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Review

Phenotypic plasticity during metastatic colonization

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Most solid cancer-related deaths result from metastasis, a multistep process in which cancer cells exit the primary site, intravasate into the bloodstream, extravasate, and colonize distant organs. Colonization is facilitated by clonal selection and the high phenotypic plasticity of cancer cells that creates reversible switching of cellular states. Cancer cell plasticity leads to intratumor heterogeneity and fitness, yielding cells with molecular and cellular programs that facilitate survival and colonization. While cancer cell plasticity is sometimes limited to the process of epithelial-to-mesenchymal transition (EMT), recent studies have broadened its definition. Plasticity arises from both cell-intrinsic and cell-extrinsic factors and is a major obstacle to efficacious anti-cancer therapies. Here, we discuss the multifaceted notion of cancer cell plasticity associated with metastatic colonization.

Highlights

Cancer cell plasticity results in tumor cells with phenotypic versatility that allows shuttling between cellular states and promotes disease progression.

During epithelial-to-mesenchymal transition, hybrid cells have the highest metastatic potential.

Dormancy of disseminated tumor cells (DTCs) and their reawakening years after arriving at distant sites is an example of cancer cell plasticity.

Metastatic cancer cells can rewire their metabolism depending on the metabolic state of the distant organ, and can adopt stem-like traits, as well as features of normal host tissue, thus favoring the colonization of the new niches.

Plasticity may generate site-specific vulnerabilities that could be exploited for novel therapeutic options.

Cancer progression based on phenotypic plasticity

Phenotypic **plasticity** (see Glossary) is a concept coined in developmental biology that describes the reversible switching of cellular states via processes of differentiation, **dedifferentiation**, and **transdifferentiation.** Recently, blocked differentiation has also been described as a new form of plasticity (Figure 1). Stem and progenitor cells are examples of cell types that exhibit phenotypic plasticity [1–3].

The precise definition of cellular states in the context of diseases such as cancer is quite challenging (Box 1). Indeed, numerous studies investigating cell plasticity have only broadened its definition. We propose that a cellular state is defined by specific genetic and epigenetic programs, characterized by the expression of particular markers, associated with biological properties, and can be interchangeable over time. Consequently, cell state switches result in phenotypic versatility of tumor cells that promotes disease progression. The mechanisms contributing to cancer cell plasticity are often divided into the two categories of cell intrinsic factors and cell extrinsic stimuli. Intrinsic factors, the stochastic accumulation of genetic and epigenetic alterations, have been shown to increase plasticity and generate tumor heterogeneity [4,5]. However, plasticity is perhaps best portrayed when cancer cells integrate external stimuli, and there is increasing evidence that plasticity is also promoted by the tumor microenvironment (TME). In the field of cancer research, the terms phenotypic plasticity and **EMT** are often used synonymously. The important contribution of EMT to cancer progression and metastasis has indeed been widely studied and reviewed [6–8]. Phenotypic plasticity may result in cancer cells shuttling between epithelial and mesenchymal states but there are several examples of phenotypic plasticity in cancer other than EMT. Any switch of cell states or phenotypes falls under the definition of phenotypic plasticity. Such cell-state switches are observed during metastatic colonization, where disseminated tumor cells (DTCs) may adopt stem-like traits, an altered metabolism, characteristics of the host tissue, or may reside in a dormant state and can potentially reawaken. This plasticity allows survival and **colonization** of

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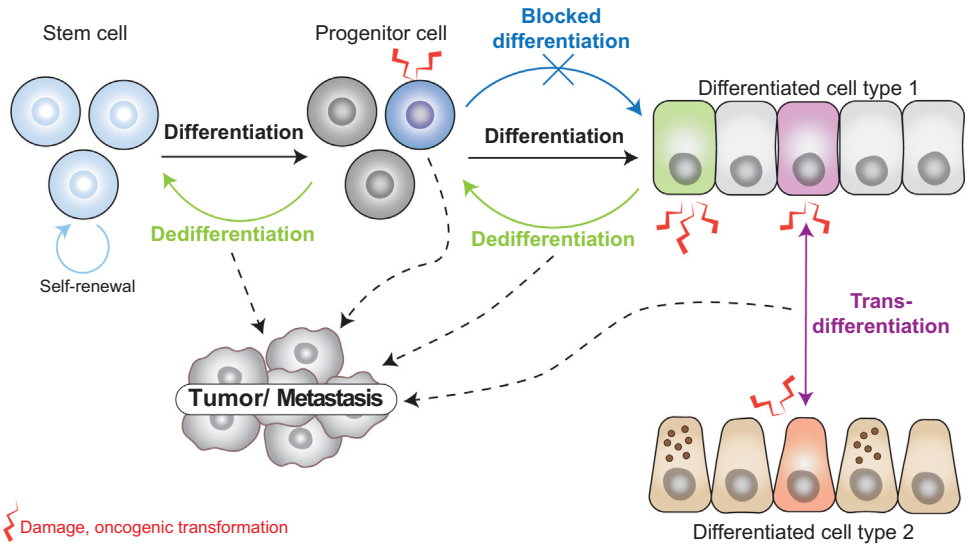


Figure 1. Differentiation, dedifferentiation, transdifferentiation, and blocked differentiation. Stem cells differentiate into progenitor cells, which differentiate into specialized cells. Upon damage or oncogenic transformation, specialized cells may revert to a progenitor-like state, a process termed dedifferentiation. Transdifferentiation describes the switch of one differentiated cell type into another.

diverse foreign, often inhospitable, environments in distant organs. In this review, we extend and harmonize the definition of phenotypic plasticity in the context of metastasis. A comprehensive awareness of the plasticity of DTCs is of paramount importance for discovering and implementing effective metastatic cancer treatments.

The different types of cancer cell plasticity

Hybrid states in EMT

During EMT, epithelial cells transiently and reversibly lose some epithelial and gain some mesenchymal features [9,10]. This process is strongly influenced by transcriptional programs, epigenetic modifications, alternative splicing, protein stability, and subcellular localization of signaling molecules and transcription factors [9,10].

Box 1. Plasticity and the definition of a cellular state

Phenotypic plasticity refers to reversible changes of cellular states. 'Cellular state' requires precise explanations, due to different interpretations in the literature. Indeed, a change in cellular state can refer to the transition from an epithelial-to-mesenchymal state (EMT) via loss of apicobasal polarity and the acquisition of motile capacities. Of note, recent evidence proposes that the nature of EMT is rather spectral than binary [7,8,13,18], which makes the notion of state change particularly fleeting in this case. Plasticity can also refer to a lineage switch of epithelial cells within the same organ. Indeed, studies in mammary gland biology have shown that basal epithelial cells can convert into luminal cells [4,111], highlighting another example of state and plasticity. Alternatively, plasticity can also describe a change of differentiated cells from one organ-specific type to another. The discovery of Yamanaka's cocktail of transcription factors that can reprogram a differentiated cell into a pluripotent embryonic stem-like state (OCT4, SOX2, KLF4, and MYC) is another example of 'forced' cellular plasticity [112,113]. Likewise, pioneering studies have shown that testis cells when transplanted into mammary fat pad differentiate into functional mammary epithelial progenitors [114]. Recent work focusing on metastasis has revealed additional cellular states, such as the dormant state (in comparison to the proliferative state), which is not simply characterized by a cell cycle arrest but also by an epigenetic landscape and a cellular program [68,70,71]. Research focusing on plasticity is of vivid interest, and in regards to the multifaceted nature of plasticity, a clear definition of cellular state is needed. We therefore propose that a cellular state can be interchangeable over time, and is defined by specific genetic and epigenetic programs, characterized by the expression of particular markers and associated with biological properties.

Glossary

Cellular dormancy: a cellular state, largely influenced by a foreign microenvironment, that results in growth arrest of DTCs. DTCs may remain dormant for years after the initial diagnosis before triggering metastatic relapse.
Colonization: metastatic colonization refers to a series of biological events that results in DTCs forming clinically relevant metastases at secondary cancer sites.
Dedifferentiation: a process resulting in differentiated cells losing specialized traits and reverting to an upstream hierarchical stem-like state.
Epithelial-to-mesenchymal transition (EMT): transition from the epithelial state to the mesenchymal state through the loss of apicobasal polarity and the acquisition of motile capacities.
Host-organ mimicry: a type of cancer cell plasticity describing the situation in which disseminated cancer cells adopt phenotypic features of the foreign host organ.
Plasticity: phenotypic plasticity describes reversible switching of cellular states via a process of transdifferentiation, dedifferentiation, or differentiation.
Stemness: defines cells that differentiate and self-renew, which thus maintains tissue homeostasis.
Transdifferentiation: the switching of one cell type to another without necessarily going through a stem-like state.

The most aggressive cancer cell phenotype is reported to lie between the purely epithelial and mesenchymal cell states. Cells that have undergone partial EMT are defined to be in a 'hybrid EMT cell state' and sometimes referred to as 'quasi-mesenchymal cells' [11,12]. Using a large panel of cell-surface markers in skin and mammary primary tumors, the expression of three proteins, namely CD61, CD51, and CD106, was shown to define distinct hybrid EMT cell states [13] (Figure 2A, Key figure). Cells in the hybrid EMT cell state showed a higher degree of plasticity and higher metastatic potential compared with purely epithelial or mesenchymal cells [13]. Notably, one of the hybrid EMT cell state markers CD106, was previously shown to be important for breast and ovarian cancer metastasis [14,15]. Yet, the metastatic potential of cancer cells correlated more with the hybrid EMT cell state than with the sole expression level of CD106 [13]. Furthermore, the potential to reverse EMT, that is, mesenchymal-to-epithelial transition, which has been proposed to be important for metastatic growth [16,17], did not correlate with the metastatic properties of cells in the hybrid EMT cell state [13].

Additional evidence supporting these results comes from a study using lineage tracing systems for hybrid and full EMT cell states in the MMTV-PyMT mouse model of metastatic breast cancer. Cells in a hybrid EMT cell state defined by the marker Tenascin C mostly contribute to metastasis compared with cells undergoing full EMT [18]. Moreover, ablation of the tumor suppressor *FAT1* promotes **stemness** and metastasis via the induction of a hybrid EMT cell state co-regulated by YAP1 and SOX2 in squamous cell carcinoma [19]. Phospho-proteomic profiling of FAT1 knock-out cells revealed new vulnerabilities that could be targeted by the SRC inhibitors dasatinib and saracatinib, thus highlighting how cell plasticity opens new therapeutic opportunities [19]. The loss of FAT1 generating hybrid EMT cell states is an insightful example of how genetic alterations contribute to plasticity and EMT. Together, these results suggest that high plasticity of DTCs correlates with effective metastatic colonization. However, the exact underlying mechanisms require further investigation.

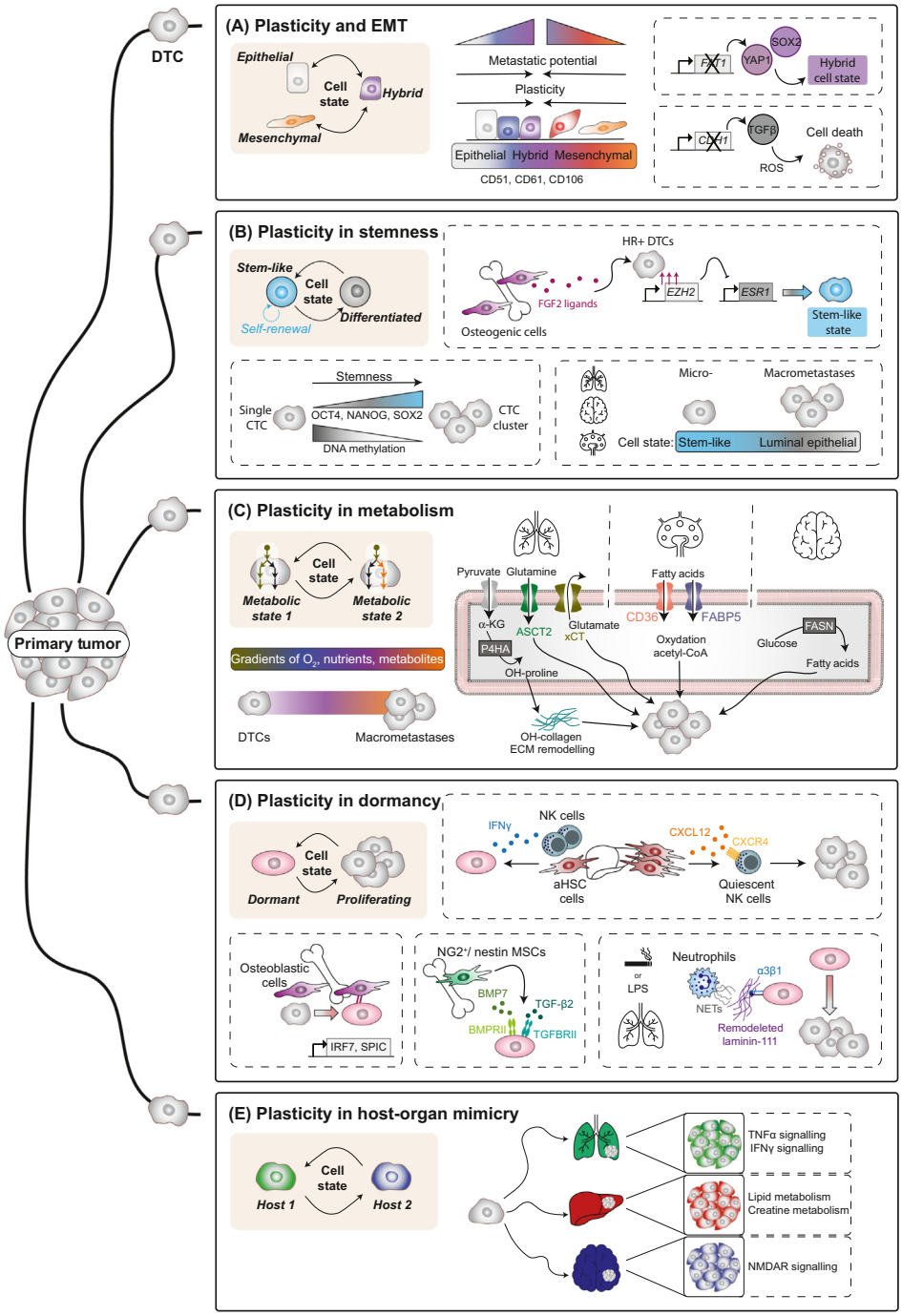
Whether EMT is required for metastasis has been debated in the field [7,11,12,20–22]. However, full EMT meaning the complete loss of epithelial features, impairs metastasis due to loss of plasticity and stemness [16,23,24]. Recently, new mechanistic insights have been gained into the underlying molecular mechanisms of full EMT impairing metastasis. Loss of E-cadherin was shown to increase transforming growth factor-β (TGFβ) signaling and accumulation of reactive oxygen species (ROS) that ultimately triggered apoptosis in DTCs and decreased metastasis [25]. In summary, cells in a hybrid EMT cell state have a high phenotypic plasticity resulting in increased metastatic potential. How the hybrid EMT and the highly plastic cell states are linked, whether one causes the other, and whether the two always co-occur, remain open questions.

Plasticity and stemness

Stemness defines the potential of a cell to differentiate and self-renew. By analogy, the term 'cancer stem cell (CSC)' was coined to describe tumorigenic cell subpopulations that (i) self-renew (i.e., form tumors when serially passaged at clonal cell doses), and (ii) give rise to a new tumor that recapitulates the phenotypic heterogeneity of the parent tumor [26,27]. Whilst a CSC model in which these cells unidirectionally give rise to both CSCs and non-CSCs holds true in some neoplasias, it appears that non-CSCs can also revert to a CSC state. Indeed, the dynamic gene regulatory networks underpinning both CSCs and non-CSCs states are sustained by feedback loops, whose epigenetic dysregulation can lead to cell state conversion [28–32]. Additional studies have suggested that CSCs may be metastatic and resistant to therapy, which poses major therapeutic challenges [30,33]. The genetic evolution and CSC models need not be mutually exclusive and a unifying model has been proposed [26].

Key figure

Phenotypic plasticity of disseminated tumor cells during metastatic colonization



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The questions of whether stemness in cancer is cancer cell- or non-cancer-cell autonomous, and of which molecular and cellular networks underpin the stem-like state are both of great interest [30]. EMT programs are associated with stem-like traits; however, recent studies have unveiled EMT-independent mechanisms that illustrate how stemness contributes to disease progression [12,34].

Reactivation of stemness properties by prevalent cancer mutations is a crucial aspect of tumorigenesis. Indeed, transgenic mouse models revealed that a key mutation in *PIK3CA* (*PIK3CA*^{H1047R}) can reactivate stemness and overcome lineage restriction in adult mammary epithelial cells, thereby generating multilineage tumors [4,5]. Corroborating this observation, *PIK3CA* mutations have been shown to correlate with stemness signatures in several cancer types [35]. *BRCA1* and *TP53* tumor suppressor deletions have also been described to increase stemness and phenotypic plasticity in breast and prostate cancer, respectively [36,37]. However, to what extent mutations, in comparison to epigenetic mechanisms, generate plasticity remains an open question. Additionally, stemness can promote metastasis. Recent work comparing the methylomes of single circulating tumor cells (CTCs) and CTC clusters revealed that binding sites for stemness and proliferation-associated transcription factors OCT4, SOX2, and NANOG are specifically hypomethylated in CTC clusters, which enhances their metastatic potential [38,39] (Figure 2B). In this context, elevated expression of cell–cell junctions may be responsible for the specific upregulation of stemness markers [39]. Reactivation of stem-like features is also a prerequisite for metastatic colonization. Studies in preclinical models of bone metastasis demonstrated that FGF2 ligands secreted from osteogenic cells signal in a paracrine manner to hormone receptor-positive DTCs which increases intrinsic EZH2 expression. Increased EZH2 expression results in H3K27me3-mediated epigenetic silencing of estrogen receptor α (ERα) and lineage-specific transcriptional programs, together with dedifferentiation of DTCs. This increased phenotypic plasticity mediates loss of endocrine therapy responsiveness in early metastatic bone lesions and enhances secondary seeding of bone-entrained metastasis [40,41]. Single cell transcriptomic profiling of luminal metastatic breast cancer patient-derived xenograft (PDX) models confirmed that early micrometastatic lesions are enriched in stem-like signature compared with macrometastatic lesions, where cells had a more luminal differentiation state, reminiscent of the primary tumor identity [42]. In a mouse colorectal cancer model, comparison of the metastatic potential of stem-

Figure 2. Disseminated tumor cells exhibit distinct types of phenotypic plasticity which generate cellular states that result in metastatic colonization. These states may arise by cell intrinsic genomic alterations or via interactions with the organ-specific tumor microenvironment (TME). (A) Cancer cell phenotypes range between the epithelial and mesenchymal states or midway as a hybrid phenotype. Cancer cells in a hybrid EMT cell state, for example, upon *FAT1* deletion, are highly metastatic. (B) Cancer cells with stem-like traits have increased metastatic colonization capabilities, highlighted by transient loss of epithelial features and acquisition of a stem-like gene signature at the early steps of colonization, or by genome-wide DNA hypomethylation in CTC clusters that promotes a stem-like transcriptional program and augments their metastatic potential. (C) Disseminated tumor cells (DTCs) rewire their metabolism depending on the metabolic state of the host organ. DTCs can use site specific metabolic substrates to fuel to their growth and to colonize distant organs. In the lung, DTCs can for example, convert pyruvate into alpha-ketoglutarate (α-KG), thereby enhancing proline hydroxylation and extracellular matrix (ECM)-stiffening and subsequent metastatic colonization. DTCs can also use fatty acids in the lymph node or convert glucose into fatty acids specifically in the brain to fuel their growth. (D) DTCs can enter dormancy and remain as such for years. The dormant state is strongly influenced by different molecular mechanisms and specialized cell types, such as mesenchymal stem cells in bone or natural killer (NK) cells in the liver, and can be released by external cues such as cigarette smoked-based neutrophil activation in the lung. (E) Metastatic colonization by DTCs can be facilitated when they possess molecular features that mimic normal host organ properties, such as creatine and lipid metabolism in the liver, tumor necrosis factor (TNF)α/NFκB signaling in the lung, or glutamate signaling in the brain. Abbreviations: aHSC, activated hepatic stellate cells; CTC, circulating tumor cell; FASN, fatty acids synthase; HR+, hormone receptor positive; IFNγ, inferferon γ; LPS, lipopolysaccharide; MSCs, mesenchymal stem cells; NETs, neutrophil extracellular traps; NMDAR, N-methyl-D-aspartate receptor; P4HA, prolyl-4-hydroxylase; ROS, reactive oxygen species; TGFβ, transforming growth factor β.

like (Lgr5$^+$) and non-stem-like (Lgr5$^-$) cells revealed that most metastases were seeded by Lgr5$^-$ cells. Notably, metastases seeded by Lgr5$^-$ cells expressed a Lgr5$^+$ subpopulation, the neutralization of which induced regression of metastatic foci. Upon paracrine signaling coming from the stem cell niche (e.g., R-Spondin, Wnt), Lgr5$^-$ cells can revert back to Lgr5$^+$ cells, illustrating a dynamic gene network rewiring and cell state conversion. Thus, plasticity supports stem-like cell maintenance at the metastatic site, which is a key factor of metastatic outgrowth [32]. In summary, the aforementioned examples demonstrate how the potential of cancer cells to re-enter a stem-like state is a critical determinant of metastatic colonization of distant organs.

Metabolic plasticity

During the metastatic cascade, DTCs encounter several environments with distinct nutrients, metabolites, and oxygen availability resulting in cell-extrinsic influence on their metabolic states [43,44]. Hence, DTCs undergo metabolic rewiring meaning changes in their metabolic dependencies, either in terms of substrate usage or enzymatic machinery. The result equals a conversion of metabolic state. These dynamic metabolic changes involve two interwoven processes: metabolic flexibility (i.e., the potential to use different nutrients for the same metabolic requirement of a specific step of the metastatic cascade) and metabolic plasticity (i.e., the potential to process one metabolic substrate in different ways and support distinct metabolic requirements imposed by different steps of the metastatic cascade) [43,44]. Here we will focus on the metabolic plasticity involving pyruvate, glutamine, and fatty acids during metastasis (Figure 2C).

During metastatic colonization, metabolic plasticity is often mediated by the prevailing nutrient availability and microenvironments within secondary organs [44,45]. For instance, DTCs that land in the lung become dependent on pyruvate, that is highly abundant in the lung interstitial fluid as compared to plasma [46]. Indeed, extracellular pyruvate uptake is essential for the metastatic outgrowth of DTCs in the lung in breast cancer mouse models [47]. Higher levels of pyruvate in the lung niche supported transamination between glutamate and pyruvate, generating alanine and α-ketoglutarate. The latter activated the enzyme collagen prolyl-4-hydroxylase resulting in extracellular matrix remodeling and metastasis [47]. Another study reported that lung metastases, but not primary breast tumors, exhibited higher mTORC1 signaling via increased activity of the serine biosynthesis pathway as a consequence of pyruvate availability in the lung [48]. Altogether, these examples show that pyruvate metabolic plasticity is critical for DTC colonization of the lung.

Changes in glutamine metabolism during DTC colonization of distant sites has been shown in a prostate cancer mouse model, where inhibition of glutamine transporter ASCT2 suppressed primary tumor and lung metastases outgrowth, but not liver metastases [49]. In a breast cancer mouse model, immunotargeting of the cystine antiporter (xCT) that secretes glutamate while importing cystine, prevented outgrowth of lung metastases by suppressing CSC self-renewal and intracellular redox balance [50]. Thus, glutamine metabolic plasticity supports metastasis in different cancer types.

A consistent body of literature describes fatty acid metabolic plasticity in colonizing DTCs. Comparative assessment of metabolic requirements between brain metastasis and breast primary tumors revealed elevated fatty acid metabolism in the brain through fatty acid synthase (FASN) overexpression, thus resulting in a site-specific dependency [51]. In an oral squamous cell carcinoma model, the high metastatic potential of DTCs in lymph nodes is mediated by their expression of fatty acid-binding protein CD36 and the subsequent enhanced uptake of fatty acids [52]. Blocking CD36 suppressed lung and lymph node metastasis, leaving primary tumor growth unaffected [52]. Another study using cervical cancer mouse models, revealed

that lymph node metastases were supported through FABP5-mediated fatty acid metabolism [53]. Moreover, co-culture of melanoma cells with lung fibroblasts induced the expression of fatty acid mono-desaturating enzyme SCD1, whose silencing decreased metastasis [54]. In addition, YAP-induced fatty acid oxidation is necessary for lymph node but not lung metastasis in melanoma mouse models [55]. Hence, metabolic plasticity is a critical part of metastatic colonization and distinct metabolic phenotypes arise in response to their surrounding niche.

Cellular dormancy of disseminated tumor cells

The concept of cancer dormancy emerged from clinical observations that patients relapse years or even decades after successful treatment of the primary disease [56–59]. Yet, analysis of primary tumor growth kinetic demonstrated that clinically perceived dormancy could equivalently reflect either slow but constant growing DTCs, or DTCs that indeed entered a period of growth arrest followed by accelerated proliferation [60]. Notably, dormant DTCs were detected in the bone marrow of patients showing no signs of metastatic disease and their prognostic significance was subsequently established [61,62]. Moreover, the unexpected observation that some patient recipients of organ transplants developed cancer that originated from the graft, suggests that dormant DTCs resided in distant organs of the donor, and were able to cause metastatic disease in the immunosuppressed host [63]. Two forms of dormancy have been observed: in tumor mass dormancy, cancer cell proliferation is offset by cell death due to immune surveillance and/or insufficient vascularization with no significant change in cell number [64–66]. By contrast, in **cellular dormancy**, DTCs are arrested in the G0 phase and are resistant to host defenses and to therapy [66,67]. The phenomenon of reawakening after a prolonged period of time identifies cellular dormancy as substantially distinct from proliferating metastatic disease and shows it to be under constant dynamic control [66,68,69]. The question whether the switch from dormancy to proliferation is an example of plasticity *per se* remains open. However, recent studies suggest that dormancy is not simply cell quiescence but rather a distinct cellular state characterized by an epigenetic landscape [70] and governed by a defined cellular program [68,69,71] (Figure 2D). DTCs can fluctuate from a dormant to a proliferative state in response to changes within their surrounding environment [72–74]. For example, recent work has shown how inflammation induced by cigarette smoke exposure or nasal instillation of lipopolysaccharide (LPS) in the lung induce substantial changes in the lung environment that result in DTCs awakening [75]. In particular, inflammation led to secretion of proteolytic enzymes neutrophil elastase (NE) and matrix metalloprotease 9 (MMP9) associated to neutrophil extracellular traps (NETs), which cleaved laminin-111 in the extracellular matrix. The newly formed laminin epitope was shown to bind to α3β1 integrin receptor expressed on dormant DTCs, eliciting a signaling cascade that converted dormant DTCs to overt lung metastases [75]. This study shows how structural changes in the surrounding niche may reprogram DTCs from a dormant to a proliferative state.

Conversely, another study revealed the contribution of the niche in inducing dormancy. Secretion of TGFβ2 and bone morphogenetic protein (BMP7) from $NG2^+$/$Nestin^+$ mesenchymal stem cells and binding to their cognate receptors on breast DTCs resulted in activation of the SMAD, p38, and p27 pathways and subsequent cancer dormancy in the bone marrow perivascular niche. Thus, DTCs can be influenced by cells from the hematopoietic stem cell niche and enter dormancy, highlighting their phenotypic plasticity [76].

Further work unraveled how the abundance and activation of natural killer (NK) cells and the activation of hepatic stellate cells dictate the state of DTCs in the liver [77]. Precisely, proliferating and dormant cells reside in two distinct tissue-specific niches. The dormant milieu comprises an increased NK cell pool that sustains dormancy via interferon (IFN)-γ signaling, while the proliferative milieu is characterized by a decrease in NK cells and concomitant expansion of activated

hepatic stellate cells. CXCL12 secreted from the latter induces NK cell quiescence through its cognate receptor CXCR4 that can no longer lock DTCs in the dormant state. These data argue that IFN-γ signaling sustains dormancy and highlight niche-mediated transcriptional reprogramming of DTCs [77]. Furthermore, an additional study showed that distinct cellular states of DTCs differ in their sensitivity to NK cell cytolysis. In particular, proliferating DTCs expressed the transcription factor SOX9, which confers resistance to NK-mediated killing via expression of MHC class I molecules [78,79].

Finally, single-cell transcriptomic profiling of dormant DTCs in prostate cancer and multiple myeloma unveiled a dormancy gene signature enriched in IFN-regulated genes [80,81]. Inhibition of these genes triggered DTCs into proliferation and metastatic growth. Intriguingly, in a model of multiple myeloma, expression of such genes as well as dormancy occurred upon contact-dependent engagement of myeloma cells with osteoblastic cells [80]. Taken together, these studies have elucidated how the microenvironment can influence DTCs to enter or exit dormancy and highlight the consequent plasticity of DTCs.

Host-organ mimicry of DTCs and the influence of the foreign environment

It is now widely accepted that different cancer types, and even different subtypes, preferentially metastasize in particular secondary organs: this phenomenon is known as organotropism [82] (Box 2). An important aim of current cancer research is to identify drivers of organ specificity and so to formulate new therapeutic strategies, but also to understand how reciprocal interactions (crosstalk) between the TME and DTCs initiate metastatic colonization. In this regard, a study profiling lung and liver metastases in the MDA-MB-231 model revealed major site-specific transcriptional differences. Interestingly, DTCs in lung overexpressed genes associated with immune inflammatory pathways [tumor necrosis factor (TNF)α, NFκB, IFNγ…] that resemble normal host tissue, the targeting of which resulted in decreased metastatic clonal heterogeneity [83]. Indeed, previous single-cell transcriptomic profiling of normal lungs revealed the prominence of the immune compartment and inflammatory pathways in this organ [84]. A key finding of this study is that the intrinsic gene signatures of MDA-MB-231 cells isolated from lung and liver metastatic sites also enabled discrimination between phenotypically normal lung and liver organs from the Genotype-Tissue Expression project (GTEx) cohort at the transcriptional level (https://gtexportal.org/home/). This observation confirms that after DTCs colonize a distant site, they share common transcriptional patterns with the normal host tissue (Figure 2E).

Box 2. Plasticity revisits the 'seed and soil' concept of Paget

Preferential patterns of metastatic colonization were reported in 1889 by Stephen Paget from autopsies of patients with breast cancer. He postulated that metastasis is not a random process but rather the result of mutual compatibility between the disseminated tumor cell, metaphorically illustrated by a 'seed', and the foreign organ, pictured as the 'soil' [115]. It is now widely accepted that different cancer types are associated with different metastatic tropisms – now known as organotropism. For example, prostate cancer predominantly metastasizes to the bones but rarely to the brain (91% vs. 1.6%), while colon cancer metastasizes to the liver rather than bone (70% vs. 8%) [82]. Such differential tropism is also observed within different subtypes of breast cancer: where hormone receptor-positive cancer mostly metastasizes to bone and lymph nodes, basal-like breast cancers are more associated with lung and brain metastases [116]. These findings have been recently supported by a statistical framework modeling the risk of recurrence of different subgroups of hormone receptor-positive and -negative patients defined by integrative clusters [117]. According to Paget, the 'seed' will only grow if the 'soil' is fertile, which suggests that the components agree in one state and are unchangeable with time. However, the molecular elucidation of metastatic dissemination and colonization refines this theory. It shows that the biochemical plasticity of DTCs adjusts them to foreign environments and leads to metastatic colonization. Paget also proposed that the properties of the foreign microenvironment determine the fate of DTCs: if the 'soil' is not congenial, the 'seed' will not 'germinate' and there will be no metastases [68]. However, we know now that DTCs may enter a dormant state and only emerge years or decades after arriving at a distant site [58], which suggests that the dynamics of metastatic disease may also depend on characteristics of the TME. Thus, recent discoveries emphasize the plasticity of DTCs and the (co-)evolving nature of the surrounding niche and refine Paget's theory.

Matched transcriptomic profiling of primary tumor cells and cancer cells from different metastatic sites using PDX models and the MDA-MB-231 cell line yielded the same observation. This work first discovered that endogenous glucocorticoid levels increase during breast cancer progression, resulting in glucocorticoid receptor activation and increased lung metastatic colonization [85]. The data also revealed that metastatic samples from distinct sites do not cluster together in principal component analyses, underlining the influence of the niche in shaping the transcriptomic landscape of DTCs. Functional annotation confirmed the upregulation of the TNFα/NFκB pathway specifically in the lung (see Table S1 in the supplemental information online). However, metastatic MDA-MB-231 cells in the liver upregulate genes associated with lipids and creatine metabolism or hepatocyte nuclear factor 3B (HNF3B) pathways, which are all associated with normal liver function (Table S1) [86–88]. Additionally, a recent study using a model of breast-to-brain metastasis demonstrated that metastatic cells react to glutamate neurotransmitter secreted from synaptic neurons by induction of N-methyl-D-aspartate receptor (NMDAR) signaling and subsequent DTC proliferation [89]. These examples of '**host-organ mimicry**' confirm that DTCs can adopt features of the foreign niche, thus uncovering a new and underappreciated plastic attribute of cancer cells that can support colonization. It is now crucial to decipher which mimicked features actively promote metastasis. For instance, creatine intake by DTCs via SCL6A8 transporter can activate SMAD2/3 and promote liver metastatic colonization, an organ highly enriched in this metabolite [90]. Given that DTCs mimic host-organ properties and become dependent on creatine metabolism, targeting this process (e.g., pharmacologically or via diet modification) might prove beneficial for patients. The mechanisms by which the plastic 'seeds' (DTCs) become compatible with the foreign 'soil' (niche) (Box 2) are incompletely understood; their elucidation should offer new therapeutic options.

Exploiting cancer cell plasticity for new therapeutic strategies

Marked phenotypic plasticity of cancer cells results in aggressiveness and fatal disease [91–93]. Identifying the molecular mechanisms of cancer cell plasticity and how they can be targeted is therefore crucial for improving patient outcome. Novel therapeutic strategies will emerge with improved definition of cellular states.

In the context of dormancy, three therapeutic strategies should be considered: (i) Locking DTCs in a dormant state, that is, preventing their reawakening. (ii) Inducing reawakening of dormant DTCs and subsequently targeting them with cytotoxic chemotherapy. (iii) Eliminating dormant DTCs [64,65,68]. An example for strategy (i) is systemic NK cell activation by injection of interleukin (IL)-15 that locks DTCs in a dormant cell state and dramatically prevents liver metastasis outgrowth, enhancing survival in preclinical models [77]. DTCs might dynamically fluctuate from one state to another in response to therapies, imposing the need of adjusting the treatment over time. While endocrine therapies prolong survival in ER^+ breast cancer patients, treatment cessation might trigger relapse [94], and patients could need additional treatments (NCT03400254[i], NCT03032406[ii]). The disadvantage of strategy (ii) is the risk of not controlling metastatic outgrowth and/or not eliminating every single DTC with chemotherapy, rendering this approach still immature to be ventured in the clinic without thorough studies aimed at refining it. Strategy (iii) requires identification of markers or pathways exclusively expressed by dormant DTCs [70,80,95]. The ones identified so far (AXL, VCAM1, SIRPA, IRF7, and IFN signaling pathways) are also found on immune cells, thus further investigation is needed to identify harbingers of the switch from dormancy to overt metastasis. Given the dependency of DTCs on the TME, the aim should be to target the interactions between DTCs and their niches, to interfere with their reactions to the microenvironment, and/or to harness the immune system to eliminate dormant DTCs or prevent their reactivation [64,68,71].

The effectiveness of targeting the metabolic state of cancer cells has been shown by the successful use of chemotherapy over decades to target nucleotide metabolism. Changes in glucose metabolism and enzyme expression in DTCs, as well as differential metabolic dependencies, present novel opportunities to treat cancers with small molecule inhibitors or diet adjustments to disrupt such metabolic states [96].

Finally, the concept of stem-like cells and plasticity has led to the idea of using differentiation and transdifferentiation therapies to reduce cancer cell plasticity and thereby aggressiveness [97,98]. Differentiation therapy has been widely successful in the treatment of acute promyelocytic leukemia (APL) by the introduction of all-trans retinoic acid [99]. However, the usefulness of differentiation therapy in the treatment of solid tumors is controversial [97]. Attempts to differentiate cancer cells into normal epithelial cells have reduced proliferation and increased sensitivity to chemotherapy [100–102]. Furthermore, a high-throughput drug screen aiming to identify agents that selectively kill dedifferentiated cancer cells revealed that salinomycin and nigericin exert specific toxicity towards CSCs by triggering intracellular ROS accumulation and lysosome membrane permeabilization, together with increased cell differentiation [103]. More recently, it was shown that cellular plasticity could be exploited to transdifferentiate breast cancer cells into functional adipocytes resulting in decreased invasion and metastasis [104]. One limitation of such approaches is the difficulty to transdifferentiate every single cancer cell resulting in the risk of fueled growth by introducing additional adipocytes to the TME [105]. Yet, this study opens the door for novel creative approaches deploying transdifferentiation therapy in cancer. In summary, knowledge of the different forms of phenotypic plasticity led to conceptualizing novel therapeutic approaches that target plasticity in cancer.

Concluding remarks

This review emphasizes the multifaceted nature of cell plasticity in the context of metastasis and delineates the different (sometimes coexisting) states of cancer cells that contribute to overt metastasis. While EMT remains the most widely described example of phenotypic plasticity, recent studies hint at many more examples of phenotypic changes that contribute to disease progression. Here we summarize the development of stem-like traits, rewiring of metabolism, awakening from dormancy, and host-organ mimicry as key features of DTCs that support colonization, the last and fatal step of cancer progression. Other forms and mechanisms of cell plasticity remain yet to be discovered and characterized (see Outstanding questions). Indeed, a colon cancer study recently revealed that primary tumor cells can hijack regenerative programs triggered upon colitis, and overexpress L1CAM, which is associated with enhanced metastatic potential [106]. The cellular and molecular mechanisms underlying wound healing and tumor growth are remarkably similar and were recently reviewed [107]. Many connections emerge from the fact that during both processes, intrinsic plasticity programs of the cells are deployed. Additionally, emerging studies on cell rheology investigate how mechanical constraints and loss of intercellular junctions regulate cell fate and impinge on plasticity [108].

The development of single cell technologies and spatial transcriptomic [109,110] greatly improved our capacity to capture tumor heterogeneity (Table 1), yet the difficulty of accessing metastatic samples in patients remains a major limitation. Comparisons of matched primary and metastatic samples within individual patients at multiple levels (i.e., epigenetic, transcriptomic, proteomic) will enhance our comprehension of the metastatic disease in an organ-specific manner and should unveil new vulnerabilities. Indeed, plasticity poses major therapeutic challenges by promoting drug tolerance and/or resistance. Plastic cells can dynamically shuttle between distinct cellular states with distinct drug responsiveness [91]. Hence, targeting cell plasticity should provide a unique opportunity to improve the efficacy of existing therapies. The variety of therapeutic targets

Outstanding questions

Which of the preclinical findings related to cancer cell plasticity can also be demonstrated in patients?

What is the contribution of genetic mutations to the different types of cancer cell plasticity?

What are the cancer cell intrinsic factors that dictate the organotropism of DTCs? What are the site-specific determinants?

What are the molecular mechanisms controlling and stabilizing the different hybrid EMT cell states promoting metastasis?

What are the organ-specific determinants of metastatic dormancy?

How does the immune system influence other facets of cancer cell plasticity?

To what extent does the metabolism of the host organ reprogram the metabolism of DTCs? What are the site-specific metabolic dependencies?

Which site-specific signaling pathways are being appropriated in DTCs and which of these enhance colonization?

Are there additional types of plasticity?

To what extent does the identity of the cell-of-origin of the cancer influence the plasticity of tumor cells?

To which extent is phenotypic plasticity inherent to a cancer cell or induced by external stimuli coming from the TME?

Can cancer cell plasticity be exploited to develop therapeutic strategies aimed at preventing metastatic relapses?

Table 1. Examples of relevant tools and models to study plasticity in the context of metastatic colonization with selected references.

Tools	Applications	Refs
Constructs		
mVenus-p27 reporter	Tag DTCs in G0	[77,118]
ERK and p38 luciferase reporter	Tag proliferating DTCs	[119]
CDK2 biosensor (DHB-mVenus)	Identify each phase of the cell cycle	[120,121]
Technologies		
Single-cell profiling	Deconvolutes the transcriptome, epigenome, and proteome of a particular cell state at the single-cell level	[39,42,122–124]
Imaging mass cytometry	Assess spatially resolved protein abundance phenotypes across single cells	[125]
Spatial transcriptomics	Assess spatially resolved transcriptome phenotypes across single cells	[126]
seqFISH (sequential Fluorescence *In Situ* Hybridization)	Integrates spatial distribution and single-cell transcriptomics	[127]
CaTCH (CRISPRa Tracing of Clones in Heterogeneous cell populations)	DNA barcoding technology that allows isolation of single clones to study if their phenotypes were acquired or pre-existent upon a specific bottleneck	[128]
Intravital imaging	Assess cell state directly in the metastatic niche	[121,129–131]
Fluorescence-activated cell sorting	Assess marker expression defining a specific cell state	[13]
Optical barcoding system	Isolate single clones on the base of 31 distinct fluorescent reporters to capture heterogeneity	[83]
Mouse models and cell lines		
Lgr5CreER/ $Kras^{LSL\text{-}G12D}$/$p53^{fl/fl}$/ $Rosa26\text{-}YFP^{+/+}$	Mouse model of skin squamous cell carcinoma that generates skin tumors undergoing spontaneous EMT	[13]
Lgr5CreER/$Kras^{LSL\text{-}G12D}$/$p53^{fl/fl}$/Rosa26-ΔNp63-IRES-GFP	Mouse model of skin squamous cell carcinoma where cancer cells are blocked in the early hybrid EMT cell state	[132]
Tnc-$CreER^{T2}$/MMTV-Flpo/RC::FrePe/MMTV-PyMT mouse	Lineage tracing of cells in the hybrid EMT cell state	[18]
Cdh2-$CreER^{T2}$/MMTV-Flpo/RC::FrePe/MMTV-PyMT mouse	Lineage tracing of cells that underwent full EMT	[18]
MMTV-PyMT; $Cdh1^{fl/fl}$	Mouse model allowing Cre recombinase inducible deletion of the epithelial marker E-cadherin in vivo	[25]
Cx3cr1-GFP;CCR2-RFP	GFP/RFP tolerized mice to model metastatic disease in an immuno-competent setting	[133,134]

range from chromatin modifying enzymes, transcription factors, kinases, to phosphatases, and ongoing clinical trials are addressing the efficacy of such approaches. In particular, several trials (currently still in Phase I) may address whether targeting plasticity using Wnt inhibitors LGK-974 and CGX1321 (NCT01351103[iii], NCT02675946[iv]) or BET inhibitors (NCT02516553[v]) alone or in combination with chemotherapies translates into improved clinical benefits for patients. To effectively implement such therapies, it is of paramount importance to unravel the molecular mechanisms promoting plasticity both at the cell-autonomous and at the micro-environment levels in an organ-specific manner. The multifaceted nature of plasticity should offer multiple and personalized therapeutic options, whose combination with pre-existing anticancer strategies may lead to long-lasting clinical responses.

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Declaration of interests

C.J., M.V., V.R., and F.R. declare no competing financial interests. M.B-A. owns equities in and has received laboratory support and compensation from Novartis, and serves as a consultant for Basilea.

Resources

[i]https://clinicaltrials.gov/ct2/show/NCT03400254

[ii]https://clinicaltrials.gov/ct2/show/NCT03032406

[iii]https://clinicaltrials.gov/ct2/show/NCT01351103

[iv]https://clinicaltrials.gov/ct2/show/NCT02675946

[v]https://clinicaltrials.gov/ct2/show/NCT02516553

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