Cancer Stem Cells and Epithelial-to-Mesenchymal Transition in Cancer Metastasis

Toni Celià-Terrassa¹ and Mohit Kumar Jolly²

¹Cancer Research Program, IMIM (Hospital del Mar Medical Research Institute), 08003 Barcelona, Spain

²Centre for BioSystems Science and Engineering, Indian Institute of Science, Bangalore 560012, India

Correspondence: acelia@imim.es; mkjolly@iisc.ac.in

The cancer stem cell (CSC) concept stands for undifferentiated tumor cells with the ability to initiate heterogeneous tumors. It is also relevant in metastasis and can explain how metastatic tumors mirror the heterogeneity of primary tumors. Cellular plasticity, including the epithelial-to-mesenchymal transition (EMT), enables the generation of CSCs at different steps of the metastatic process including metastatic colonization. In this review, we update the concept of CSCs and provide evidence of the existence of metastatic stem cells (MetSCs). In addition, we highlight the nuanced understanding of EMT that has been gained recently and the association of mesenchymal-to-epithelial transition (MET) with the acquisition of CSCs properties during metastasis. We also comment on the computational approaches that have profoundly influenced our understanding of CSCs and EMT; and how these studies and new experimental technologies can yield a deeper understanding of the biological aspects of metastasis.

Solid cancers are heterogeneous masses of malignant cells with genomic and phenotypic heterogeneity. Within this heterogeneity, and mirroring the hierarchical organization of normal tissues, tumors can maintain a hierarchical phenotypic organization, with stem-cell-like cells at the apex and more differentiated cells toward the bottom of this hierarchy. Such tumor cells with stem-cell-like properties are termed what we call “cancer stem cells” (CSCs), because of their capacity to give rise to more differentiated progenies with limited proliferative potential, but also to initiate tumor growth and “reproduce” such tumors when cells seed distant organs to develop metastasis (Reya et al. 2001; Clevers 2011; Visvader and Lindeman 2012;

Battle and Clevers 2017). Different types of tumors have varying percentage of CSCs, and often higher content of CSCs implicates poorly differentiated tumors, which is characteristic of tumor aggressiveness and poor clinical prognosis (Ben-Porath et al. 2008).

Metastasis is the deadliest process of cancer, it accounts for >90% of cancer-related deaths (Gupta and Massague 2006; Mehlen and Puisieux 2006). It is a dynamic multistep and complex process that includes the escape from the primary tumor, dissemination throughout the systemic circulation, extravasation at distant tissues, organ-seeding, and final metastatic colonization with the tumor outgrowth (Lambert et al. 2017). The repertoire of obstacles and the

Editors: Jeffrey W. Pollard and Yibin Kang

Additional Perspectives on Metastasis: Mechanism to Therapy available at www.perspectivesinmedicine.org

Copyright © 2020 Cold Spring Harbor Laboratory Press; all rights reserved; doi: 10.1101/cshperspect.a036905

Cite this article as *Cold Spring Harb Perspect Med* 2020;10:a036905

T. Celià-Terrassa and M.K. Jolly

unfavorable ever-changing conditions demands high adaptability and cellular plasticity to tumor cells to overcome the odds. Fortunately, these difficulties make metastasis an inefficient process, in which most cells die in circulation or especially on arrival at the distant tissue, as evidenced in experimental studies (Fidler 1970; Luzzi et al. 1998; Cameron et al. 2000). The later steps of metastasis initiation and colonization are the major roadblocks for disseminated tumor cells (DTCs) to develop into overt metastases (Cameron et al. 2000; Chambers et al. 2002). Thus, only a minority of DTCs are capable of initiating tumor growth at distant tissues, these are the “metastasis-initiating cells” (MICs) with high adaptability and CSC-like properties (Celià-Terrassa and Kang 2016), also called “metastatic stem cells” (MetSCs) (Oskarsson et al. 2014). These cells are reminiscent of CSCs of the primary tumor, with the ability to reinitiate tumor growth in different distant tissues, thus they harbor additional survival strategies to adapt their metabolism, undergo stromal cooption, evade the immune system, show therapy resistance, and others, as previously compiled (Celià-Terrassa and Kang 2016). Moreover, metastasis and CSCs have been found to be connected through one additional common axis—that of epithelial-mesenchymal transition (EMT) and its reverse mesenchymal-epithelial transition (MET) (Mani et al. 2008; Ocaña et al. 2012; Esposito et al. 2019; Pastushenko and Blanpain 2019).

Stem cell properties play a key role during metastasis; however, the study of CSCs in metastasis is still an immature field owing to the technical challenges to faithfully follow-up the course of CSCs, or de novo CSCs during metastasis. Here, we will comment on new supporting evidence of MetSCs, new markers, and new experimental technologies coupled with advanced computational methods to capture the dynamics of epithelial-mesenchymal plasticity (EMP) and stem-cell-like phenotypes during metastasis. Indeed, during the past few years, mathematical models have revealed intriguing aspects about CSCs, EMT/MET, and metastasis (Lu et al. 2013; Boareto et al. 2015; Celià-Terrassa et al. 2018; Bocci et al. 2019a). Therefore, we

stress the necessity to exploit and couple new computational and experimental technologies to further decode the biology and dynamics of MetSCs during metastasis.

THE CANCER STEM CELL CONCEPT

The origin of the CSC concept dates back to the beginning of the 20th century (1907), when the pathologist Max Askanazy postulated that differentiated ovarian teratomas arise from a multipotential type of cell (Kleinsmith and Pierce 1964; Maehle 2011). In addition, single cell transplantations of leukemic and ascites cells were shown to give rise to heterogeneous tumors (Furth et al. 1937; Makino 1956). The first strong experimental evidence for this theory was reported by Barry Pierce’s studies in teratocarcinomas (Kleinsmith and Pierce 1964), in which tumorigenic cells could give rise to multiple differentiated nontumorigenic cells. Additional studies in solid tumors and the hematopoietic system further showed that only small subsets of tumor cells are capable to form colonies in vitro and tumors in vivo (Southam and Brunschwig 1961; Bruce and Van Der Gaag 1963; Fidler and Kripke 1977; Fidler and Hart 1982). However, these observations were contemporary to the discovery of the genetic causes of cancer, drifting the attention to the clonal evolution model as proposed by Nowell (1976). Years after, Dick and colleagues isolated heterogeneous populations from acute myeloid leukemias (AML), and showed the existence of a unique population with tumor-initiating ability in AML, the $CD34^+CD38^-$ cells (Lapidot et al. 1994; Bonnet and Dick 1997). This brought back the idea that not all the tumor cells of a heterogeneous population can initiate tumor growth, but only CSCs can. Remarkably, in 2003, Clarke’s group isolated CSCs from human breast tumors using $Lineage^-/CD24^{low}/CD44^+$ markers with tumor-initiating abilities in immunocompromised mice in vivo (Al-Hajj et al. 2003), thus showing and identifying a particular subpopulation of cancer cells that can regenerate tumor growth after transplantation. Similar studies showed the existence of different populations of CSCs in other cancer types using

different markers (Singh et al. 2004; Dalerba et al. 2007; Ricci-Vitiani et al. 2007). However, not all tumor types may follow the CSC model, as observed for melanoma where most of cells grew tumors on transplantation assays in vivo (Quintana et al. 2008, 2010), although the ABCB5⁺ cells were considered as melanoma CSCs in other studies (Schatton et al. 2008).

To identify CSCs, the most common markers are CD24^{low/-}/CD44⁺, CD133⁺, and ALDH⁺, which have been found in breast, colon, pancreas, prostate, and other organs (Medema 2013). Yet, the specificity of these populations is not absolute and varies among tumor types, as these markers are not necessarily functionally participating in the stem cell phenotype. This variability and the operational use of immuno-

compromised mice to define CSCs have raised controversy about their existence. However, few years ago, the concept was robustly supported by using lineage tracing in unperturbed conditions in spontaneous mouse tumor models (Driessens et al. 2012; Schepers et al. 2012), and also by showing that specific depletion of CSCs led to a lack of tumor initiation (Chen et al. 2012).

The current CSC model (Fig. 1) redefines some old-established misconceptions about CSCs based on the new knowledge and the use of new technologies. First, CSCs do not need to be a rare minority within the tumor population; they can form an abundant proportion (Batlle and Clevers 2017). This frequency may depend on the type and stage of the tumor, such that poorly differentiated tumors contain high

Figure 1. Cancer stem cells (CSCs) and epithelial-to-mesenchymal transition (EMT) plasticity during metastasis. The CSC model in the primary tumor follow a hierarchical organization with CSCs at the apex of this structure. CSCs differentiate into transit amplifying cells (TA) and further into differentiated tumor cells without self-renewal ability. According to the updated knowledge on CSCs, differentiated tumor cells can become CSCs. This cellular plasticity includes processes such as the EMT and its reversion by mesenchymal-to-epithelial transition (MET). The intermediate EMT states—also called hybrid EMT or partial EMT (pMET)—can acquire stem cell properties with self-renewal ability, which is essential for tumor propagation. However, the extreme states are more differentiated and lack tumor-initiating ability. During metastasis, heterogeneous tumors release different type of individual E (epithelial-like) and M (mesenchymal-like) cells into the bloodstream, but also as mixed clusters of circulating tumor cells (CTCs), increasing metastasis. Therefore, CSCs, either in E, M, or pEMT, can travel with other cells and seed distant organs, however, only the CSCs are capable to form metastatic—at this step called metastatic stem cells (MetSCs). This can be the result of E-CSCs with M-cell trailblazers, de novo generation of CSCs from differentiated cells; and/or the seeding of M-CSCs leading micrometastasis with final reversion by MET to form proliferative overt metastasis.

T. Celià-Terrassa and M.K. Jolly

frequencies of CSCs. Second, the CSC concept does not imply that the origin of these cells are normal stem cells. Indeed, they can derive from any type of adult somatic cell and be reprogrammed with a malignant stem-cell-like phenotype (Visvader and Lindeman 2012; Kreso and Dick 2014). Third, CSCs are not a fixed population; instead, stemness is a dynamic cell trait, many studies have shown how non-CSCs can become CSCs, and vice-versa. This has been shown in vitro (Chaffer et al. 2011; Gupta et al. 2011; Phillips et al. 2014), but also in vivo by intravital imaging lineage tracing (Zomer et al. 2013). Moreover, the return from a senescent state is associated with the acquisition of stem cell phenotype (Milanovic et al. 2018), as another example of dramatic plasticity. Therefore, CSCs are dynamic populations with high cellular plasticity, which is critical for metastasis and chemoresistance (Celià-Terrassa and Kang 2016). Fourth, CSCs can be highly proliferative; they do not need to reside in a quiescent state as often assumed, and thus both quiescent and nonquiescent CSCs may coexist, as it is the case for normal adult stem cells in many tissues (Li and Clevers 2010). Indeed, the original definition of CSCs by B.G. Pierce described undifferentiated proliferative malignant stem cells and differentiated tumor cells with limited proliferative capacity (Pierce and Speers 1988). Furthermore, one of the first studies isolating CSCs showed no cell cycle differences among breast CSCs and non-CSCs (Al-Hajj et al. 2003).

EVIDENCE OF CSCs LEADING METASTASIS

With their ability to initiate tumors and cellular plasticity, CSCs are believed to be the cells responsible for repopulating metastatic outgrowths. Similar to the observations in primary tumors, not all cells are capable to form experimental metastasis. In fact, metastasis has been proven to be an extremely inefficient process with <0.1% of administered cells able to grow metastasis in experimental/preclinical settings (Fidler 1970; Luzzi et al. 1998; Cameron et al. 2000), suggesting that only few special cells with self-renewal ability are able to drive the tumorigenesis at distance (Fig. 1). Therefore, the CSC

model is aligned with these experimental observations and many studies have shown that only certain tumor populations are capable to form metastasis when injected either orthotopically or directly into systemic circulation (Hermann et al. 2007; Charafe-Jauffret et al. 2009, 2010; Davis et al. 2010; Liu et al. 2010; Pang et al. 2010; Malanchi et al. 2012), which is the same operational definition of CSCs in the primary tumor, but for metastasis.

Primary and metastatic tumors have similar hierarchically organized structures in different types of human cancer samples (Ma et al. 2003; Ramaswamy et al. 2003; Weigelt et al. 2003; Dalerba et al. 2011; Merlos-Suárez et al. 2011), presumably owing to CSCs being able to recreate the primary tumor in a distant site. These observations are consistent with recent single-cell transcriptomic approaches comparing primary and metastatic tumors of head and neck cancer (Puram et al. 2017). Studies in the breast cancer field are also good examples of MetSC driving hierarchically organized metastases, in which the basal-like DTCs seeding distant organs can generate metastatic tumors with luminal-like differentiated traits showing their pluripotency (Lawson et al. 2015; Ye et al. 2015). To support this notion of CSCs as the root of metastasis, colon cancer metastases might be exclusively driven by a tumor-initiating cell subpopulation with long-term self-renewal as a requirement for these cells to metastasize (Dieter et al. 2011).

Many signaling pathways regulating self-renewal in normal stem cells have been reported to promote stemness and malignant progression, such as Notch signaling, Sonic hedgehog (Shh), Wnt, and others (Reya et al. 2001; Reya and Clevers 2005; Oskarsson et al. 2014). Moreover, many cell fate determinants and embryonic-related genes are critical for the metastatic establishment. Stem cell-related genes like SOX2, NANOG, KLF4, OCT4, SNAI2, SNAI1, SOX9, and others, are important promoters of stemness and increase metastasis in different cancer types (Celià-Terrassa and Kang 2016), and also observed in clinical metastasis (Malta et al. 2018). A recent study in breast cancer has shown that clusters of circulating tumor cells

(CTCs) with metastatic ability display hypomethylation of stemness transcription factors SOX2, NANOG, OCT4, and SIN3A, and their methylation suppresses metastasis because of a loss of expression of these genes, which are functionally implicated in stemness (Gkountela et al. 2019). In addition, the methylation profile of these genes in CTC-clusters correlate with poor prognosis. Other examples are the SOX2/SOX9 expression in DTCs leading metastasis initiation after latency of breast cancer (Malladi et al. 2016), the increased metastasis by SLUG/SOX9 (Guo et al. 2012), and by the miR-199a stemness regulator in normal and cancer cells that favors tumor and metastasis initiation (Celià-Terrassa et al. 2017).

MetSCs also share common markers of CSCs (Table 1): CD24^{low/-}/CD44⁺, ALDH⁺, or CD133⁺ (Al-Hajj et al. 2003; Sato et al. 2010; Reuben et al. 2011; Rodriguez-Torres and Allan 2016). Human breast cancer CD44⁺ cells can be spontaneously metastatic to lungs after injection into the mammary fat pad (Liu et al. 2010) and

are also found as a population of CSCs in the metastatic tumors. Moreover, ALDH⁺ CSCs were shown to be more metastatic to bone, lung, soft tissues, and muscle than ALDH⁻ cells, which barely form metastasis (Charafe-Jauffret et al. 2009, 2010). Another group, using the MMTV-PyMT breast cancer mouse model, identified a CSC population based on the CD24⁺/Thy1⁺ markers with lung metastasis ability, but not the CD24⁻/Thy1⁻ counterparts (Malanchi et al. 2012). In pancreatic cancer metastasis (Hermann et al. 2007) and in colorectal cancer (CRC) metastasis (Müller et al. 2001; Zhang et al. 2012), the CD133⁺CXCR4⁺ population has been shown to behave as MetSCs with self-renewal and drug resistance ability, similar to that seen in CD133⁺CD26⁺ cells (Pang et al. 2010) in CRC metastasis. In addition, the drug depletion of the CXCR4⁺ population in CRC eliminates liver metastasis, indicating this population as a MetSC population and suggesting a possible therapeutic strategy for metastasis (Céspedes et al. 2018). Moreover, Epcam⁺CD44⁺

Table 1. Metastatic stem cell (MetSC) populations

Identified MetSCs	Primary tumor of origin	Site of metastasis	Exclusive for MetSCs
CD24 ^{low/-} /CD44 ⁺ (Liu et al. 2010)	Breast cancer	Lungs	No
ALDH ⁺ (Charafe-Jauffret et al. 2009, 2010)	Breast cancer	Bone, lungs, soft tissues, and muscle	No
CD24 ⁺ /Thy1 ⁺ (Malanchi et al. 2012)	Breast cancer	Lungs	No
CD133 ⁺ CD44 ⁺ (Chen et al. 2011)	Colorectal cancer	Liver	No
CD133 ⁺ CXCR4 ⁺ (Müller et al. 2001; Hermann et al. 2007)	Pancreatic and colorectal cancer	Liver; lungs	No
CD133 ⁺ CD26 ⁺ (Pang et al. 2010)	Colorectal cancer	Liver	No
Epcam ⁺ CD44 ⁺ CD47 ⁺ MET ⁺ (Baccelli et al. 2013)	Breast cancer CTCs	Bone, lungs, and liver	Yes
CD44 ⁺ CD36 ⁺ (Pascual et al. 2017)	Oral squamous cell carcinoma	Lymph nodes	Yes
Lgr5 ⁺ (de Sousa e Melo et al. 2017)	Colorectal cancer	Liver	Yes
EpCAM ⁻ CD106 ⁺ (Pastushenko et al. 2018)	Skin squamous cell carcinoma and breast cancer	Lungs	Yes

T. Celià-Terrassa and M.K. Jolly

CD47⁺MET⁺ CTCs have shown metastasis-initiating capacity in the bone marrow, suggesting them as a circulating population of MetSCs (Baccelli et al. 2013). Interestingly, there are particular subsets of markers unique of metastatic initiation (Table 1) that strikingly do not affect the primary tumor growth, most likely because of the extraordinary requirements imposed by the host distant organs (Celià-Terrassa and Kang 2016). This is the case of the CD44⁺CD36⁺ population of oral squamous cell carcinoma (OSCC) that only promotes tumor initiation in metastatic lymph nodes (Pascual et al. 2017) where they use high lipid consumption to grow. Similarly, the Lgr5⁺ CSCs depletion in CRC impedes the metastasis formation to liver, but not the primary tumor growth (de Sousa e Melo et al. 2017). Remarkably, a recent study has shown that EpCAM⁺CD106⁺ skin and mammary carcinoma cells display high plasticity and increased tumor-initiating abilities in the lung, but not in the primary tumor site (Pastushenko et al. 2018). Further research needs to focus on identifying such type of unique markers associated with specific MIC properties, to identify and eventually halt the root of metastasis.

These studies show experimental evidence of the CSC model in metastasis; however, only few studies have isolated and characterized CSC from metastatic clinical samples (Al-Hajj et al. 2003; Shipitsin et al. 2007), because of the difficulty to obtain tumor metastasis samples. More efforts need to be aligned with medical oncologists to obtain metastatic clinical samples and the molecular/functional analysis of clinical MetSCs.

EPITHELIAL-TO-MESENCHYMAL PLASTICITY (EMP) AS AN ENGINE FORCE OF CSCs DURING METASTASIS

Epithelial-to-mesenchymal transition (EMT) is a typical process of the embryonic development that is aberrantly awakened in cancer, fibrosis, and wound healing (Kalluri and Weinberg 2009). During EMT, epithelial cells suffer dramatic cytoskeleton changes losing cell polarity, cell adhesion molecules (i.e., E-cadherin), and detach from the epithelial layer gaining migratory

properties. The process is regulated by a complex network of genes that trigger the *trans*-differentiation into a mesenchymal-like phenotype. Remarkably, it is a reversible process, such that cells that undergo an EMT can undergo MET. Cycles of EMT–MET occur for cell type specialization when cells arrive to the final destination in the embryo (Nakajima et al. 2000; Thiery et al. 2009), and we can find similar cycles of EMT–MET dynamics in metastasis (Celià-Terrassa and Kang 2016; Nieto et al. 2016). This dynamic reversibility has recently received the name of epithelial-to-mesenchymal plasticity (EMP) or epithelial plasticity (Nieto 2013; Varga and Greten 2017; Gupta et al. 2019). Importantly, EMT has been proposed as one of the most important biological process inducing stem cell properties (Mani et al. 2008); initially, this observation was made in breast cancer; and many other studies showed it across different cancer types (Nieto et al. 2016). This aspect is particularly relevant for metastasis since EMT has long been linked to increase invasiveness, favoring the escape from the primary tumor site and thus metastasis (Batlle et al. 2000; Cano et al. 2000; Thiery et al. 2009). Hence, besides immune evasion (Kudo-Saito et al. 2009; Terry et al. 2017) and chemoresistance (Fischer et al. 2015; Zheng et al. 2015), EMT can converge two of the most important properties to promote metastasis: invasiveness and stemness.

Today, we know that there are many intermediate EMT states in which cells can acquire stemness (Dongre and Weinberg 2019; Pastushenko and Blanpain 2019), but “extreme” EMT, that is, acquisition of a mesenchymal phenotype, can lead to loss of stemness, plasticity, and tumor initiation (Celià-Terrassa et al. 2012). A hybrid epithelial/mesenchymal (E/M) phenotype was reported to have tumor-initiation ability and cell plasticity to differentiate into different lineages (Strauss et al. 2011). Therefore, the window of stemness is most likely to be found in between epithelial and mesenchymal phenotypes, as predicted by computational models (Fig. 1) (Jolly et al. 2015a,b). Follow-up studies that categorized cells into three, instead of two EMT phenotypes, revealed that the hybrid E/M cells (CD24⁺/CD44⁺) formed 10 times more mammo-

spheres in vitro as compared with epithelial (CD24⁺/CD44⁻) or mesenchymal (CD44⁺/CD24⁻) ones (Grosse-Wilde et al. 2015). The aggressive behavior of such “double positive” cells was also witnessed in vivo (Goldman et al. 2015). Remarkably, two main studies have recently identified new markers of these predicted intermediate EMT stem cell states. Blanpain’s group found EpCAM⁻ CD106⁺ cells as showing an intermediate state of spontaneous EMT in in vivo models of skin squamous cell carcinoma (SCC) and the MMTV-PyMT mouse model of breast cancer (Pastushenko et al. 2018). This intermediate state is more plastic, stem-like, and metastatic than the extreme epithelial or mesenchymal states. In addition, Weinberg’s group has recently identified a new population within the CD44⁺ cells in breast cancer, which are CD104⁺ and display a hybrid E/M phenotype of tumor-initiating CSCs (Kröger et al. 2019). These CD104⁺/CD44⁺ cells were shown to initiate tumors in vivo, a behavior that cannot be obtained even by simultaneously injecting “extremely epithelial” and “extremely mesenchymal” cells (Kröger et al. 2019). Together, these studies corroborate the observations that a spatiotemporal regulation of EMP is critical during metastasis, and the cells that get “locked” in a “fully mesenchymal” state may have lost the plasticity and stemness required for the metastatic colonization (Celià-Terrassa et al. 2012; Ocaña et al. 2012; Tsai et al. 2012). Although the markers for identifying hybrid E/M phenotypes and the hybrid E/M CSCs are starting to be identified, computational models in future studies can contribute to mapping the traits of the EMT spectrum in terms of other hallmarks of metastasis, such as metabolic adaptation, immune evasion, self-renewal, anoikis resistance, and drug resistance (Celià-Terrassa and Kang 2016; Tripathi et al. 2016; Jolly et al. 2019b). It should be noted that the association of hybrid E/M and CSCs does not need to be one-to-one, that is, there may be CSCs that are not hybrid E/M, and there may be hybrid E/M cells that do not behave as CSCs. Indeed, CSCs across cancer types were found to lie along various positions in the EMT spectrum, based on their transcriptomic profiling (Fig. 1) (Bocci et al. 2018).

Some studies suggest the existence of multiple subsets of CSCs, the EMT-like CSCs, which are slow cycling and invasive, and the epithelial-like CSCs, which are more proliferative and thus can initiate the tumor growth (Liu et al. 2014; Poleszczuk et al. 2015). This observation is aligned with the fact that EMT is important for escape the primary tumor site, survive in circulation, and seed distant metastasis in which they can remain latent using self-renewal for long periods of time in slow-cycling mode. On the other hand, MET is crucial to form proliferative E-like CSCs within the window of stemness leading to metastatic colonization (Fig. 1). CD24^{low/-}/CD44⁺ markers are associated with an EMT-like phenotype and ALDH⁺ with an E-like (Liu et al. 2014) or hybrid E/M phenotype (Colacino et al. 2018). Thus, future studies need to consider these possibilities and exploit new technologies, especially single-cell analysis to study the dynamics of metastasis (Lawson et al. 2018), and how the different type of CSCs populations may coexist and/or coevolve, driving metastasis and their critical roles in different aspects of the metastatic process.

Recent studies have also uncovered new nuances of EMT that may be crucial, such as spontaneous switching among various EMT phenotypes (Tripathi et al. 2019), the existence of distinct EMT gene programs with different migratory modes (Aiello et al. 2018), and distinct EMT dynamics (Celià-Terrassa et al. 2018). Strikingly, different EMT dynamics can generate distinct EMT gene-programs with similar invasive abilities but different metastatic potential associated with gain of stem cell properties (Celià-Terrassa et al. 2018). Thus, the implications of EMT in cancer metastasis are likely to be more complex than initially thought, depending on the intracellular/intercellular dynamics, intermediate states, and cellular context (Bocci et al. 2019b).

THE JOURNEY OF CSCs AND EMP DURING METASTASIS

During the past decades, many conceptual models have been proposed to explain the course of metastasis: the all cell metastasis model; the

T. Celià-Terrassa and M.K. Jolly

subpopulation evolution model; the clonal dominance model; the tumor heterogeneity of metastatic variants model; the parallel acquisition of mutations model; the circulating oncogene model that transform distant organ tumor cells; and the CSCs model, which are well-reviewed elsewhere (see Weigelt et al. 2005). Many of these models overlap with each other and converge to an integrative model centered around the existence of CSCs propagating metastasis. These CSCs may accumulate mutations, deal with new encountered environments, establish heterogeneous tumors, and can explain the clinical course of metastasis and distant relapse (Weigelt et al. 2005).

The metastatic journey starts when tumor cells escape from the primary tumor site. For this first step, the acquisition of invasive ability by EMT is relevant for most of the cancer types. Millions of cells are released into circulation with heterogeneous phenotypes: epithelial-like CTCs and mesenchymal-like CTCs are found in blood patient samples. Cells in one or more hybrid E/M phenotype(s) have been observed in individual and clustered CTCs, and in tumor buds across cancer types (Armstrong et al. 2011; Yu et al. 2013; Sarioglu et al. 2015; Grigore et al. 2016; Chebouti et al. 2017; Seroczynska et al. 2019), indicating their potential functional roles in metastasis. CTC clusters can generate a disproportionately higher percentage of metastasis than individual CTCs (Aceto et al. 2014; Cheung et al. 2016), and recent evidence show how stem cell gene profiles of these clusters increase metastasis (Gkountela et al. 2019). In addition, mathematical models have identified crucial cell-to-cell communication molecules such as JAG1 that may contribute to their formation (Boareto et al. 2016). Indeed, JAG1 was found to be among the top five differentially expressed genes in cells positive for K14—a proposed marker for collective cell migration (Cheung et al. 2016). Knocking down JAG1 led to a reduction in tumor emboli formation in inflammatory breast cancer cells SUM159, without affecting cell viability or proliferation, suggesting a role of JAG1/Notch signaling in maintaining stemness (Bocci et al. 2019a). Such cell-to-cell communication may affect the

frequency and size distribution of CTC clusters as experimentally observed in multiple cancer types in mouse models and patients (Bocci et al. 2019b).

Recently, lineage tracing and barcoding studies have shown the polyclonal origin of metastasis (Reeves et al. 2018), opening the possibility of clonal cooperation among E and M tumor cells. Indeed, a recent study using barcoding sequencing in triple-negative breast cancer suggests that the invasive trailblazer cells—suggestive of EMT-like cells—do not persist in the metastatic site and other cells seed the successful metastasis (Merino et al. 2019). In such scenario, extravasation would be favored by the invasive ability of EMT-like cells and then opportunistic epithelial-like CSCs can take advantage of the opened track to colonize and grow in distant tissues (Fig. 1), which is according to previous observations (Tsuji et al. 2008; Celià-Terrassa et al. 2012; Chapman et al. 2014; Westcott et al. 2015). Another common model is that EMT-like cells extravasate and seed distant organs, establish relationships with the metastatic niche, evade immune surveillance and after a variable latency periods, the metastatic niche signals or other stimuli, induce their MET to initiate the distant growth maintaining their tumor-initiating ability and stemness (Fig. 1) (Celià-Terrassa and Kang 2018). This schema has been proposed on observation of many studies describing the requirement of MET to colonize distant tissues (Ocaña et al. 2012; Tsai et al. 2012; Lawson et al. 2015), and is specifically well-shown in a recent study reporting how the bone niche endothelium E-selecting induce MET and stemness in bone metastatic cells engaging the Wnt pathway (Esposito et al. 2019). Together, these studies suggest that the most important property to successfully form metastasis is the tumor-initiating ability/stemness.

COMPUTATIONAL MODELING OF CSCs/EMP NETWORKS AND THEIR PREDICTIONS

Advances in mapping the regulatory networks governing the EMP and/or CSC state of a cell have facilitated the development of computational models to decode their emergent dynam-

ics, drive novel hypotheses, and identify targets to restrict this cellular plasticity (Fig. 2). For instance, a hybrid E/M state was largely considered to be “metastable,” that is, cells cannot maintain it for extended periods, and a terminal mesenchymal state was believed to be the end point of a transition (Thiery 2002; Lee et al. 2006; Chaffer and Weinberg 2011; Scheel and Weinberg 2012). Recent mathematical models predicted that EMT is not an “all-or-none” process, instead cells may stably maintain one or more hybrid E/M phenotypes (Jolly et al. 2017a). In vitro and in vivo evidence for the stability of hybrid E/M phenotypes has since accumulated across cancer types (Jolly et al. 2016; Pastushenko et al. 2018; Kröger et al. 2019). Cells in one or more hybrid E/M phenotypes may be more stem-like, drug-resistant, and aggressive than those in “purely mesenchymal” or “purely epithelial” ones; hence, the frequency of hybrid

E/M cells correlate with poor clinical outcomes (Jolly et al. 2019a). Besides predicting the existence of one or more stable hybrid E/M phenotypes, another prediction that the mathematical models of EMT networks have made is these hybrid E/M phenotype(s) may coexist alongside epithelial and/or mesenchymal cells in a given isogenic population (Jolly and Levine 2017). Such phenotypic heterogeneity has been observed in vitro in multiple cell lines in which cells coexpressing various epithelial and mesenchymal markers have been found alongside ones predominantly expressing only epithelial or only mesenchymal markers (Grosse-Wilde et al. 2015; Andriani et al. 2016; George et al. 2017; Celià-Terrassa et al. 2018). Such coexistence of phenotypes is a hallmark of multistability, which can emerge as a result various feedback loops regulating EMT (Jia et al. 2017), as has been well-studied in other biological

Figure 2. Iterative computational–experimental approach to decode the dynamics of metastasis. The interplay between experimental data and computational modeling: models can help to infer the underlying gene networks of epithelial–mesenchymal plasticity (EMP) and metastatic stem cells (MetSCs) from dynamic/static experimental data; these networks can then be simulated to identify their dynamical properties; specific predictions from such models can be tested experimentally in vitro and in vivo, hence contributing to gaining new biological insights.

T. Celià-Terrassa and M.K. Jolly

systems (Guantes and Poyatos 2008). Such coexistence may enable clonal cooperation during metastasis.

In addition to making predictions based on “bottom-up” mechanism-based models, computational approaches can also help navigate through the deluge of high-dimensional single-cell RNA-Seq data being generated currently, that is, a “top-down” approach of identifying patterns/subsets without any a priori information about the underlying mechanisms. Single-cell RNA-Seq analysis has been applied to stratify cancer subtypes too; intriguingly, this analysis found that most tumors contain cells of multiple molecular subtypes—a feature that cannot be extracted from bulk analysis (Patel et al. 2014). For instance, in metastatic melanoma, each sample could be classified as “MITF-high” or “AXL-high” at a bulk level, but every tumor contained cells from both of these transcriptional states (Tirosh et al. 2016). Single-cell expression data can be analyzed using nonlinear dimensional reduction methods such as t-distributed stochastic neighbor embedding (t-SNE) to unravel the underlying transcriptomic heterogeneity in cancer cell population (Saadatpour et al. 2014). Such methods can also help analyze data from other single-cell methods such as mass cytometry (CyTOF). For instance, CyTOF data was collected from multiple time points for 28 markers as lung cancer cells were exposed to transforming growth factor (TGF β) and then allowed to revert to being epithelial after withdrawal of TGF β . The dynamic data generated an EMT–MET state map, onto which the lung cancer specimens were projected to identify the presence of various EMP phenotypes (Karacosta et al. 2019). One of the common criticisms of single-cell analysis is the lack of functional implications (Lawson et al. 2018), but insights gained from such high-dimensional analysis can unearth new mechanistic aspects too. For instance, the EMT–MET state map revealed that inducing MET may not need be the same as inhibiting EMT (Karacosta et al. 2019), as cells may take different paths in the multidimensional morphological and molecular landscape defining EMP (Jolly et al. 2017b). Gene expression pro-

files being collected at various timepoints during EMT (Meyer-Schaller et al. 2019) and/or MET (Stylianou et al. 2018) has helped unravel the gene signatures unique to MET, thus underscoring the asymmetry between EMT/MET.

Another limitation of single-cell RNA-Seq methods is that the data collected tends to be sparse, typically capturing a small number of molecules (Krishnaswamy et al. 2018). This limitation can at least partially, be overcome by imputation and data denoising methods such as MAGIC (Markov-affinity based graph imputation of cells) (van Dijk et al. 2018). Applying pseudotemporal algorithms such as Wanderlust on the RNA-Seq data can yield insights into dynamics of EMP by tracking the strengths of different edges in the network over a period of time (Krishnaswamy et al. 2018). Application of MAGIC to TGF β -treated HMLE suggested that 80% of cells showed one or more hybrid E/M phenotypes, and identified many genes whose expression peaked during these hybrid phenotypes while maintained low levels in both epithelial and mesenchymal states (van Dijk et al. 2018). Identification of those markers unique to hybrid E/M phenotypes (Bocci et al. 2019c) may reveal novel MetSC markers. Thus, computational analysis of static/dynamic single-cell expression data can possibly discover novel regulatory networks for EMP/CSC and pinpoint potential therapeutic vulnerabilities (Fig. 2).

Similar to EMP heterogeneity, single-cell analysis of hepatocellular carcinoma revealed transcriptomic, molecular, and functional heterogeneity of CSC subsets (Zheng et al. 2018), reminiscent of observations in human breast stem and progenitor cells (Colacino et al. 2018; Giraddi et al. 2018). A stemness index derived using one-class logistic regression was observed to be higher in individual metastatic breast cancer cells compared with primary tumors (Malta et al. 2018), suggesting a putative MetSC-specific signature. Similar logistic regression models have also been used to quantify EMP (George et al. 2017), and concluded that, unlike the previously long-held notion, EMT did not always correlate with worse patient survival, an observation that was also shown through using another EMT scoring metric (Tan et al. 2014).

Furthermore, a partial-EMT signature identified from single-cell transcriptomics can predict nodal metastasis and adverse pathological features in head and neck squamous carcinoma (Puram et al. 2017). Thus, single-cell RNA-Seq analysis can unravel new signatures associated with clinical parameters. Put together, mathematical models, combined with the accumulation of high-throughput single-cell data can yield valuable insights into functionally relevant open questions: (1) how many EMP and CSC states exist and how do they correlate?; (2) what are the hallmarks of those EMP/CSC states?; and (3) what vulnerabilities of the underlying networks for these cell states can be potentially exploited clinically for designing adaptive, combinatorial, and/or differentiation therapies? (Jolly et al. 2019a).

CONCLUDING REMARKS

The current CSC model includes the notion of MetSCs as a dynamic plastic population with the ability to adapt and recreate heterogeneous tumors in distant sites similar to the primary tumor. In this model, cellular plasticity stands as a core condition of MetSCs and essential to understand the biology of CSCs. In addition, the EMP refers to the bidirectional reversibility of EMT and the intermediate states generated that have been recently identified with stem-cell-like properties and the most aggressive for metastasis. The spatiotemporal control of EMP and CSCs should be a matter of intense investigation during the next years.

Emerging new single-cell-omic technologies, mass cytometry (CyTOF), intravital imaging, lineage tracing models, and other sophisticated tools are valuable methods to provide unprecedented insights into the contributions of spatiotemporal dynamics and the functional relevance of heterogeneity in establishing metastases. Advanced and dedicated computational models can be used to process such high-dimensional data to characterize the dynamics and heterogeneity of EMP and/or CSC traits, and to generate new predictions that can be experimentally tested. Thus, an iterative interaction among mathematical modeling, func-

tional assays, and clinical data will be crucial in elucidating the nonlinear dynamics of the multifaceted nature of MetSCs.

ACKNOWLEDGMENTS

We apologize to the investigators whose important studies could not be cited here because of space limitations. The work was supported by the Instituto de Salud Carlos III-FSE (MS17/00037; PI18/00014) and the Cancer Research Institute CLIP grant to T.C.-T.; and by Ramamujan Fellowship (SB/S2/RJN-049/2018) provided by SERB, Department of Science and Technology, Government of India to M.K.J.

REFERENCES

- Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, Yu M, Pely A, Engstrom A, Zhu H, et al. 2014. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell* **158**: 1110–1122. doi:10.1016/j.cell.2014.07.013
- Aiello NM, Maddipati R, Norgard RJ, Balli D, Li J, Yuan S, Yamazoe T, Black T, Sahmoud A, Furth EE, et al. 2018. EMT subtype influences epithelial plasticity and mode of cell migration. *Dev Cell* **45**: 681–695.e4. doi:10.1016/j.devcel.2018.05.027
- Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. 2003. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci* **100**: 3983–3988. doi:10.1073/pnas.0530291100
- Andriani F, Bertolini G, Facchinetti F, Baldoli E, Moro M, Casalini P, Caserini R, Milione M, Leone G, Pelosi G, et al. 2016. Conversion to stem-cell state in response to micro-environmental cues is regulated by balance between epithelial and mesenchymal features in lung cancer cells. *Mol Oncol* **10**: 253–271. doi:10.1016/j.molonc.2015.10.002
- Armstrong AJ, Marengo MS, Oltean S, Kemeny G, Bitting RL, Turnbull JD, Herold CI, Marcom PK, George DJ, Garcia-Blanco MA. 2011. Circulating tumor cells from patients with advanced prostate and breast cancer display both epithelial and mesenchymal markers. *Mol Cancer Res* **9**: 997–1007. doi:10.1158/1541-7786.MCR-10-0490
- Baccelli I, Schneeweiss A, Riethdorf S, Stenzinger A, Schillert A, Vogel V, Klein C, Saini M, Bäuerle T, Wallwiener M, et al. 2013. Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay. *Nat Biotechnol* **31**: 539–544. doi:10.1038/nbt.2576
- Batlle E, Clevers H. 2017. Cancer stem cells revisited. *Nat Med* **23**: 1124–1134. doi:10.1038/nm.4409
- Batlle E, Sancho E, Francí C, Domínguez D, Monfar M, Baulida J, García de Herreros A. 2000. The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. *Nat Cell Biol* **2**: 84–89. doi:10.1038/35000034

T. Celià-Terrassa and M.K. Jolly

- Ben-Porath I, Thomson MW, Carey VJ, Ge R, Bell GW, Regev A, Weinberg RA. 2008. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. *Nat Genet* **40**: 499–507. doi:10.1038/ng.127
- Boaretto M, Jolly MK, Ben-Jacob E, Onuchic JN. 2015. Jagged mediates differences in normal and tumor angiogenesis by affecting tip-stalk fate decision. *Proc Natl Acad Sci* **112**: E3836–E3844. doi:10.1073/pnas.1511814112
- Boaretto M, Jolly MK, Goldman A, Pietilä M, Mani SA, Sengupta S, Ben-Jacob E, Levine H, Onuchic JN. 2016. Notch-Jagged signalling can give rise to clusters of cells exhibiting a hybrid epithelial/mesenchymal phenotype. *J R Soc Interface* **13**: 20151106. doi:10.1098/rsif.2015.1106
- Bocci F, Jolly MK, George JT, Levine H, Onuchic JN. 2018. A mechanism-based computational model to capture the interconnections among epithelial-mesenchymal transition, cancer stem cells and Notch-Jagged signaling. *Oncotarget* **9**: 29906–29920. doi:10.18632/oncotarget.25692
- Bocci F, Gearhart-Serna L, Boaretto M, Ribeiro M, Ben-Jacob E, Devi GR, Levine H, Onuchic JN, Jolly MK. 2019a. Toward understanding cancer stem cell heterogeneity in the tumor microenvironment. *Proc Natl Acad Sci* **116**: 148–157. doi:10.1073/pnas.1815345116
- Bocci F, Kumar Jolly M, Onuchic JN. 2019b. A biophysical model uncovers the size distribution of migrating cell clusters across cancer types. *Cancer Res*. doi:10.1158/0008-5472.CAN-19-1726
- Bocci F, Tripathi SC, Vilchez Mercedes SA, George JT, Casabar JP, Wong PK, Hanash SM, Levine H, Onuchic JN, Jolly MK. 2019c. NRF2 activates a partial epithelial-mesenchymal transition and is maximally present in a hybrid epithelial/mesenchymal phenotype. *Integr Biol (Camb)* **11**: 251–263. doi:10.1093/intbio/zyz021
- Bonnet D, Dick JE. 1997. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* **3**: 730–737. doi:10.1038/nm0797-730
- Bruce WR, Van Der Gaag H. 1963. A quantitative assay for the number of murine lymphoma cells capable of proliferation in vivo. *Nature* **199**: 79–80. doi:10.1038/199079a0
- Cameron MD, Schmidt EE, Kerkvliet N, Nadkarni KV, Morris VL, Groom AC, Chambers AF, MacDonald IC. 2000. Temporal progression of metastasis in lung: cell survival, dormancy, and location dependence of metastatic inefficiency. *Cancer Res* **60**: 2541–2546.
- Cano A, Pérez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, Del Barrio MG, Portillo F, Nieto MA. 2000. The transcription factor Snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. *Nat Cell Biol* **2**: 76–83. doi:10.1038/35000025
- Celià-Terrassa T, Kang Y. 2016. Distinctive properties of metastasis-initiating cells. *Genes Dev* **30**: 892–908. doi:10.1101/gad.277681.116
- Celià-Terrassa T, Kang Y. 2018. Metastatic niche functions and therapeutic opportunities. *Nat Cell Biol* **20**: 868–877. doi:10.1038/s41556-018-0145-9.
- Celià-Terrassa T, Meca-Cortés Ó, Mateo F, Martínez de Paz A, Rubio N, Arnal-Estapé A, Ell BJ, Bermudo R, Díaz A, Guerra-Rebollo M, et al. 2012. Epithelial-mesenchymal transition can suppress major attributes of human epithelial tumor-initiating cells. *J Clin Invest* **122**: 1849–1868. doi:10.1172/JCI59218
- Celià-Terrassa T, Liu DD, Choudhury A, Hang X, Wei Y, Zamalloa J, Alfaro-Aco R, Chakrabarti R, Jiang YZ, Koh BI, et al. 2017. Normal and cancerous mammary stem cells evade interferon-induced constraint through the MIR-199a-LCOR axis. *Nat Cell Biol* **19**: 711–723. doi:10.1038/ncb3533
- Celià-Terrassa T, Bastian C, Liu D, Ell B, Aiello NM, Wei Y, Zamalloa J, Blanco AM, Hang X, Kunisky D, et al. 2018. Hysteresis control of epithelial-mesenchymal transition dynamics conveys a distinct program with enhanced metastatic ability. *Nat Commun* **9**: 5005. doi:10.1038/s41467-018-07538-7
- Céspedes MV, Unzueta U, Aviñó A, Gallardo A, Álamo P, Sala R, Sánchez-Chardi A, Casanova I, Mangues MA, Lopez-Pousa A, et al. 2018. Selective depletion of metastatic stem cells as therapy for human colorectal cancer. *EMBO Mol Med* **10**: e8772. doi:10.15252/emmm.201708772
- Chaffer CL, Weinberg RA. 2011. A perspective on cancer cell metastasis. *Science* **331**: 1559–1564. doi:10.1126/science.1203543
- Chaffer CL, Brueckmann I, Scheel C, Kaestli AJ, Wiggins PA, Rodrigues LO, Brooks M, Reinhardt F, Su Y, Polyak K, et al. 2011. Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. *Proc Natl Acad Sci* **108**: 7950–7955. doi:10.1073/pnas.1102454108
- Chambers AF, Groom AC, MacDonald IC. 2002. Dissemination and growth of cancer cells in metastatic sites. *Nat Rev Cancer* **2**: 563–572. doi:10.1038/nrc865
- Chapman A, Fernandez del Ama L, Ferguson J, Kamarashev J, Wellbrock C, Hurlstone A. 2014. Heterogeneous tumor subpopulations cooperate to drive invasion. *Cell Rep* **8**: 688–695. doi:10.1016/j.celrep.2014.06.045
- Charafe-Jauffret E, Ginestier C, Iovino F, Wicinski J, Cervera N, Finetti P, Hur MH, Diebel ME, Monville F, Dutcher J, et al. 2009. Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature. *Cancer Res* **69**: 1302–1313. doi:10.1158/0008-5472.CAN-08-2741
- Charafe-Jauffret E, Ginestier C, Iovino F, Tarpin C, Diebel M, Esterni B, Houvenaeghel G, Extra J, Bertucci F, Jacquemier J, et al. 2010. Aldehyde dehydrogenase 1-positive cancer stem cells mediate metastasis and poor clinical outcome in inflammatory breast cancer. *Clin Cancer Res* **16**: 45–55. doi:10.1158/1078-0432.CCR-09-1630
- Chebouti I, Kasimir-Bauer S, Buderath P, Wimberger P, Hauch S, Kimmig R, Kuhlmann JD. 2017. EMT-like circulating tumor cells in ovarian cancer patients are enriched by platinum-based chemotherapy. *Oncotarget* **8**: 48820–48831.
- Chen KL, Pan F, Jiang H, Chen JF, Pei L, Xie FW, Liang HJ. 2011. Highly enriched CD133⁺ CD44⁺ stem-like cells with CD133⁺ CD44 high metastatic subset in HCT116 colon cancer cells. *Clin Exp Metastasis* **28**: 751–763. doi:10.1007/s10585-011-9407-7
- Chen J, Li Y, Yu TS, McKay RM, Burns DK, Kernie SG, Parada LF. 2012. A restricted cell population propagates glioblastoma growth after chemotherapy. *Nature* **488**: 522–526. doi:10.1038/nature11287

- Cheung KJ, Padmanaban V, Silvestri V, Schipper K, Cohen JD, Fairchild AN, Gorin MA, Verdone JE, Pienta KJ, Bader JS, et al. 2016. Polyclonal breast cancer metastases arise from collective dissemination of keratin 14-expressing tumor cell clusters. *Proc Natl Acad Sci* **113**: E854–E863. doi:10.1073/pnas.1508541113
- Clevers H. 2011. The cancer stem cell: premises, promises and challenges. *Nat Med* **17**: 313–319. doi:10.1038/nm.2304
- Colacino JA, Azizi E, Brooks MD, Harouaka R, Fouladdel S, McDermott SP, Lee M, Hill D, Madden J, Boerner J, et al. 2018. Heterogeneity of human breast stem and progenitor cells as revealed by transcriptional profiling. *Stem Cell Rep* **10**: 1596–1609. doi:10.1016/j.stemcr.2018.03.001
- Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, Hoey T, Gurney A, Huang EH, Simeone DM, et al. 2007. Phenotypic characterization of human colorectal cancer stem cells. *Proc Natl Acad Sci* **104**: 10158–10163. doi:10.1073/pnas.0703478104
- Dalerba P, Kalisky T, Sahoo D, Rajendran PS, Rothenberg ME, Leyrat AA, Sim S, Okamoto J, Johnston DM, Qian D, et al. 2011. Single-cell dissection of transcriptional heterogeneity in human colon tumors. *Nat Biotechnol* **29**: 1120–1127. doi:10.1038/nbt.2038
- Davis SJ, Divi V, Owen JH, Bradford CR, Carey TE, Papanerakis S, Prince MEP. 2010. Metastatic potential of cancer stem cells in head and neck squamous cell carcinoma. *Arch Otolaryngol Neck Surg* **136**: 1260–1266. doi:10.1001/archoto.2010.219
- de Sousa e Melo F, Kurtova AV, Harnoss JM, Kljavin N, Hoek JD, Hung J, Anderson JE, Storm EE, Modrusan Z, Koeppen H, et al. 2017. A distinct role for Lgr5⁺ stem cells in primary and metastatic colon cancer. *Nature* **543**: 676–680. doi:10.1038/nature21713
- Dieter SM, Ball CR, Hoffmann CM, Nowrouzi A, Herbst F, Zavidij O, Abel U, Arens A, Weichert W, Brand K, et al. 2011. Distinct types of tumor-initiating cells form human colon cancer tumors and metastases. *Cell Stem Cell* **9**: 357–365. doi:10.1016/j.stem.2011.08.010
- Dongre A, Weinberg RA. 2019. New insights into the mechanisms of epithelial–mesenchymal transition and implications for cancer. *Nat Rev Mol Cell Biol* **20**: 69–84. doi:10.1038/s41580-018-0080-4
- Driessens G, Beck B, Caauwe A, Simons BD, Blanpain C. 2012. Defining the mode of tumour growth by clonal analysis. *Nature* **488**: 527–530. doi:10.1038/nature11344
- Esposito M, Mondal N, Greco TM, Wei Y, Spadazzi C, Lin SC, Zheng H, Cheung C, Magnani JL, Lin SH, et al. 2019. Bone vascular niche E-selectin induces mesenchymal–epithelial transition and Wnt activation in cancer cells to promote bone metastasis. *Nat Cell Biol* **21**: 627–639. doi:10.1038/s41556-019-0309-2
- Fidler IJ. 1970. Metastasis: quantitative analysis of distribution and fate of tumor emboli labeled. *J Natl Cancer Inst* **45**: 773–782.
- Fidler IJ, Hart IR. 1982. Biological diversity in metastatic neoplasms: origins and implications. *Science* **217**: 998–1003. doi:10.1126/science.7112116
- Fidler IJ, Kripke ML. 1977. Metastasis results from preexisting variant cells within a malignant tumor. *Science* **197**: 893–895. doi:10.1126/science.887927
- Fischer KR, Durrans A, Lee S, Sheng J, Li F, Wong STC, Choi H, El Rayes T, Ryu S, Troeger J, et al. 2015. Epithelial–to–mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. *Nature* **527**: 472–476. doi:10.1038/nature15748
- Furth J, Kahn MC, Breedis C. 1937. The transmission of leukemia of mice with a single cell. *Am J Cancer* **31**: 276–282.
- George JT, Jolly MK, Xu S, Somarelli JA, Levine H. 2017. Survival outcomes in cancer patients predicted by a partial EMT gene expression scoring metric. *Cancer Res* **77**: 6415–6428. doi:10.1158/0008-5472.CAN-16-3521
- Girardi RR, Chung CY, Heinz RE, Balcioglu O, Novotny M, Trejo CL, Dravis C, Hagos BM, Mehrabad EM, Rodewald LW, et al. 2018. Single-cell transcriptomes distinguish stem cell state changes and lineage specification programs in early mammary gland development. *Cell Rep* **24**: 1653–1666.e7. doi:10.1016/j.celrep.2018.07.025
- Gkoutela S, Castro-Giner F, Szczerba BM, Vetter M, Landin J, Scherrer R, Krol I, Scheidmann MC, Beisel C, Stirnimann CU, et al. 2019. Circulating tumor cell clustering shapes DNA methylation to enable metastasis seeding. *Cell* **176**: 98–112.e14. doi:10.1016/j.cell.2018.11.046
- Goldman A, Majumder B, Dhawan A, Ravi S, Goldman D, Kohandel M, Majumder PK, Sengupta S. 2015. Temporally sequenced anticancer drugs overcome adaptive resistance by targeting a vulnerable chemotherapy-induced phenotypic transition. *Nat Commun* **6**: 6139. doi:10.1038/ncomms7139
- Grigore A, Jolly M, Jia D, Farach-Carson M, Levine H. 2016. Tumor budding: the name is EMT. Partial EMT. *J Clin Med* **5**: 51. doi:10.3390/jcm5050051
- Grosse-Wilde A, Fouquier d' Hérouël A, McIntosh E, Ertaylan G, Skupin A, Kuestner RE, del Sol A, Walters KA, Huang S. 2015. Stemness of the hybrid epithelial/mesenchymal state in breast cancer and its association with poor survival. *PLoS ONE* **10**: e0126522. doi:10.1371/journal.pone.0126522
- Guantes R, Poyatos JF. 2008. Multistable decision switches for flexible control of epigenetic differentiation. *PLoS Comput Biol* **4**: e1000235. doi:10.1371/journal.pcbi.1000235
- Guo W, Keckesova Z, Donaher JL, Shibue T, Tischler V, Reinhardt F, Itzkovitz S, Noske A, Zurrer-Härdi U, Bell G, et al. 2012. Slug and Sox9 cooperatively determine the mammary stem cell state. *Cell* **148**: 1015–1028. doi:10.1016/j.cell.2012.02.008
- Gupta GP, Massagué J. 2006. Cancer metastasis: building a framework. *Cell* **127**: 679–695. doi:10.1016/j.cell.2006.11.001
- Gupta PB, Fillmore CM, Jiang G, Shapira SD, Tao K, Kuperwasser C, Lander ES. 2011. Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. *Cell* **146**: 633–644. doi:10.1016/j.cell.2011.07.026
- Gupta PB, Pastushenko I, Skibinski A, Blanpain C, Kuperwasser C. 2019. Phenotypic plasticity: driver of cancer initiation, progression, and therapy resistance. *Cell Stem Cell* **24**: 65–78. doi:10.1016/j.stem.2018.11.011
- Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, Bruns CJ, Heeschen C. 2007. Distinct populations of cancer stem cells determine tumor growth and

T. Celià-Terrassa and M.K. Jolly

- metastatic activity in human pancreatic cancer. *Cell Stem Cell* **1**: 313–323. doi:10.1016/j.stem.2007.06.002
- Jia D, Jolly MK, Kulkarni P, Levine H. 2017. Phenotypic plasticity and cell fate decisions in cancer: insights from dynamical systems theory. *Cancers (Basel)* **9**: E70.
- Jolly MK, Levine H. 2017. Computational systems biology of epithelial-hybrid-mesenchymal transitions. *Curr Opin Syst Biol* **3**: 1–6. doi:10.1016/j.coisb.2017.02.004
- Jolly M, Boareto M, Huang B, Jia D, Lu M, Ben-Jacob E, Onuchic JN, Levine H. 2015a. Implications of the hybrid epithelial/mesenchymal phenotype in metastasis. *Front Oncol* **5**: 155. doi:10.3389/fonc.2015.00155
- Jolly MK, Jia D, Boareto M, Mani SA, Pienta KJ, Ben-Jacob E, Levine H. 2015b. Coupling the modules of EMT and stemness: a tunable “stemness window” model. *Oncotarget* **6**: 25161–25174. doi:10.18632/oncotarget.4629
- Jolly M, Tripathi SC, Jia D, Mooney SM, Celiktas M, Hanash SM, Mani SA, Pienta KJ, Ben-Jacob E, Levine H. 2016. Stability of the hybrid epithelial/mesenchymal phenotype. *Oncotarget* **7**: 27067–27084. doi:10.18632/oncotarget.8166
- Jolly MK, Tripathi SC, Somarelli JA, Hanash SM, Levine H. 2017a. Epithelial–mesenchymal plasticity: how have quantitative mathematical models helped improve our understanding? *Mol Oncol* **11**: 739–754. doi:10.1002/1878-0261.12084
- Jolly MK, Ware KE, Gilja S, Somarelli JA, Levine H. 2017b. EMT and MET: necessary or permissive for metastasis? *Mol Oncol* **11**: 755–769. doi:10.1002/1878-0261.12083
- Jolly MK, Somarelli JA, Sheth M, Biddle A, Tripathi SC, Armstrong AJ, Hanash SM, Bapat SA, Rangarajan A, Levine H. 2019a. Hybrid epithelial/mesenchymal phenotypes promote metastasis and therapy resistance across carcinomas. *Pharmacol Ther* **194**: 161–184. doi:10.1016/j.pharmthera.2018.09.007
- Jolly MK, Ware KE, Xu S, Gilja S, Shetler S, Yang Y, Wang X, Austin RG, Runyambo D, Hish AJ, et al. 2019b. E-cadherin represses anchorage-independent growth in sarcomas through both signaling and mechanical mechanisms. *Mol Cancer Res* **17**: 1391–1402. doi:10.1158/1541-7786.MCR-18-0763
- Kalluri R, Weinberg RA. 2009. The basics of epithelial–mesenchymal transition. *J Clin Invest* **119**: 1420–1428. doi:10.1172/JCI39104
- Karacosta LG, Anchang B, Ignatiadis N, Kimmey SC, Benson JA, Shrager JB, Tibshirani R, Bendall SC, Plevritis SK. 2019. Mapping lung cancer epithelial–mesenchymal transition states and trajectories with single-cell resolution. bioRxiv doi: 10.1101/570341.
- Kleinsmith LJ, Pierce GB. 1964. Multipotentiality of single embryonal carcinoma cells. *Cancer Res* **24**: 1544–1551.
- Kreso A, Dick JE. 2014. Evolution of the cancer stem cell model. *Cell Stem Cell* **14**: 275–291. doi:10.1016/j.stem.2014.02.006
- Krishnaswamy S, Zivanovic N, Sharma R, Pe’Er D, Bodenmiller B. 2018. Learning time-varying information flow from single-cell epithelial to mesenchymal transition data. *PLoS ONE* **13**: e0203389. doi:10.1371/journal.pone.0203389
- Kröger C, Afeyan A, Mraz J, Eaton EN, Reinhardt F, Khodor YL, Thiru P, Bieri B, Ye X, Burge CB, et al. 2019. Acquisition of a hybrid E/M state is essential for tumorigenicity of basal breast cancer cells. *Proc Natl Acad Sci* **116**: 7353–7362. doi:10.1073/pnas.1812876116.
- Kudo-Saito C, Shirako H, Takeuchi T, Kawakami Y. 2009. Cancer metastasis is accelerated through immunosuppression during snail-induced EMT of cancer cells. *Cancer Cell* **15**: 195–206. doi:10.1016/j.ccr.2009.01.023
- Lambert AW, Pattabiraman DR, Weinberg RA. 2017. Emerging biological principles of metastasis. *Cell* **168**: 670–691. doi:10.1016/j.cell.2016.11.037
- Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA, Dick JE. 1994. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* **367**: 645–648.
- Lawson DA, Bhakta NR, Kessenbrock K, Prummel KD, Yu Y, Takai K, Zhou A, Eyob H, Balakrishnan S, Wang CY, et al. 2015. Single-cell analysis reveals a stem-cell program in human metastatic breast cancer cells. *Nature* **526**: 131–135. doi:10.1038/nature15260
- Lawson DA, Kessenbrock K, Davis RT, Pervolarakis N, Werb Z. 2018. Tumour heterogeneity and metastasis at single-cell resolution. *Nat Cell Biol* **20**: 1349–1360. doi:10.1038/s41556-018-0236-7
- Lee JM, Dedhar S, Kalluri R, Thompson EW. 2006. The epithelial–mesenchymal transition: new insights in signaling, development, and disease. *J Cell Biol* **172**: 973–981. doi:10.1083/jcb.200601018
- Li N, Clevers H. 2010. Coexistence of quiescent and active adult stem cells in mammals. *Science* **327**: 542–545. doi:10.1126/science.1180794
- Liu H, Patel MR, Prescher JA, Patsialou A, Qian D, Lin J, Wen S, Chang YF, Bachmann MH, Shimono Y, et al. 2010. Cancer stem cells from human breast tumors are involved in spontaneous metastases in orthotopic mouse models. *Proc Natl Acad Sci* **107**: 18115–18120. doi:10.1073/pnas.1006732107
- Liu S, Cong Y, Wang D, Sun Y, Deng L, Liu Y, Martin-Trevino R, Shang L, McDermott SP, Landis MD, et al. 2014. Breast cancer stem cells transition between epithelial and mesenchymal states reflective of their normal counterparts. *Stem Cell Rep* **2**: 78–91. doi:10.1016/j.stemcr.2013.11.009
- Lu M, Jolly MK, Levine H, Onuchic JN, Ben-Jacob E. 2013. MicroRNA-based regulation of epithelial–hybrid–mesenchymal fate determination. *Proc Natl Acad Sci* **110**: 18144–18149. doi:10.1073/pnas.1318192110
- Luzzi KJ, MacDonald IC, Schmidt EE, Kerkvliet N, Morris VL, Chambers AF, Groom AC. 1998. Multistep nature of metastatic inefficiency. *Am J Pathol* **153**: 865–873. doi:10.1016/S0002-9440(10)65628-3
- Ma X-J, Salunga R, Tuggle JT, Gaudet J, Enright E, McQuary P, Payette T, Pistone M, Stecker K, Zhang BM, et al. 2003. Gene expression profiles of human breast cancer progression. *Proc Natl Acad Sci* **100**: 5974–5979. doi:10.1073/pnas.0931261100
- Maehle AH. 2011. Ambiguous cells: the emergence of the stem cell concept in the nineteenth and twentieth centuries. *Notes Rec R Soc Lond* **65**: 359–378. doi:10.1098/rsnr.2011.0023

- Makino S. 1956. Further evidence favoring the concept of the stem cell in ascites tumors of rats. *Ann NY Acad Sci* **63**: 818–830. doi:10.1111/j.1749-6632.1956.tb50894.x
- Malanchi I, Santamaria-Martínez A, Susanto E, Peng H, Lehr HA, Delaloye JF, Huelsken J. 2012. Interactions between cancer stem cells and their niche govern metastatic colonization. *Nature* **481**: 85–89. doi:10.1038/nature10694
- Malladi S, MacAlinao DG, Jin X, He L, Basnet H, Zou Y, De Stanchina E, Massagué J. 2016. Metastatic latency and immune evasion through autocrine inhibition of WNT. *Cell* **165**: 45–60. doi:10.1016/j.cell.2016.02.025
- Malta TM, Sokolov A, Gentles AJ, Burzykowski T, Poisson L, Weinstein JN, Kamińska B, Huelsken J, Omberg L, Gevaert O, et al. 2018. Machine learning identifies stemness features associated with oncogenic dedifferentiation. *Cell* **173**: 338–354.e15. doi:10.1016/j.cell.2018.03.034
- Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, et al. 2008. The epithelial–mesenchymal transition generates cells with properties of stem cells. *Cell* **133**: 704–715. doi:10.1016/j.cell.2008.03.027
- Medema JP. 2013. Cancer stem cells: the challenges ahead. *Nat Cell Biol* **15**: 338–344. doi:10.1038/ncb2717
- Mehlen P, Puisieux A. 2006. Metastasis: a question of life or death. *Nat Rev Cancer* **6**: 449–458. doi:10.1038/nrc1886
- Merino D, Weber TS, Serrano A, Vaillant F, Liu K, Pal B, Di Stefano L, Schreuder J, Lin D, Chen Y, et al. 2019. Barcoding reveals complex clonal behavior in patient-derived xenografts of metastatic triple negative breast cancer. *Nat Commun* **10**: 766. doi:10.1038/s41467-019-08595-2
- Merlos-Suárez A, Barriga FM, Jung P, Iglesias M, Céspedes MV, Rossell D, Sevillano M, Hernando-Momblona X, Da Silva-Diz V, Muñoz P, et al. 2011. The intestinal stem cell signature identifies colorectal cancer stem cells and predicts disease relapse. *Cell Stem Cell* **8**: 511–524. doi:10.1016/j.stem.2011.02.020
- Meyer-Schaller N, Cardner M, Diepenbruck M, Saxena M, Tiede S, Lüönd F, Ivanek R, Beerenwinkel N, Christofori G. 2019. A hierarchical regulatory landscape during the multiple stages of EMT. *Dev Cell* **48**: 539–553.e6. doi:10.1016/j.devcel.2018.12.023
- Milanovic M, Fan DNY, Belenki D, Däbritz JHM, Zhao Z, Yu Y, Dörr JR, Dimitrova L, Lenze D, Monteiro Barbosa IA, et al. 2018. Senescence-associated reprogramming promotes cancer stemness. *Nature* **553**: 96–100. doi:10.1038/nature25167
- Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, et al. 2001. Involvement of chemokine receptors in breast cancer metastasis. *Nature* **410**: 50–56. doi:10.1038/35065016
- Nakajima Y, Yamagishi T, Hokari S, Nakamura H. 2000. Mechanisms involved in valvuloseptal endocardial cushion formation in early cardiogenesis: roles of transforming growth factor (TGF)- β and bone morphogenetic protein (BMP). *Anat Rec* **258**: 119–127. doi:10.1002/(SICI)1097-0185(20000201)258:2<119::AID-AR1>3.0.CO;2-U
- Nieto MA. 2013. Epithelial plasticity: a common theme in embryonic and cancer cells. *Science* **342**: 1234850. doi:10.1126/science.1234850
- Nieto MA, Huang RYYJ, Jackson RAA, Thiery JPP. 2016. EMT. 2016. *Cell* **166**: 21–45. doi:10.1016/j.cell.2016.06.028
- Nowell PC. 1976. The clonal evolution of tumor cell populations. *Science* **194**: 23–28. doi:10.1126/science.959840
- Ocaña OH, Córcoles R, Fabra Á, Moreno-Bueno G, Acloque H, Vega S, Barrallo-Gimeno A, Cano A, Nieto MA. 2012. Metastatic colonization requires the repression of the epithelial–mesenchymal transition inducer Prrxl. *Cancer Cell* **22**: 709–724. doi:10.1016/j.ccr.2012.10.012
- Oskarsson T, Batlle E, Massagué J. 2014. Metastatic stem cells: sources, niches, and vital pathways. *Cell Stem Cell* **14**: 306–321. doi:10.1016/j.stem.2014.02.002
- Pang R, Law WL, Chu ACY, Poon JT, Lam CSC, Chow AKM, Ng L, Cheung LWH, Lan XR, Lan HY, et al. 2010. A subpopulation of CD26⁺ cancer stem cells with metastatic capacity in human colorectal cancer. *Cell Stem Cell* **6**: 603–615. doi:10.1016/j.stem.2010.04.001
- Pascual G, Avgustinova A, Mejetta S, Martín M, Castellanos A, Attolini CSO, Berenguer A, Prats N, Toll A, Huetto JA, et al. 2017. Targeting metastasis-initiating cells through the fatty acid receptor CD36. *Nature* **541**: 41–45. doi:10.1038/nature20791
- Pastushenko I, Blanpain C. 2019. EMT transition states during tumor progression and metastasis. *Trends Cell Biol* **29**: 212–226. doi:10.1016/j.tcb.2018.12.001
- Pastushenko I, Brisebarre A, Sifrim A, Fioramonti M, Revenco T, Boumahdi S, Van Keymeulen A, Brown D, Moers V, Lemaire S, et al. 2018. Identification of the tumour transition states occurring during EMT. *Nature* **556**: 463–468. doi:10.1038/s41586-018-0040-3
- Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, Wakimoto H, Cahill DP, Nahed B V, Curry WT, Martuza RL, et al. 2014. Single-cell RNA-Seq highlights intratumoral heterogeneity in primary glioblastoma. *Science* **344**: 1396–1401. doi:10.1126/science.1254257
- Phillips S, Prat A, Sedic M, Proia T, Wronski A, Mazumdar S, Skibinski A, Shirley SH, Perou CM, Gill G, et al. 2014. Cell-state transitions regulated by SLUG are critical for tissue regeneration and tumor initiation. *Stem Cell Rep* **2**: 633–647. doi:10.1016/j.stemcr.2014.03.008
- Pierce GB, Speers WC. 1988. Tumors as caricatures of the process of tissue renewal: prospects for therapy by directing differentiation. *Cancer Res* **48**: 1996–2004.
- Poleszczuk J, Hahnfeldt P, Enderling H. 2015. Evolution and phenotypic selection of cancer stem cells. *PLoS Comput Biol* **11**: e1004025. doi:10.1371/journal.pcbi.1004025
- Puram SV, Tirosh I, Parikh AS, Patel AP, Yizhak K, Gillespie S, Rodman C, Luo CL, Mroz EA, Emerick KS, et al. 2017. Single-cell transcriptomic analysis of primary and metastatic tumor ecosystems in head and neck cancer. *Cell* **171**: 1611–1624.e24. doi:10.1016/j.cell.2017.10.044
- Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. 2008. Efficient tumour formation by single human melanoma cells. *Nature* **456**: 593–598. doi:10.1038/nature07567
- Quintana E, Shackleton M, Foster HR, Fullen DR, Sabel MS, Johnson TM, Morrison SJ. 2010. Phenotypic heterogeneity among tumorigenic melanoma cells from patients that is reversible and not hierarchically organized. *Cancer Cell* **18**: 510–523. doi:10.1016/j.ccr.2010.10.012

T. Celià-Terrassa and M.K. Jolly

- Ramaswamy S, Ross KN, Lander ES, Golub TR. 2003. A molecular signature of metastasis in primary solid tumors. *Nat Genet* **33**: 49–54. doi:10.1038/ng1060
- Reeves MQ, Kandyba E, Harris S, Del Rosario R, Balmain A. 2018. Multicolour lineage tracing reveals clonal dynamics of squamous carcinoma evolution from initiation to metastasis. *Nat Cell Biol* **20**: 699–709. doi:10.1038/s41556-018-0109-0
- Reuben JM, Lee BN, Gao H, Cohen EN, Mego M, Giordano A, Wang X, Lodhi A, Krishnamurthy S, Hortobagyi GN, et al. 2011. Primary breast cancer patients with high risk clinicopathologic features have high percentages of bone marrow epithelial cells with ALDH activity and CD44⁺CD24^{lo} cancer stem cell phenotype. *Eur J Cancer* **47**: 1527–1536. doi:10.1016/j.ejca.2011.01.011
- Reya T, Clevers H. 2005. Wnt signalling in stem cells and cancer. *Nature* **434**: 843–850. doi:10.1038/nature03319
- Reya T, Morrison SJ, Clarke MF, Weissman IL. 2001. Stem cells, cancer, and cancer stem cells. *Nature* **414**: 105–111. doi:10.1038/35102167
- Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, De Maria R. 2007. Identification and expansion of human colon-cancer-initiating cells. *Nature* **445**: 111–115. doi:10.1038/nature05384
- Rodriguez-Torres M, Allan AL. 2016. Aldehyde dehydrogenase as a marker and functional mediator of metastasis in solid tumors. *Clin Exp Metastasis* **33**: 97–113. doi:10.1007/s10585-015-9755-9
- Saadatpour A, Guo G, Orkin SH, Yuan GC. 2014. Characterizing heterogeneity in leukemic cells using single-cell gene expression analysis. *Genome Biol* **15**: 525. doi:10.1186/s13059-014-0525-9
- Sarioglu AF, Aceto N, Kojic N, Donaldson MC, Zeinali M, Hamza B, Engstrom A, Zhu H, Sundaresan TK, Miyamoto DT, et al. 2015. A microfluidic device for label-free, physical capture of circulating tumor cell clusters. *Nat Methods* **12**: 685–691. doi:10.1038/nmeth.3404
- Sato A, Sakurada K, Kumabe T, Sasajima T, Beppu T, Asano K, Ohkuma H, Ogawa A, Mizoi K, Tominaga T, et al. 2010. Association of stem cell marker CD133 expression with dissemination of glioblastomas. *Neurosurg Rev* **33**: 175–184. doi:10.1007/s10143-010-0239-8
- Schatton T, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, Gasser M, Zhan Q, Jordan S, Duncan LM, Weishaupt C, et al. 2008. Identification of cells initiating human melanomas. *Nature* **451**: 345–349. doi:10.1038/nature06489
- Scheel C, Weinberg RA. 2012. Cancer stem cells and epithelial-mesenchymal transition: concepts and molecular links. *Semin Cancer Biol* **22**: 396–403. doi:10.1016/j.semcancer.2012.04.001
- Schepers AG, Snippert HJ, Stange DE, Van Den Born M, Van Es JH, Van De Wetering M, Clevers H. 2012. Lineage tracing reveals Lgr5⁺ stem cell activity in mouse intestinal adenomas. *Science* **337**: 730–735. doi:10.1126/science.1224676
- Seroczynska B, Stokowy T, Welnicka-Jaskiewicz M, Markiewicz A, Topa J, Nagel A, Skokowski J, Zaczek AJ. 2019. Spectrum of epithelial-mesenchymal transition phenotypes in circulating tumour cells from early breast cancer patients. *Cancers (Basel)* **11**: 59. doi:10.3390/cancers11010059
- Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloushtain-Qimron N, Yao J, Nikolskaya T, Serebryiskaya T, Beroukhi R, Hu M, et al. 2007. Molecular definition of breast tumor heterogeneity. *Cancer Cell* **11**: 259–273. doi:10.1016/j.ccr.2007.01.013
- Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. 2004. Identification of human brain tumour initiating cells. *Nature* **432**: 396–401. doi:10.1038/nature03128
- Southam CM, Brunschwig A. 1961. Quantitative studies of autotransplantation of human cancer. *Cancer* **14**: 971–978. doi:10.1002/1097-0142(196109/10)14:5<971::AID-CNCR2820140510>3.0.CO;2-O
- Strauss R, Li ZY, Liu Y, Beyer I, Persson J, Sova P, Möller T, Pesonen S, Hemminki A, Hamerlik P, et al. 2011. Analysis of epithelial and mesenchymal markers in ovarian cancer reveals phenotypic heterogeneity and plasticity. *PLoS ONE* **6**: e16186. doi:10.1371/annotation/8c637352-3614-406c-89dc-e78d10fa069c
- Stylianou N, Lehman ML, Wang C, Fard AT, Rockstroh A, Fazli L, Jovanovic L, Ward M, Sadowski MC, Kashyap AS, et al. 2018. A molecular portrait of epithelial-mesenchymal plasticity in prostate cancer associated with clinical outcome. *Oncogene* **38**: 913–934. doi:10.1038/s41388-018-0488-5
- Tan TZ, Miow QH, Miki Y, Noda T, Mori S, Huang RYJ, Thiery JP. 2014. Epithelial-mesenchymal transition spectrum quantification and its efficacy in deciphering survival and drug responses of cancer patients. *EMBO Mol Med* **6**: 1279–1293. doi:10.15252/emmm.201404208
- Terry S, Savagner P, Ortiz-Cuaran S, Mahjoubi L, Saintigny P, Thiery JP, Chouaib S. 2017. New insights into the role of EMT in tumor immune escape. *Mol Oncol* **11**: 824–846. doi:10.1002/1878-0261.12093
- Thiery JP. 2002. Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer* **2**: 442–454. doi:10.1038/nrc822
- Thiery JP, Acloque H, Huang RYJ, Nieto MA. 2009. Epithelial-mesenchymal transitions in development and disease. *Cell* **139**: 871–890. doi:10.1016/j.cell.2009.11.007
- Tirosh I, Izar B, Prakadan SM, Wadsworth MH, Treacy D, Trombetta JJ, Rotem A, Rodman C, Lian C, Murphy G, et al. 2016. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-Seq. *Science* **352**: 189–196. doi:10.1126/science.aad0501
- Tripathi SC, Peters HL, Taguchi A, Katayama H, Wang H, Momin A, Jolly MK, Celiktas M, Rodriguez-Canales J, Liu H, et al. 2016. Immunoproteasome deficiency is a feature of non-small cell lung cancer with a mesenchymal phenotype and is associated with a poor outcome. *Proc Natl Acad Sci* **113**: E1555–E1564. doi:10.1073/pnas.1521812113
- Tripathi S, Levine H, Jolly MK. 2019. A mechanism for epithelial-mesenchymal heterogeneity in a population of cancer cells. bioRxiv doi: 10.1101/592691.
- Tsai JH, Donaher JL, Murphy DA, Chau S, Yang J. 2012. Spatiotemporal regulation of epithelial-mesenchymal transition is essential for squamous cell carcinoma metastasis. *Cancer Cell* **22**: 725–736. doi:10.1016/j.ccr.2012.09.022
- Tsuji T, Ibaragi S, Shima K, Hu MG, Katsurano M, Sasaki A, Hu GF. 2008. Epithelial-mesenchymal transition induced

- by growth suppressor p12^{CDK2-AP1} promotes tumor cell local invasion but suppresses distant colony growth. *Cancer Res* **68**: 10377–10386. doi:10.1158/0008-5472.CAN-08-1444
- van Dijk D, Sharma R, Nainys J, Yim K, Kathail P, Carr AJ, Burdziak C, Moon KR, Chaffer CL, Pattabiraman D, et al. 2018. Recovering gene interactions from single-cell data using data diffusion. *Cell* **174**: 716–729.e27. doi:10.1016/j.cell.2018.05.061
- Varga J, Greten FR. 2017. Cell plasticity in epithelial homeostasis and tumorigenesis. *Nat Cell Biol* **19**: 1133–1141. doi:10.1038/ncb3611
- Visvader JE, Lindeman GJ. 2012. Cancer stem cells: current status and evolving complexities. *Cell Stem Cell* **10**: 717–728. doi:10.1016/j.stem.2012.05.007
- Weigelt B, Glas AM, Wessels LFA, Witteveen AT, Peterse JL, van't Veer LJ. 2003. Gene expression profiles of primary breast tumors maintained in distant metastases. *Proc Natl Acad Sci* **100**: 15901–15905. doi:10.1073/pnas.2634067100
- Weigelt B, Peterse JL, van't Veer LJ. 2005. Breast cancer metastasis: markers and models. *Nat Rev Cancer* **5**: 591–602. doi:10.1038/nrc1670
- Westcott JM, Precht AM, Maine EA, Dang TT, Esparza MA, Sun H, Zhou Y, Xie Y, Pearson GW. 2015. An epigenetically distinct breast cancer cell subpopulation promotes collective invasion. *J Clin Invest* **125**: 1927–1943. doi:10.1172/JCI77767
- Ye X, Tam WL, Shibue T, Kaygusuz Y, Reinhardt F, Ng Eaton E, Weinberg RA. 2015. Distinct EMT programs control normal mammary stem cells and tumour-initiating cells. *Nature* **525**: 256–260. doi:10.1038/nature14897
- Yu M, Bardia A, Wittner BS, Stott SL, Smas ME, Ting DT, Isakoff SJ, Ciciliano JC, Wells MN, Shah AM, et al. 2013. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. *Science* **339**: 580–584. doi:10.1126/science.1228522
- Zhang S, Han Z, Jing Y, Tao S, Li T, Wang H, Wang Y, Li R, Yang Y, Zhao X, et al. 2012. CD133⁺ CXCR4⁺ colon cancer cells exhibit metastatic potential and predict poor prognosis of patients. *BMC Med* **10**: 85. doi:10.1186/1741-7015-10-85
- Zheng X, Carstens JL, Kim J, Scheible M, Kaye J, Sugimoto H, Wu CC, Lebleu VS, Kalluri R. 2015. Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer. *Nature* **527**: 525–530. doi:10.1038/nature16064
- Zheng H, Pomyen Y, Hernandez MO, Li C, Livak F, Tang W, Dang H, Greten TF, Davis JL, Zhao Y, et al. 2018. Single-cell analysis reveals cancer stem cell heterogeneity in hepatocellular carcinoma. *Hepatology* **68**: 127–140. doi:10.1002/hep.29778
- Zomer A, Ellenbroek SIJ, Ritsma L, Beerling E, Vriskoop N, Van Rheenen J. 2013. Brief report: intravital imaging of cancer stem cell plasticity in mammary tumors. *Stem Cells* **31**: 602–606. doi:10.1002/stem.1296

Cancer Stem Cells and Epithelial-to-Mesenchymal Transition in Cancer Metastasis

Toni Celià-Terrassa and Mohit Kumar Jolly

Cold Spring Harb Perspect Med 2020; doi: 10.1101/cshperspect.a036905 originally published online September 30, 2019

Subject Collection [Metastasis: Mechanism to Therapy](#)

Cellular Plasticity during Metastasis: New Insights Provided by Intravital Microscopy

Andreia S. Margarido, Laura Bornes, Claire Vennin, et al.

The Etiology and Impact of Muscle Wasting in Metastatic Cancer

Anup K. Biswas and Swarnali Acharyya

Normal Aging and Its Role in Cancer Metastasis

Mitchell Fane and Ashani T. Weeraratna

Zebrafish In Vivo Models of Cancer and Metastasis

Katy R. Astell and Dirk Sieger

Extracellular Vesicles and Metastasis

Shizhen Emily Wang

The Different Facets of Liquid Biopsy: A Kaleidoscopic View

Zahra Eslami-S, Luis Enrique Cortés-Hernández, Laure Cayrefourcq, et al.

Myeloid Cells in Metastasis

Agnieszka Swierczak and Jeffrey W. Pollard

Cancer Cell Dormancy in Metastasis

Matthew A. Summers, Michelle M. McDonald and Peter I. Croucher

Premetastasis

Yoshiro Maru

Bone Tropism in Cancer Metastases

Hai Wang, Weijie Zhang, Igor Bado, et al.

Impact of Immunometabolism on Cancer Metastasis: A Focus on T Cells and Macrophages

Nina C. Flerin, Sotiria Pinioti, Alessio Menga, et al.

New Approaches on Cancer Immunotherapy

Jong-Ho Cha, Li-Chuan Chan, Min Sup Song, et al.

Cancer Stem Cells and Epithelial-to-Mesenchymal Transition in Cancer Metastasis

Toni Celià-Terrassa and Mohit Kumar Jolly

Clinical Perspectives in Brain Metastasis

Krutika Deshpande, Ian Buchanan, Vahan Martirosian, et al.

Brain Metastasis Organotropism

Arseniy E. Yuzhalin and Dihua Yu

The Immune Microenvironment and Cancer Metastasis

Asmaa El-Kenawi, Kay Hänggi and Brian Ruffell

For additional articles in this collection, see <http://perspectivesinmedicine.cshlp.org/cgi/collection/>