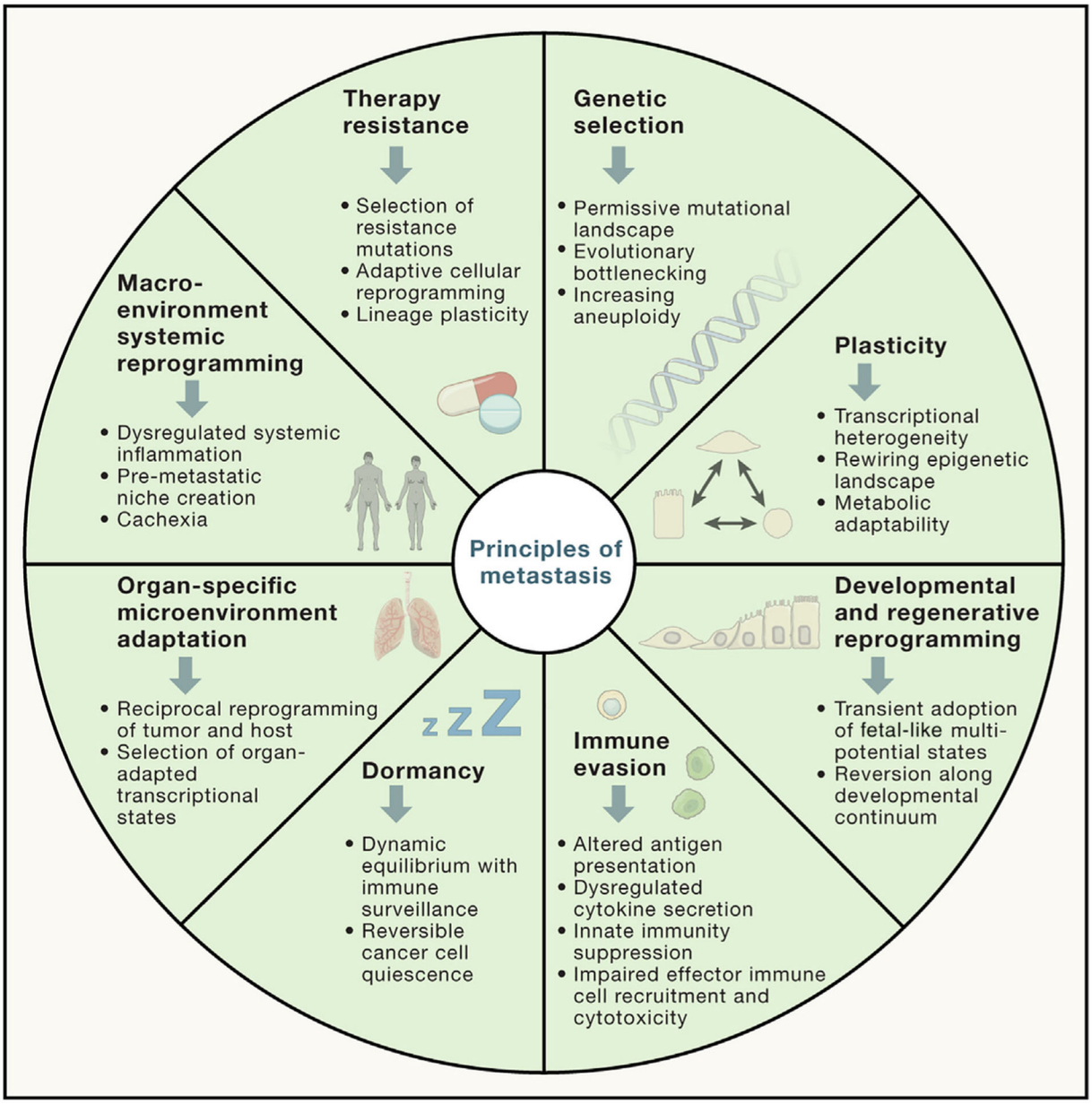

Figure 2. Principles of metastasis

MICs acquire a set of functional abilities that enable them to disseminate, colonize, and survive multiple stressors in a hostile environment, summarized here as the principles of metastasis.

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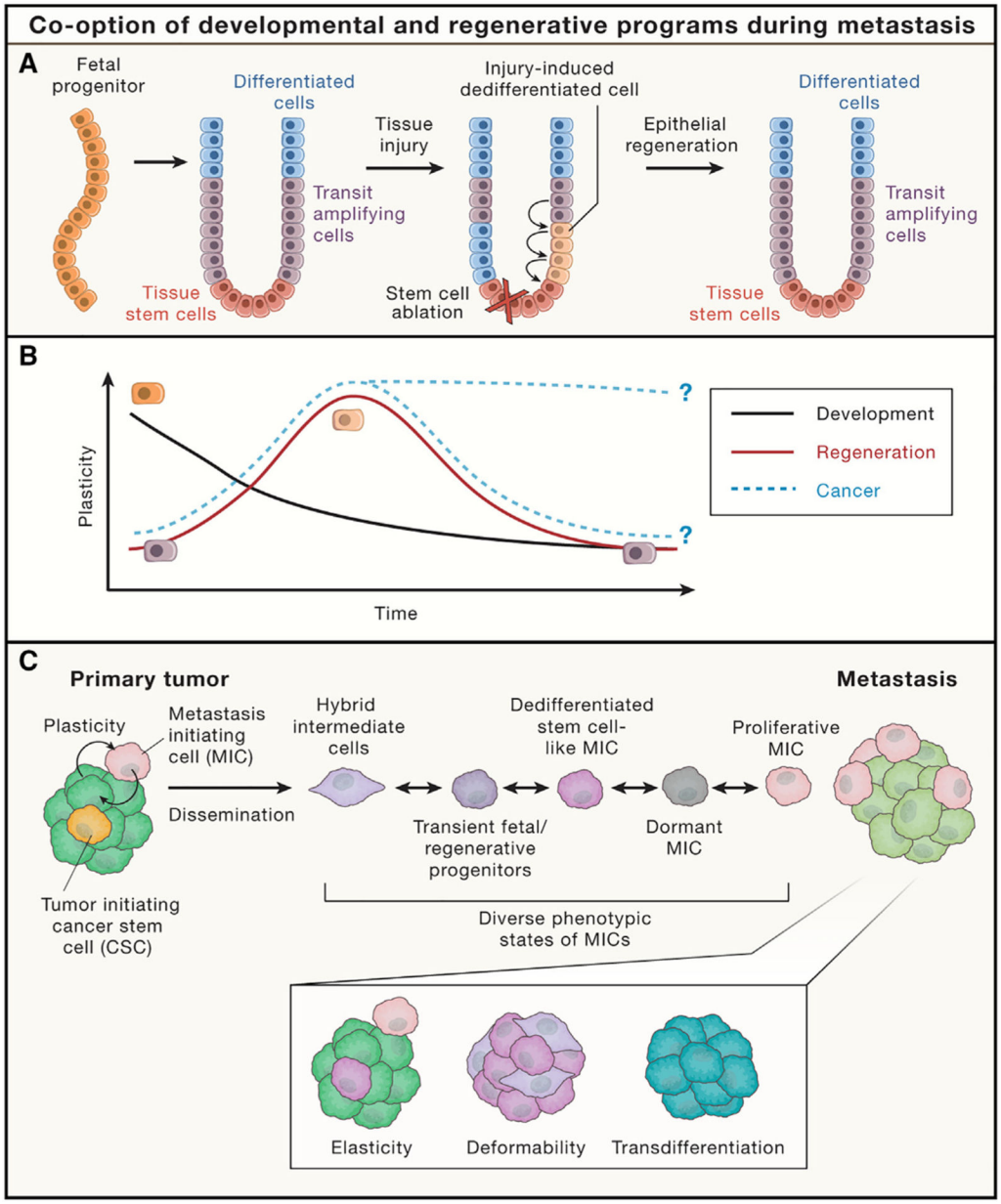

Figure 3. Co-option of developmental and regenerative programs during metastasis

Metastatic cells redeploy developmental and regenerative programs of normal embryonic development and wound healing.

(A) During homeostasis, tissue-specific stem cells continuously generate transit-amplifying progenitors and mature differentiated cells. Upon tissue injury, differentiated epithelial cells dedifferentiate to re-enter tissue fetal-like, damage-associated transient progenitor states that can differentiate into tissue stem cells and then diverse differentiated cells, restoring epithelial integrity.

(B) Cell plasticity and fate become progressively restricted during embryonic development. Upon tissue injury, fate-restricted differentiated cells undergo transient increases in plasticity. Cancer cells co-opt programs employed by developmental and regenerative

progenitors in adaptation to stresses during tumor progression, although whether cells in macrometastases remain highly plastic or become fate-restricted remains unclear.
(C) Disseminated MICs adopt high-plasticity states. These include hybrid EMT states, damage-associated transient progenitor-like states or immune-evasive dormant states. During metastatic colonization, MICs can regenerate phenotypically heterogeneous macrometastatic tumors that can enter dormancy or initiate tumor growth, re-enter states similar to the primary tumor (elasticity), remain trapped in MIC-like states (deformability), or undergo lineage plasticity into new cell states not found in the primary tumor (transdifferentiation).

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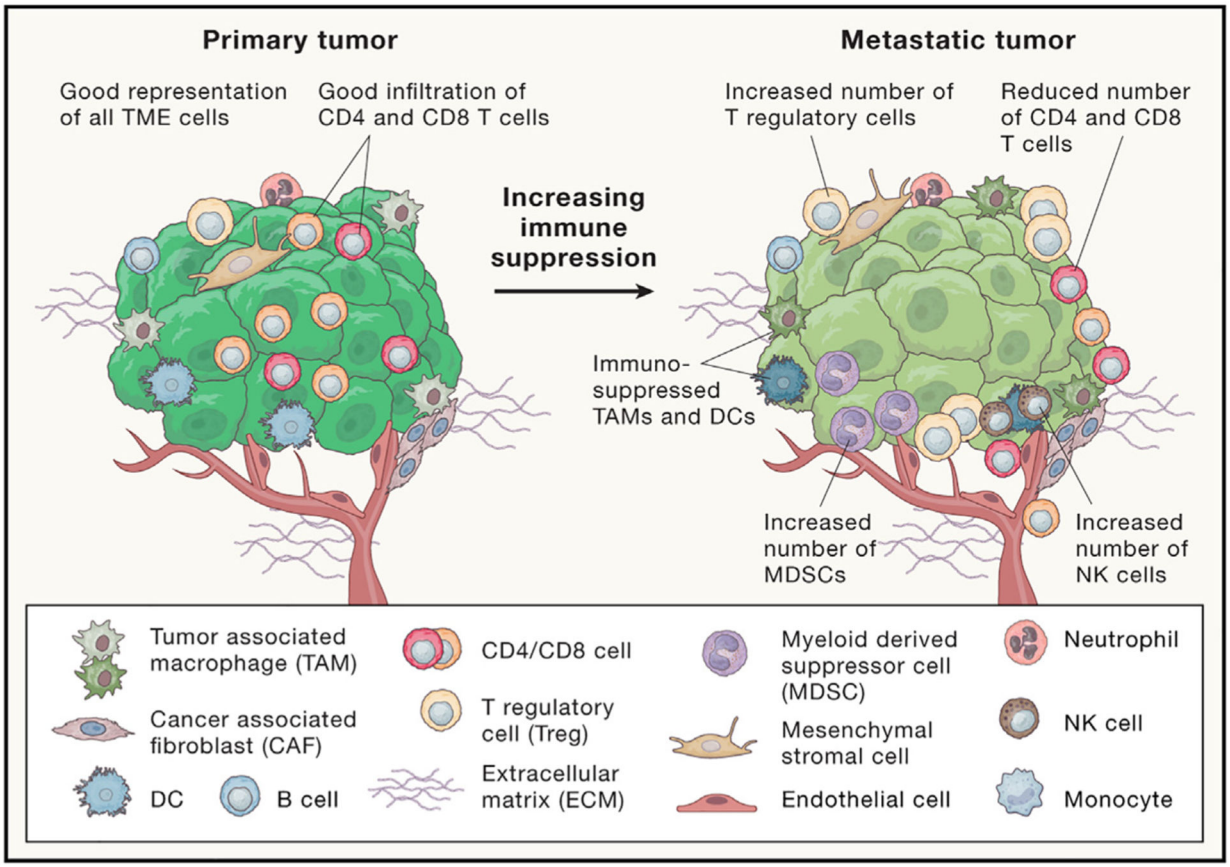

Figure 4. The metastatic tumor microenvironment

The composition and co-option of the TME is essential for tumor growth and progression. Main components of the TME are components of the innate and adaptive immune system as well as stromal cells: tissue-resident and bone-marrow-derived macrophages, polarized into immunosuppressive TAMs, monocytes, myeloid-derived suppressor cells, T cells, NK cells, dendritic cells, blood and lymphatic vessels, cancer-associated fibroblasts, and components of the ECM. The environment of immune cells in the TME and expression of immune regulatory receptors becomes more immunosuppressed in metastatic tumors.

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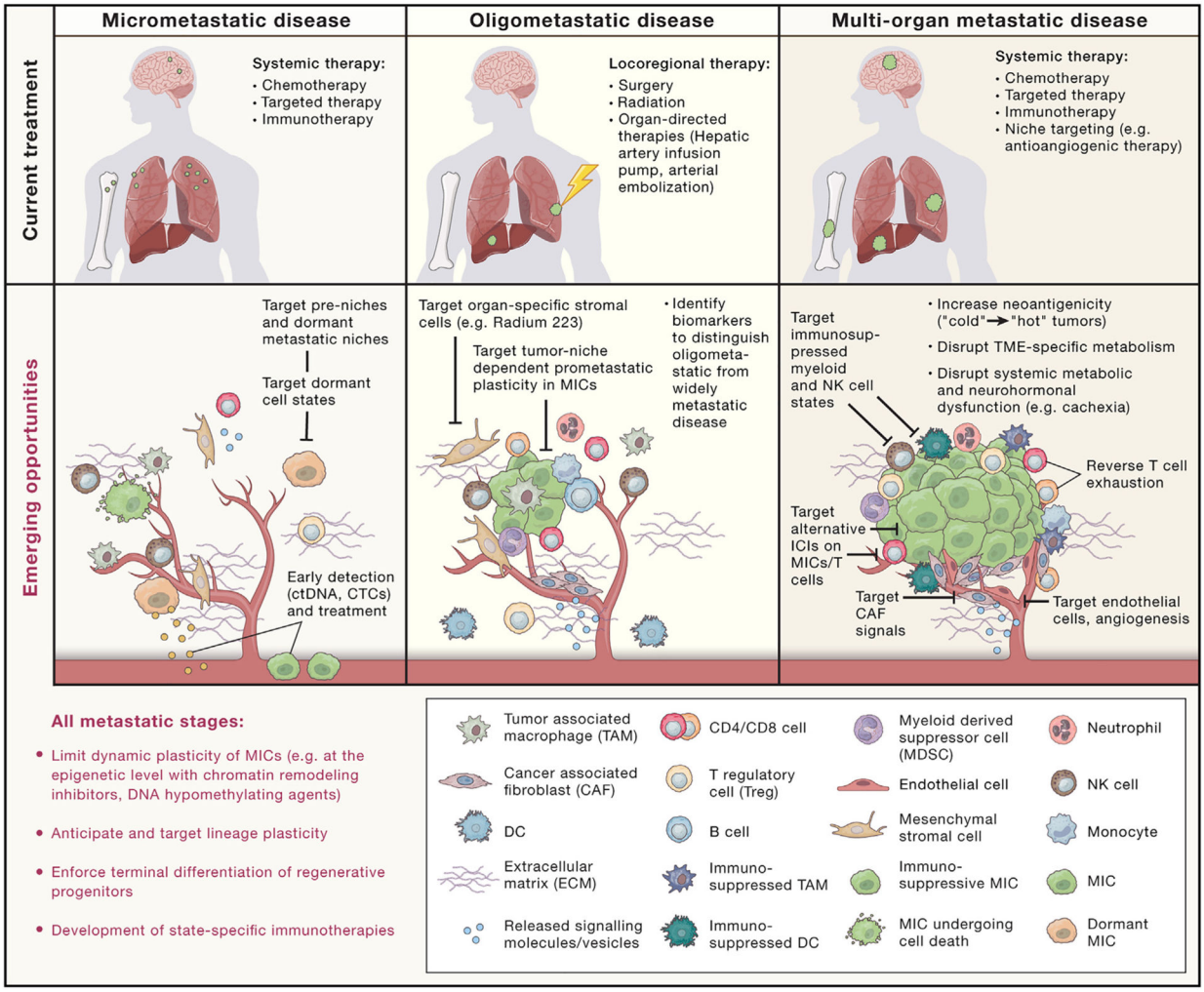

Figure 5. Current and emerging therapeutic strategies for metastatic disease

(A) Metastatic disease is treated in three contexts: Micrometastatic disease is suspected in the (neo-)adjuvant therapeutic setting when metastatic disease cannot be detected by standard imaging and screening technologies. Although multi-organ macrometastasis is largely incurable, selective local therapy of oligometastatic disease can prolong life and sometimes be curative for several cancers. Multi-organ metastatic disease is generally treated with systemic therapy, including chemotherapy, targeted therapy (e.g., small-molecule inhibitors, antibodies, or antibody-drug-conjugates), and immunotherapy.

(B) Opportunities for therapeutic modalities that target cancer cells or their TME to maximize elimination of metastatic cells are highlighted. In micrometastasis, MICs are in dynamic equilibrium with immune surveillance. Proliferating cells are frequently eliminated by tissue-resident or circulating immune cells, whereas dormant cells evade immune destruction. In oligometastasis, small tumors are infiltrated by TME resident cells and recruited immune cells. In multi-organ metastasis, the TME becomes increasingly immunosuppressive, expelling tumor-reactive immune cells or co-opting them into immunosuppressive states.