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## *Review*

# Metastatic dormancy: a complex network between cancer stem cells and their microenvironment

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**Summary.** Metastasis represents the major threat of cancer progression and generally emerges years after the detection of the primary tumor. An important rate-limiting step resides in cellular dormancy, where a disseminated tumor cell remains in a quiescent state at a remote organ. Herein we review the molecular mechanisms leading to tumor dormancy, mainly in regards to cellular quiescence and the tumor microenvironment. Based on the current published literature, we provide evidence that links the cancer stem cell (CSC) theory with dormancy and metastasis. Once a disseminated tumor cell reaches a target tissue, a tight regulation imposed by the foreign microenvironment will dictate the fate of these cells, which implies a balance in the secretion of soluble factors, modulation of the extracellular matrix and the angiogenic switch. We investigate thoroughly whether the CSC theory could also apply to metastasis initiation. In fact, the resistance of CSCs to therapy, leading to the minimal residual disease and cellular quiescence phenotypes, predisposes for the development of metastases. Finally, we describe the new technologies available for the identification of circulating tumor cells (CTCs), as well as their clinical relevance in dormancy of metastatic cancer patients.

**Key words:** Metastatic niche, Extracellular matrix, Therapy resistance

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### Metastasis, a multisep process

Metastasis is responsible for more than 90% of tumor-associated mortality in patients and has become the most feared complication of widespread cancer (Chaffer and Weinberg, 2011). Indeed, because of the systemic spread of the disease and its extremely high resistance to current therapies, patients diagnosed with metastasis are generally incurable. Looking at the pathogenesis, metastasis involves a cascade of events in which a subset of cancer cells disseminates from a primary tumor to eventually colonize distant secondary organs or tissues. These complex events have been diligently detailed in numerous reviews (Nguyen et al., 2009; Valastyan and Weinberg, 2011). Briefly, a primary tumor cell seems to undergo first an epithelial to mesenchymal transition (EMT), leading to increased motility and invasion ability (Scheel and Weinberg, 2012). This change in cell behavior is usually accompanied by the degradation of the basement membrane and extracellular matrix. Proteins of the Matrix Metalloprotease (MMP) family figure as main actors in this active proteolysis, which then permits the cells to enter the stroma (Shuman Moss et al., 2012). Interestingly, stroma cells such as fibroblasts and mesenchymal cells have the potential to foster the aggressiveness of the invasive cells. The modifications in the tumor microenvironment subsequently allow the intravasation of tumor cells into the circulation or the lymphatic system. Specific interactions with the pericyte and endothelium compartments are needed for cells to cross the vessel walls. Binding of tumor-related adhesion molecules to their corresponding receptors on

endothelial cells facilitates transmigration of tumor cells (Desgrosellier and Cheresh, 2010). Once in circulation, cancer cells must then survive and escape the immune system attack. Another major stress event for a circulating tumor cell (CTC) to face is anoikis, a cell death program induced by the loss of integrin-mediated cell-matrix contact (Yu et al., 2011). When these threats are overcome, the CTC is then free to extravasate and invade the parenchyma of a distant tissue. Signals from the new metastatic niche will allow the growth of the solitary tumor cell, a process also named colonization (Psaila and Lyden, 2009). The final steps comprise micrometastasis development, activation of the angiogenic switch and formation of macrometastasis (Valastyan and Weinberg, 2011). It is to be noted that each step differs from one cancer type to another and is endowed with defined gene alterations. Thus, the genetic profile of the tumor of origin and the target organ will largely influence the course of metastasis (Steeg, 2006).

Extravasation and colonization of a remote organ from a primary tumor type appear to be specific phenomena (Gupta and Massague, 2006). Among the various target sites, the lung, bone and liver are particularly receptive to metastasis development. Defined gene signatures within the primary cancer have been identified that clinically correlate with metastasis development and poor-prognosis in patients. The results of these studies have been extensively detailed in previous reviews. In the case of bone metastasis, the colonization of tumor cells into the bone marrow (BM) is common, reaching up to 60-75% in patients with breast and prostate cancer at late stages (Roodman, 2012). Interestingly, the BM can also serve as a transit for disseminated tumor cells (DTC), i.e. before arrival at their final destination (Nguyen et al., 2009; Hussein and Komarova, 2011). As we will describe further in this review, the BM provides a microenvironment particularly suitable for cellular dormancy. Overall, metastasis is undoubtedly a highly complex process in which both the primary tumor and the receptive organ will dictate the fate of a migratory tumor cell.

## The controversial existence of Cancer Stem Cells and their relationship with metastasis

As previously described, metastases from a primary tumor are thought to originate from a few tumor cells endowed with the ability to transform, adapt to different microenvironments and to colonize a new organ. Recent studies suggest that such a tumor subpopulation with metastatic initiating ability is linked to the cancer stem cell (CSC) population. Accumulating evidence suggests that a tumor enriched for stem cell-like characteristics is responsible for cancer initiation and sustainment as well as resistance to treatment. These tumor-initiating cells (TIC) have been defined as "cells within a tumor that possess the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor" (Clarke et al., 2006). The CSC hypothesis could then provide a better understanding of each phase of cancer development and of diverse concepts such as minimal residual disease and drug resistance. Nonetheless, it remains a topic of controversy because it challenges the stochastic (or clonal evolution) model, which postulates that all the cancer cells possess tumorigenic potential with the ability to repopulate and regenerate the tumor itself (Nowell, 1976). Interestingly, the CSC hypothesis suggests that these cells may arise from normal stem cells, since they share common biological characteristics with the presence of similar cell surface markers. The longevity of stem cells makes them susceptible to the accumulation of genetic and epigenetic alterations involved in cancer progression. This acquisition is sufficient over time to induce carcinogenesis and sustain tumor growth (Jamieson et al., 2004; Passegue et al., 2004). Conversely, accumulation of mutations in differentiated cells can also give rise to stem-like properties (Krivtsov et al., 2006, Jordan, 2009; Scheel et al., 2011). Overall, the most important feature related to CSCs is their ability to grow after transplantation into mice, where they can recapitulate the original tumor heterogeneity and give rise to all the different cell types that can be found in the original tumor.

Extensive efforts have been undertaken to identify biomarkers able to recognize CSCs among highly abundant differentiated cancer cells. The first pioneering studies identified CSCs in acute myeloid leukaemia (AML) as the CD34+CD38- subpopulation, which is able to differentiate and initiate tumour growth into immunodeficient NOD/SCID mice (Lapidot et al., 1994; Bonnet and Dick, 1997). Ever since this initial discovery, CSCs have been isolated from a wide variety of human cancers including brain (CD133+ cells) (Singh et al., 2003), breast (CD44+CD24- cells) (Al-Hajj et al., 2003), prostate (CD44+CD24- cells) (Hurt et al., 2008), pancreas (CD44+CD24+ESA+ cells) (Li et al., 2007) and colon (CD133+, EpCAM+CD44+ cells) (Dalerba et al., 2007; Ricci-Vitiani et al., 2007). CSCs can also be isolated on the basis of their functional properties. The side population (SP) phenotype identifies a cell subset with the ability to exclude fluorescent dyes and drugs through ABCG2 transporter activity (Bleau et al., 2009a). Sorted SP cells have been shown to contain the TIC population with sphere-forming ability (Bleau et al., 2009b). Similarly, Aldehyde dehydrogenase (ALDH) activity has been commonly used to isolate TIC from various tumor types (Ma and Allan, 2011). Moreover, these tumors generally present increased expression and activity of ALDH enzymes, which are associated with cancer relapse (Sullivan et al., 2010; Visus et al., 2011).

Usually, CSCs purified with the methods described above have the ability to generate tumors in xenografts with a much higher frequency than the non-CSC population. Some recent studies, however, demonstrated that these markers are not universal as they may not be stable during the course of tumor progression, and may vary among patients with the same disease (Taussig et

al., 2008; Tirino et al., 2013). As a consequence of CSC plasticity, the specificity of these techniques for isolating CSCs is continuously under scrutiny, generating criticisms and discussions. Some of the reported CSC biomarkers might be relevant in some stages of tumor progression but obsolete in others. The general understanding relies on the idea that each method cannot cover the whole CSC population and that the combination of CSC markers and functional assays might help us to better identify CSCs (Brescia et al., 2012).

Advanced and aggressive tumors are frequently enriched in CSCs, and cells isolated from distant metastases often display a CSC phenotype. Therefore, it has been proposed that CSCs may have a role in mediating cancer metastasis and thus be considered as the metastatis-initiating cells (MICs). Evidence indicating that MICs might be found within CSCs subpopulations relies on the idea that CSCs possess tumor-initiating capacity, mandatory to establish a secondary tumor to distant organs. Supporting this hypothesis, CSCs have been shown to express EMT markers (Mani et al., 2008), which are associated with migrating ability. Several reports suggest that MICs share some markers of CSCs. In breast cancer, CD44+ cells have been shown to be particularly pro-metastatic (Liu et al., 2010), and downregulation of this protein markedly suppressed tumorigenicity and bone metastases in nude mice (Hiraga et al., 2013). In line with this, CD44+/CXCR4+ cells in pancreatic cancer are considered as the putative cells related to metastasis formation (Hermann et al., 2007). CD133, in combination with other markers (CD26 (Diehn and Majeti, 2010; Pang et al., 2010), CD44 (Bellizzi et al., 2013), and CXCR4 (Zhang et al., 2012)), has been used to isolate metastatic colon cancer cell. Similar findings were made for ALDH activity in several tumor types, where ALDH-positive cells displayed high metastasis-initiating capacity (Charafe-Jauffret et al., 2010; Marcato et al., 2011; Mu et al., 2013).

### CSCs and resistance to treatment

Due to their quiescence, efficient DNA repair, expression of multidrug-resistant transporters and impaired apoptosis, CSCs are thought to play an important role in drug resistance, tumor relapse and metastasis (Singh and Settleman, 2010). Conventional chemotherapy treatments are generally directed against the highly proliferative cells of the tumor bulk, and it is likely that they do not target CSCs, which would remain alive and retain their capacity to regenerate the tumor and give rise to metastases (Vinogradov and Wei, 2012). The CSC theory could therefore offer an explanation for cancer relapse in patients, despite an initial response to treatments. An increasing number of studies show that CSCs are involved in resistance to chemo/radiotherapy in various tumor types, such as lung (Gottschling et al., 2012), breast (Ginestier et al., 2007), colon (Meng et al., 2009), ovarian (Zhang et al., 2008), glioma (Ulasov et al., 2011), etc.

Using an orthotopic hepatocellular carcinoma model we found that, despite the effectiveness of metronomic cyclophosphamide-based chemotherapy, few isolated

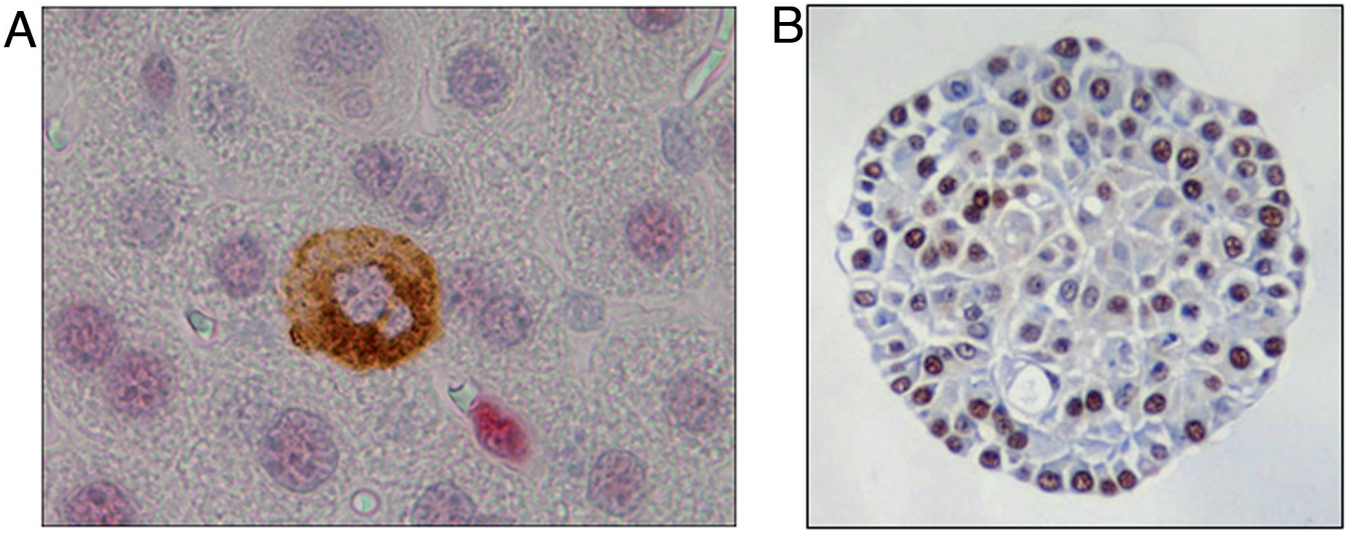


**Fig. 1.** Quiescent cancer stem cells are resistant to chemotherapy. **A.** Double immunohistochemistry for hAFP (brown) and PCNA (fuchsine-red) in a mouse liver affected with hepatocellular carcinoma. After treatment (low dose metronomic cyclophosphamide), the resistant cancer stem cells present in the tissue are positive for hAFP but remain in a quiescent state (PCNA negative). x 1,000. **B.** Immunohistochemistry for PCNA performed on sectioned tumor spheres shows that sphere cultures isolated from a mouse lung adenocarcinoma model present a population of resting (PCNA negative) and proliferating (PCNA positive) tumor cells.

tumor cells or small tumor clusters positive for α-fetoprotein (hAFP+) were still present in the liver of treated mice. We observed that these remaining cells were positive for the liver CSC marker CD13 (Haraguchi et al., 2010), but negative for PCNA, suggesting that they were in a resting state (Martin-Padura et al., 2012) (Fig. 1A). This situation, referred to as tumor dormancy, occurs as a result of cell-cycle arrest and can take place at the primary cancer location, in metastatic sites, or after therapy administration (Giancotti, 2013). This evidence, together with other data from the literature, points out that CSCs are involved in tumor recurrence after therapy.

Other reports using experimental models have also shown that chemotherapy is particularly inefficient in CSCs. For example, the group of Hermann et al. demonstrated that the administration of gemcitabine in a mouse model of orthotopic pancreatic cancer resulted in a marked enrichment in CD133+ CSCs, whereas the CD133- population was eliminated (Hermann et al., 2007). Along with this, glioma xenografts irradiated *in vivo* were enriched in CD133+ cells relative to untreated xenografts (Bao et al., 2006). In addition, targeting CD44+ cells induced a delay in leukaemia progression in mouse models (Jin et al., 2006). Our group and others have also demonstrated that the drug salinomycin (a P-glycoprotein MDR1/ABCB1 substrate and inhibitor of potassium channels) targets specifically CSCs, which are otherwise resistant to conventional chemotherapy (Larzabal et al., 2013). Interestingly, in NSCLC models, we found that salinomycin strongly reduces the ALDH+ CSC population and decreases metastatic lesions, but not primary tumors. Such an effect was accompanied by a drop in the expression of the cancer migratory-related molecules CXCR4/SFD-1 in metastatic nodules (Larzabal et al., 2013). These data also suggest that CSC-targeted drugs may have an impact on metastatic spread.

Studies conducted in patient samples also documented the resistance of CSCs to treatment. In a series of patients with metastatic breast cancer treated with neoadjuvant chemotherapy (paclitaxel plus epirubicin-based schedule), the proportion of ALDH+ CSCs increased significantly, as compared to untreated tumors (Tanei et al., 2009). Other reports have shown that expression of ALDH1A1 is associated with resistance to temozolomide in glioblastoma (Tanei et al., 2009; Schafer et al., 2012). Moreover, expression of ALDH1A1 has been correlated with poor prognosis (Sullivan et al., 2010; Charafe-Jauffret et al., 2010). In NSCLC, the number of CD133+ cells increased after cisplatin treatment, suggesting that the CD133+ CSC fraction may confer drug resistance (Bertolini et al., 2009).

Development of new therapeutic strategies based on the CSC model has therefore become an important research avenue. Current approaches under investigation include: (I) targeting CSC-specific pathways or properties (such as drug efflux); (II) targeting the CSC niche; (III) inducing CSC differentiation. A number of signaling pathways have been linked to CSC chemoresistance and provide interesting avenues for therapy (Sun et al., 2012), like those mediated by WNT/β-catenin (Noda et al., 2009), Notch (Meng et al., 2009), NF-κB (Alvero et al., 2009) and BCL-2 pathways (Konopleva et al., 2002). However, further investigation is needed to better understand the mechanism by which these molecules target CSCs (Gupta et al., 2009). Finally, some authors, including us, have suggested the convenience of co-targeting CSC and non-CSC populations to treat cancer. Although our experiments combining salinomycin and paclitaxel in NSCLC models did not result in an increased efficacy compared to single therapies (Larzabal et al., 2013), other drug combinations may be effective. Indeed, salinomycin synergizes with gemcitabine to impair tumor growth in a model of pancreatic cancer (Zhang et al., 2012). Therefore, targeting CSCs has become a hot topic in cancer research, but future studies are needed to determine whether implementation of this therapeutic modality in patients will increase survival.

## Cellular dormancy in metastasis

As previously described, new reports suggest that CSCs exhibit enhanced metastatic ability, although their relative contribution still remains uncertain. Their ability to survive and transit between a quiescent and self-renewal state in a specific microenvironment are thought to contribute to some particular traits of metastasis initiation. In spite of being the main cause of patient death, metastasis is an ineffective process, since less than 0.01% of CTCs will be able to complete the late events of metastasis (Zhe et al., 2011). A major rate-limiting step implies the dormancy phenotype, which takes place between extravasation and metastasis outgrowth. Clinically overt metastasis usually includes a period of latency in patients, ranging from years to decades, in which a DTC remains in a dormant state, waiting for the appropriate signals to re-initiate proliferation (Aguirre-Ghiso et al., 2013). For this reason, metastasis has always been thought to be a late event in carcinogenesis, although evidence now indicates that tumor dissemination actually occurs at early stages.

Mechanisms of cancer dormancy are poorly understood and there is still no consensus on what dormancy really implies. Nonetheless, it is generally recognized as cellular dormancy as well as angiogenic dormancy (Aguirre-Ghiso, 2007). Cellular dormancy would imply that a solitary tumor cell in a remote organ remains in a quiescent state, as defined by an arrest in the G0-G1 phase of the cell cycle (Aguirre-Ghiso, 2007). Cellular mechanisms of dormancy include a decrease in uPAR (metastasis-associated urokinase receptor) expression level and inactivation of the α5β1 integrin (Allgayer and Aguirre-Ghiso, 2008). This leads to a reduction in phosphorylated ERK (extracellular

signaling-regulated kinase) and activation of p38 protein. Consequently, hallmarks for cellular dormancy generally imply low pERK/p38 ratio, activated stress-related pathways, and increased nuclear levels of p16 and p27 (Sosa et al., 2013).

Autophagy, a process of self-digestion that involves the degradation of unnecessary cellular components to ensure survival during starvation, has also been proposed to contribute to cellular dormancy (Nechemia-Arbely et al., 2008). A particular way of response to stress may induce autophagy of solitary dormant cells. This process includes a detachment from the extracellular matrix (ECM) by either impaired β1-integrin signaling (Fung et al., 2008), or through increased PERK kinase (Protein kinase RNA- like endoplasmic reticulum kinase) activity, which then inhibit mTORC1-p70(S6K) to provide protection from anoikis (Avivar-Valderas et al., 2013). Autophagy may also favor resistance of dormant tumor cells to apoptotic stimuli, such as those mediated by TRAIL (Han et al., 2008). Although autophagy and apoptosis are two distinct processes, there is an obvious crosstalk in their signaling pathways, where both could eventually lead to cell death (Mukhopadhyay et al., 2014). In this regard, autophagy induced by Rottlerin, a plant-derived chemopreventive drug, was shown to trigger apoptosis of breast cancer stem cells by suppressing Akt and mTOR phosphorylation (Kumar et al., 2013). Further studies on the mechanisms regulating autophagy could offer novel alternatives to specifically eradicate dormant CSCs.

Angiogenic dormancy, which limits *de novo* vascular formation, prevents a micrometastasis from evolving into a macrometastasis. Such a phenomenon also refers to tumor mass dormancy and results from low blood supply and nutrient deprivation (Bergers and Benjamin, 2003; Naumov et al., 2006). In this context, a proliferative tumor mass can be maintained at a limited size due to high apoptotic rate. The balance between pro- and anti-angiogenic factors will then determine whether the angiogenic switch is turned on, or whether dormancy is maintained. VEGF figures as a master player in this shift, while thrombospondin and p53 repress neovascularization (Kang and Watnick, 2008). Interestingly, CSCs have been shown to stimulate blood vessel formation through the secretion of VEGF (Bao et al., 2006). Consequently, anti-VEGF therapy was reported to strongly reduce the CSC population and tumor growth (Calabrese et al., 2007). Hypoxia and the sense of low oxygen levels can also promote blood vessel formation through induction of hypoxia-inducible factor 1 alpha (HIF-1α) (Krock et al., 2011). Moreover, such a hypoxic environment has been associated with increased motility and invasiveness of tumor cells (Lu et al., 2012).

Whether CSC characteristics relate to metastatic dormancy is one of the foremost new topics in the field. To date there are still no general markers available to isolate and characterize dormant CSCs. Besides cell cycle distribution, dye retention assays with fluorescent dyes, such as PKH26, have been used to track stem cells. This dye, which becomes diluted after each cell division, accumulates in quiescent cells (Martin-Padura et al., 2012). Such label-retaining cells have been identified in a sub-population of CD44+/CD24-/ESA+ breast cancer cells, and these particular cells were endowed with resistance to chemotherapy (Fillmore and Kuperwasser, 2008). Recently, the presence of label-retaining cells has been shown not only to result from slow cycling cells, but also from CSCs undergoing asymmetric cell division; most importantly, such cells presented tumor-initiating ability (Xin et al., 2012).

Tumor cells grown as spheroids have been reported to display a quiescent phenotype that can be reverted upon attachement to susbtrates (Correa et al., 2012). We found that spheres isolated from a mouse lung adenocarcinoma (Bleau et al., 2014) present a mixed population of proliferating (PCNA-positive) and non-proliferating (PCNA-negative) cells (Fig. 1B). Such an observation suggests the existence of quiescent cells within tumor spheres. Recently, low levels of reactive oxygen species have been used to isolate quiescent leukemia CSCs. These cells showed a pronounced cell cycle arrest (into the G0/G1 phase) and could be eradicated by inhibition of BCL-2 (Lagadinou et al., 2013).

Recently, a robust investigation demonstrated that the quiescent phenotype of CSCs might function as a tumor suppressor in squamous cell carcinomas (White et al., 2014). In this tumor model, hair follicle stem cells were incapable of initiating tumorigenesis while remaining in a quiescent state. Curiously, quiescence acted as a dominant event over the activation of the Ras oncogene or the loss of the tumor suppressor p53. The mechanism of dormancy in this context involved PTEN activity, which maintained the quiescent CSCs.

## The metastatic niche and its relationship with cell dormancy

Similarly to normal stem cells, CSCs have been found to reside in specific niches, generally in close proximity to blood vessels (Borovski et al., 2011). The niche is composed of the vascular network, ECM components and the stroma, which include mesenchymal and immune cells. Factors secreted within this microenvironment will regulate stemness and self-renewal ability. In metastasis, the "seed and soil" hypothesis postulates that a hospitable micro-environment at a primary site is essential to allow for the colonization and growth of a tumor cell at a distant site (Paget, 1989). The parenchymal tissue of a target organ was proposed to adapt before the settling down of the first tumor cell, possibly through the secretion of factors like VEGF by the primary tumor. Upon reaching the target organ, these soluble factors then attract bone marrow-derived hematopoietic progenitor cells (HPC) to produce chemokines (Psaila and Lyden, 2009). This will lead to an increase in fibronectin expression by resident

fibroblasts, providing a suitable microenvironment for the homing of tumor cells. Another important pathway in which the CSC population participates is the SDF-1-CXCR4 (stromal cell-derived factor-1/C-X-C chemokine receptor type 4) axis (Teicher and Fricker, 2010). The SDF-1 cytokine permits the recruitment of endothelial progenitor cells as well as tumor cells at the remote organ for metastasis outgrowth. As described for the CSC niche, the premetastatic niche presents a complex architecture composed of stroma cells, ECM proteins, non-malignant cells, and the signaling molecules they produce (Borovski et al., 2011). Since DTCs were proposed to remain in a dormant state at the metastatic niche, the microenvironment is critical for the switch from a dormant metastatis state to a proliferative one. The deregulation of the reciprocal interactions between the microenvironment and the tumor cells might decide whether they will survive, become dormant or progressively grow to form fulminant metastases (Bragado et al., 2012).

The EMC is a dynamic structure that can be remodelled and degraded by different enzymes, including MMPs. It is found in immediate contact with the tumor cells, and the resulting interactions are critical to metastatic development. Changes in ECM components, such as the production and organization of collagen type I and fibronectin, contribute to the formation of a permissive niche for the transition from dormancy to metastatic growth (Naumov et al., 2003; Tran et al., 2011). Stromal MMPs may also contribute to the release of cytokines like bFGF or VEGF and angiogenic factors that are sequestered in the ECM and initiate the angiogenic switch (Tlsty and Coussens, 2006). Dormant cells have been shown to present atypical cytoskeletal organization with temporary adhesion to the ECM. Such contact leads to cytoskeletal reorganization and generation of actin stress fibers through β1 integrin signaling that allow the transition from quiescence to activation of proliferation (Barkan et al., 2008). Similarly, a recent study demonstrated that the formation of filopodia-like projections that harbor integrin β1 allows the micrometastasis to evolve into a macrometastasis. In this process, focal adhesion kinase (FAK) activation into the newly formed adhesion plaques induces ERK and tumor cell proliferation (Shibue et al., 2012). Finally, carcinoma-associated fibroblasts have newly been proposed to awake cancer cells from metastatic dormancy (Mukhopadhyay et al., 2014). These data indicate that to escape tumor dormancy, a tumor cell needs to interact with ECM components.

Growing evidence supports a role for TGFβ1 signaling in the regulation of cellular dormancy and stem cell homeostasis, which most likely results from a balance in the concentration of TGFβ family members and pro-mitogenic cytokines. Recently, TGFβ2 has been shown to foster cellular dormancy in the BM via the activation of TGF-β-RII and p38α/β signaling (Bragado et al., 2013). Still in the bone, the BM microenvironment was found to promote leukemia cell dormancy by secretion of the extracellular matrix protein Osteopontin (OPN) (Boyerinas et al., 2013). In this context, acute lymphoblastic leukemia cells can adhere to OPN within the osteoblastic niche to further secrete this protein, which is then detected near dormant tumor cells. Of relevance, blocking of OPN signaling induced cell proliferation and metastasis development. Prostate cancer cells have been shown to also remain dormant in the BM by binding to osteoblasts in the niche (Kim et al., 2013). Such an interaction led to induction of TBK1 expression in these cells, which then inhibited mTOR function to trigger cell cycle arrest. In a different study, secretion of the soluble vascular cell adhesion protein 1 (VCAM-1) was reported to induce the exit of breast cancer bone micrometastasis from tumor dormancy (Lu et al., 2011). In the lung, which provides a more permissive microenvironment for metastasis outgrowth, DTCs are maintained in a dormant state through BMP secretion by cellular components of the normal lung. Only the DTCs that express the BMP inhibitor Coco (and are able to block BMP activity) can re-enter the cell cycle and generate metastasis (Gao et al., 2012). Interestingly, Coco has also been linked to the CSC phenotype due to its ability to promote sphere formation and sustain the expression of stem cell transcription factors. These data imply that the biological composition of the ECM and changes in the tumor microenvironment could regulate the entry or exit in the dormant state.

Additional microenvironmental factors might influence the dormant state of CTCs. During the "wound healing" process, several cytokines can induce the migration and growth of tumor cells. Interestingly, wound-healing gene expression signatures, which consist of genes related to extensive remodelling of the ECM, predicted metastasic relapse in patients with breast cancer (Chang et al., 2005). Furthermore, several metastasis suppressor genes that respond to microenvironmental stress may regulate the dormant state. These genes include MKK4, KISS1, MKK6, BHLHLB3/Sharp-1 and Nm23-H, among others (Paez et al., 2012). They can influence dormancy through different mechanisms, but all of them exert an overall effect on regulation of the p38/ERK1/2 signalling ratio, a hallmark of cellular dormancy. Interestingly, the coagulation system, which mediates tissue responses to injury and that is often found disrupted in cancer, has been identified as a new regulator of tumor dormancy (Boyerinas et al., 2013). In this study, deficiency in tissue factor (TF), the cancer cell-associated initiator of the coagulation system, kept glioma cells in a permanent dormant state, while overexpression of TF induced a transition into a proliferative state.

The CSC niche is likely to be one of the most crucial targets in cancer treatment. Therefore, targeting this niche (the microenvironment supporting CSCs maintenance) may represent an important step towards tumor eradication. For example, inhibition of angiogenesis might be an approach to destabilize CSC

survival. Indeed, it has been demonstrated that the CSC niche is highly vascularised and that inhibition of vessel growth with anti-VEGF antibodies, like bevacizumab, may destroy the niche and reduce the number of CSCs (Yang and Wechsler-Reya, 2007). An elegant study from Bao and collegues has shown that glioblastoma CSCs can generate pericytes through the SDF-1/CXCR4 axis in order to sustain vessel function and tumor growth (Cheng et al., 2013). Interestingly, elimination of these pericytes strongly reduced tumor growth by disrupting the neovasculature.

To further investigate the relationship between the metastatic niche and cellular dormancy, the lab of G. Dontu developed a novel 3D coculture system to model bone metastasis dormancy from breast cancer cells in mice (Marlow et al., 2013). This new model uses cocultivation of bone marrow cells with breast cancer cell lines in a 3D-collagen biomatrix. Modulation of key signaling pathways involved in dormancy (such as p38 and TGF-β) in the matrix microenvironment allows to dictate between a supportive or inhibitory dormant niche. By using a similar 3D *in vitro* system, Green's group demonstrated that pharmacological inhibition of Src family kinase signaling prevents dormant breast cancer cells from re-entering the cell cycle through translocation of the p27 protein (El Touny et al., 2014). As ERK1/2 activation was required for these cells to proliferate, co-treatment with a MEK1/2 inhibitor produced apoptosis with a prominent delay in metastasis outgrowth. Overall, new models for *in vivo* dormancy will allow for the development of new strategies to target dormant solitary tumor cells by preventing their "re-awakening" during tumorigenesis.

## CTCs, CSCs and dormancy in metastatic cancer patients

Clinical cancer dormancy is characterized by the latency of recurrence after the initial diagnosis. Some tumor types may have a long lasting latency period, including breast, melanoma and renal carcinoma, which may remain without clinical manifestations for many years (Goss and Chambers, 2010). In a study of 36 breast cancer patients with no evidence of clinical disease (clinically dormant), at least 36% showed CTCs 8 to 22 years after mastectomy, presumably coming from micrometastasis (Meng et al., 2004). As previously mentioned in this review, tumor relapse would require the existence of quiescent dormant isolated or clustered cancer cells that, due to a yet unknown reason awake, proliferate and switch angiogenesis on. Although it remains to be elucidated whether these cells may correspond to CSCs in patients, experiments in animals suggest that this could be the case.

Clinical observations have shown that cancer patients even at very early stages of tumorigenesis may have disseminated cells in the lymph nodes, blood or the bone marrow (Pantel et al., 2009). The lymph node status is a critical factor to determine prognosis in many solid tumors (Galimberti and Cole, 2013), but several studies have also shown the clinical relevance of detecting CTCs in patients. Extensive work conducted by Cristofanilli's group (and other research groups) in metastatic breast cancer (mBrCa) patients showed that the presence of 5 or more CTCs per 7.5 mL in peripheral blood before therapy and at the first follow up post-treatment examination was an independent predictor of progression-free and overall survival (Cristofanilli et al., 2004). Moreover, the number of CTCs in mBrCa patients appears to have superior prognostic potential than radiological image techniques (Budd et al., 2006). Unfavourable prognosis has also been reported for patients with CTCs in other tumor types, such as prostate, colon and lung (Pantel et al., 2009). In prostate cancer (PrCa) patients, the number of CTCs after treatment was a better prognostic indicator of survival than a 50% reduction in PSA (Prostate-Specific Antigen) levels (Scher et al., 2009). Several clinical trials are currently determining the potential value of CTCs as biomarkers of response in AR-targeted therapies (Aviraterone or MDV3100) in castration resistant PrCa patients that had progressed to docetaxel treatment (Danila et al., 2011).

A variety of methods have been developed for the identification and isolation of CTCs; however, none of them has yet been implemented in clinical practice. Basically, these methods rely on techniques such as immunomagnetic separation, size-based filtration or centrifugation, microfluidic devices, flow cytometry and PCR-based techniques (Alix-Panabieres and Pantel, 2014). The CellSearch® technology (Johnson and Johnson), based on immunodetection of $EpCAM^+$ cells followed by immunostaining for cytokeratins, is the most clinically advanced detection system and has received FDA approval for mBrCa patients. Several ongoing clinical trials are trying to validate the prognostic usefulness and predictive values of this technology in different types of cancers (Pantel et al., 2009). One of its limitations is that the CellSearch method can underestimate the number of CTCs as it leaves behind the $EpCAM^-$ cells that may have undergone EMT. This situation could be particularly relevant for the highly aggressive triple negative ($ER^-$/$PR^-$/$HER2^-$) dedifferentiated breast tumors (Yu et al., 2013). Using a combination of methodologies, Giordano et al. (2012) have shown that patients with Her-$2^+$ mBrCa display a heterogeneous population of epithelial cells with $EpCAM^+$ CTCs detected by CellSearch and a population of CTCs with EMT/CSC features that lack EpCAM expression and cannot be detected by CellSearch. Similarly, in NSCLC, the existence of hybrid CTCs with EMT phenotype has been described (Lecharpentier et al., 2011).

Some studies have addressed the question whether CTCs harbor molecular alterations that may represent a step ahead in carcinogenesis towards metastasis. This appears to be the case for CTCs from breast cancer patients that express lower levels of ERα/PR and higher

levels of Her-2 than the primary breast cancer cells (Rack et al., 2012). In CTCs from prostate cancer patients, androgen receptor (AR) genomic amplification and copy number gain have been reported (Leversha et al., 2009). Moreover, patients with androgen-responsive prostate tumors present with a strong "AR-on" signalling in their CTCs that becomes "AR-mixed" in more advanced and AR-insensitive tumors (Miyamoto et al., 2012). Although it remains uncertain whether specific molecular alterations in CTCs may serve to predict and monitor response to targeted therapies, there are high expectations as an increasing number of publications is addressing this issue.

The possibility that CTCs correspond to MIC has recently been investigated by Baccelli et al. (2013). These authors have demonstrated that a particular population of CTCs from mBrCa patients is able to initiate metastasis when xenotransplanted in mice: CTCs with an $EpCAM^+/CD44^+/CD47^+/MET^+$ phenotype found in some patients were able to produce tumors at distant sites, thus showing that this population corresponds to the one that initiates metastasis. Histologically, these tumors resembled those obtained in patients from whom CTCs had been isolated. In addition, the number of $EpCAM^+/CD44^+/CD47^+/MET^+$ cells increased in parallel to clinical progression (unlike the bulk of $EpCAM^+$ CTCs), which suggests that this population may resist treatment (a typical property of CSCs) (Baccelli et al., 2013). It is unknown though whether $EpCAM^+/CD44^+/CD47^+/MET^+$ cells display CSC features, such as the ability to self-renew and differentiate.

A more precise molecular characterization of therapy resistant cells (presumably with CSC phenotype and in dormant state) is a key issue in our efforts at preventing recurrence after therapy. In patients, $ER^+$ breast cancer and $AR^+$ prostate cancer may serve as paradigm to investigate tumor dormancy and relapse after long-term therapy. Administration of antiestrogenic or antiandrogenic therapies for 5 years reduces the risk of recurrence, although clinically dormant metastases may be still present (Early Breast Cancer Trialists' Collaborative Group (EBCTCG), 2005). On the contrary, administration of antiestrogenic therapy for longer periods of time may favour the appearance of ER- breast cancer cells and provides no clear evidence of improved survival (Goss and Chambers, 2010). Thus, while the continuous pressure exerted by the drug on tumor cells (even at low doses) may keep them in a dormant state, it may also force a molecular switch towards the acquisition of a more aggressive and therapy resistant phenotype. In fact, in patients with AR-sensitive prostate tumors subjected to antiandrogen therapy, AR+ CTCs switch their phenotype to an "AR-off" state (Miyamoto et al., 2012). These data indicate that there is a critical need for extended clinical trials aimed at evaluating the efficacy of prolonged therapy with respect to tumor recurrence. Here, the discovery of an early biomarker of tumor awakening, measurable in blood, would also represent a major breakthrough to this end.

## Conclusions and future perspectives

Increasing experimental evidence suggests that CSCs are responsible for metastasis initiation and relapse after chemotherapy and/or radiotherapy. These cells, which display EMT features, appear to be able to leave the tumor and circulate in blood as CTCs, together with an epithelial EpCAM+ cancer cell population. It is also possible that, due to their plasticity, differentiated cancer cells become undifferentiated CSCs in circulation or in the metastatic niche. Upon treatment, therapy resistant metastatic cells with CSC features in a dormant state can give rise to metastasis outgrowth under specific circumstances that involve changes in the extracellular matrix. A more comprehensive understanding on how these different phenotypes and biological processes take place will facilitate the development of novel antitumor strategies. This may also help to prevent tumor relapse in patients that no longer show evidence of cancer (based on radiological tests), and who have been off therapy even for several years.

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## References

Aguirre-Ghiso J.A. (2007). Models, mechanisms and clinical evidence for cancer dormancy. Nat. Rev. Cancer 7, 834-846.

Aguirre-Ghiso J.A., Bragado P. and Sosa M.S. (2013). Metastasis awakening: Targeting dormant cancer. Nat. Med. 19, 276-277.

Al-Hajj M., Wicha M.S., Benito-Hernandez A., Morrison S.J. and Clarke M.F. (2003). Prospective identification of tumorigenic breast cancer cells. Proc. Natl. Acad. Sci. USA 100, 3983-3988.

Alix-Panabieres C. and Pantel K. (2014). Technologies for detection of circulating tumor cells: Facts and vision. Lab. Chip 14, 57-62.

Allgayer H. and Aguirre-Ghiso J.A. (2008). The urokinase receptor (u-par)--a link between tumor cell dormancy and minimal residual disease in bone marrow? APMIS 116, 602-614.

Alvero A.B., Chen R., Fu H.H., Montagna M., Schwartz P.E., Rutherford T., Silasi D.A., Steffensen K.D., Waldstrom M., Visintin I. and Mor G. (2009). Molecular phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemoresistance. Cell Cycle 8, 158-166.

Avivar-Valderas A., Bobrovnikova-Marjon E., Alan Diehl J., Bardeesy N., Debnath J. and Aguirre-Ghiso J.A. (2013). Regulation of autophagy during ecm detachment is linked to a selective inhibition of mtorc1 by perk. Oncogene 32, 4932-4940.

Baccelli I., Schneeweiss A., Riethdorf S., Stenzinger A., Schillert A., Vogel V., Klein C., Saini M., Bauerle T., Wallwiener M., Holland-Letz T., Hofner T., Sprick M., Scharpff M., Marme F., Sinn H.P., Pantel K., Weichert W. and Trumpp A. (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.

Bao S., Wu Q., McLendon R.E., Hao Y., Shi Q., Hjelmeland A.B., Dewhirst M.W., Bigner D.D. and Rich J.N. (2006). Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 444, 756-760.

Barkan D., Kleinman H., Simmons J.L., Asmussen H., Kamaraju A.K., Hoenorhoff M.J., Liu Z.Y., Costes S.V., Cho E.H., Lockett S., Khanna C., Chambers A.F. and Green J.E. (2008). Inhibition of metastatic outgrowth from single dormant tumor cells by targeting the cytoskeleton. Cancer Res. 68, 6241-6250.

Bellizzi A., Sebastian S., Ceglia P., Centonze M., Divella R., Manzillo E.F., Azzariti A., Silvestris N., Montemurro S., Caliandro C., De Luca R., Cicero G., Rizzo S., Russo A., Quaranta M., Simone G. and Paradiso A. (2013). Co-expression of cd133(+)/cd44(+) in human colon cancer and liver metastasis. J. Cell. Physiol. 228, 408-415.

Bergers G. and Benjamin L.E. (2003). Tumorigenesis and the angiogenic switch. Nat. Rev. Cancer 3, 401-410.

Bertolini G., Roz L., Perego P., Tortoreto M., Fontanella E., Gatti L., Pratesi G., Fabbri A., Andriani F., Tinelli S., Roz E., Caserini R., Lo Vullo S., Camerini T., Mariani L., Delia D., Calabro E., Pastorino U. and Sozzi G. (2009). Highly tumorigenic lung cancer cd133+ cells display stem-like features and are spared by cisplatin treatment. Proc. Natl. Acad. Sci. USA 106, 16281-16286.

Bleau A.M., Huse J.T. and Holland E.C. (2009a). The abcg2 resistance network of glioblastoma. Cell Cycle 8, 2936-2944.

Bleau A.M., Hambardzumyan D., Ozawa T., Fomchenko E.I., Huse J.T., Brennan C.W. and Holland E.C. (2009b). Pten/pi3k/akt pathway regulates the side population phenotype and abcg2 activity in glioma tumor stem-like cells. Cell Stem Cell 4, 226-235.

Bleau A., Freire F., Pajares M.J., Zudaire I., Anton I., Nistal-Villán E., Redrado M., Garmendia I., Ajona D., Blanco D., Pio R., Lecanda F., Calvo A. and Montuenga L. (2014). New syngeneic inflammatory-related lung cancer metastatic model harboring double kras/wwox alterations. Int. J. Cancer (Accepted Article).

Bonnet D. and Dick J.E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat. Med. 3, 730-737.

Borovski T., De Sousa E.M.F., Vermeulen L. and Medema J.P. (2011). Cancer stem cell niche: The place to be. Cancer Res. 71, 634-639.

Boyerinas B., Zafrir M., Yesilkanal A.E., Price T.T., Hyjek E.M. and Sipkins D.A. (2013). Adhesion to osteopontin in the bone marrow niche regulates lymphoblastic leukemia cell dormancy. Blood 121, 4821-4831.

Bragado P., Sosa M.S., Keely P., Condeelis J. and Aguirre-Ghiso J.A. (2012). Microenvironments dictating tumor cell dormancy. Recent Results Cancer Res. 195, 25-39.

Bragado P., Estrada Y., Parikh F., Krause S., Capobianco C., Farina H.G., Schewe D.M. and Aguirre-Ghiso J.A. (2013). Tgf-beta2 dictates disseminated tumour cell fate in target organs through tgf-beta-riii and p38alpha/beta signalling. Nat. Cell. Biol. 15, 1351-1361.

Brescia P., Richichi C. and Pelicci G. (2012). Current strategies for identification of glioma stem cells: Adequate or unsatisfactory? J. Oncol. 2012, 376894.

Budd G.T., Cristofanilli M., Ellis M.J., Stopeck A., Borden E., Miller M.C., Matera J., Repollet M., Doyle G.V., Terstappen L.W. and Hayes D.F. (2006). Circulating tumor cells versus imaging--predicting overall survival in metastatic breast cancer. Clin. Cancer Res. 12, 6403-6409.

Calabrese C., Poppleton H., Kocak M., Hogg T.L., Fuller C., Hamner B., Oh E.Y., Gaber M.W., Finklestein D., Allen M., Frank A., Bayazitov I.T., Zakharenko S.S., Gajjar A., Davidoff A. and Gilbertson R.J. (2007). A perivascular niche for brain tumor stem cells. Cancer Cell 11, 69-82.

Chaffer C.L. and Weinberg R.A. (2011). A perspective on cancer cell metastasis. Science 331, 1559-1564.

Chang H.Y., Nuyten D.S., Sneddon J.B., Hastie T., Tibshirani R., Sorlie T., Dai H., He Y.D., van't Veer L.J., Bartelink H., van de Rijn M., Brown P.O. and van de Vijver M.J. (2005). Robustness, scalability, and integration of a wound-response gene expression signature in predicting breast cancer survival. Proc. Natl. Acad. Sci. USA 102, 3738-3743.

Charafe-Jauffret E., Ginestier C., Iovino F., Tarpin C., Diebel M., Esterni B., Houvenaeghel G., Extra J.M., Bertucci F., Jacquemier J., Xerri L., Dontu G., Stassi G., Xiao Y., Barsky S.H., Birnbaum D., Viens P. and Wicha M.S. (2010). Aldehyde dehydrogenase 1-positive cancer stem cells mediate metastasis and poor clinical outcome in inflammatory breast cancer. Clin. Cancer Res. 16, 45-55.

Cheng L., Huang Z., Zhou W., Wu Q., Donnola S., Liu J.K., Fang X., Sloan A.E., Mao Y., Lathia J.D., Min W., McLendon R.E., Rich J.N. and Bao S. (2013). Glioblastoma stem cells generate vascular pericytes to support vessel function and tumor growth. Cell 153, 139-152.

Clarke M.F., Dick J.E., Dirks P.B., Eaves C.J., Jamieson C.H., Jones D.L., Visvader J., Weissman I.L. and Wahl G.M. (2006). Cancer stem cells--perspectives on current status and future directions: Aacr workshop on cancer stem cells. Cancer Res. 66, 9339-9344.

Correa R.J., Peart T., Valdes Y.R., DiMattia G.E. and Shepherd T.G. (2012). Modulation of akt activity is associated with reversible dormancy in ascites-derived epithelial ovarian cancer spheroids. Carcinogenesis 33, 49-58.

Cristofanilli M., Budd G.T., Ellis M.J., Stopeck A., Matera J., Miller M.C., Reuben J.M., Doyle G.V., Allard W.J., Terstappen L.W. and Hayes D.F. (2004). Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N. Engl. J. Med. 351, 781-791.

Dalerba P., Dylla S.J., Park I.K., Liu R., Wang X., Cho R.W., Hoey T., Gurney A., Huang E.H., Simeone D.M., Shelton A.A., Parmiani G., Castelli C. and Clarke M.F. (2007). Phenotypic characterization of human colorectal cancer stem cells. Proc. Natl. Acad. Sci. USA 104, 10158-10163.

Danila D.C., Fleisher M. and Scher H.I. (2011). Circulating tumor cells as biomarkers in prostate cancer. Clin. Cancer Res. 17, 3903-3912.

Desgrosellier J.S. and Cheresh D.A. (2010). Integrins in cancer: Biological implications and therapeutic opportunities. Nat. Rev. Cancer 10, 9-22.

Diehn M. and Majeti R. (2010). Metastatic cancer stem cells: An opportunity for improving cancer treatment? Cell Stem Cell 6, 502-503.

Early Breast Cancer Trialists Collaborative Group (EBCTCG). (2005). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: An overview of the randomised trials. Lancet 365, 1687-1717.

El Touny L.H., Vieira A., Mendoza A., Khanna C., Hoenerhoff M.J. and Green J.E. (2014). Combined sfk/mek inhibition prevents metastatic outgrowth of dormant tumor cells. J. Clin. invest. 124, 156-168.

Fillmore C.M. and Kuperwasser C. (2008). Human breast cancer cell lines contain stem-like cells that self-renew, give rise to

phenotypically diverse progeny and survive chemotherapy. Breast Cancer Res. 10, R25.

Fung C., Lock R., Gao S., Salas E. and Debnath J. (2008). Induction of autophagy during extracellular matrix detachment promotes cell survival. Mol. Biol. Cell 19, 797-806.

Galimberti V. and Cole B.F. (2013). Axillary versus sentinel-lymph-node dissection for micrometastatic breast cancer--authors' reply. Lancet Oncol.14, e251-252.

Gao H., Chakraborty G., Lee-Lim A.P., Mo Q., Decker M., Vonica A., Shen R., Brogi E., Brivanlou A.H. and Giancotti F.G. (2012). The bmp inhibitor coco reactivates breast cancer cells at lung metastatic sites. Cell 150, 764-779.

Giancotti F.G. (2013). Mechanisms governing metastatic dormancy and reactivation. Cell 155, 750-764.

Ginestier C., Hur M.H., Charafe-Jauffret E., Monville F., Dutcher J., Brown M., Jacquemier J., Viens P., Kleer C.G., Liu S., Schott A., Hayes D., Birnbaum D., Wicha M.S. and Dontu G. (2007). Aldh1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell 1, 555-567.

Giordano A., Gao H., Anfossi S., Cohen E., Mego M., Lee B.N., Tin S., De Laurentiis M., Parker C.A., Alvarez R.H., Valero V., Ueno N.T., De Placido S., Mani S.A., Esteva F.J., Cristofanilli M. and Reuben J.M. (2012). Epithelial-mesenchymal transition and stem cell markers in patients with her2-positive metastatic breast cancer. Mol. Cancer Therap. 11, 2526-2534.

Goss P.E. and Chambers A.F. (2010). Does tumour dormancy offer a therapeutic target? Nat. Rev. Cancer 10, 871-877.

Gottschling S., Schnabel P.A., Herth F.J. and Herpel E. (2012). Are we missing the target? Cancer stem cells and drug resistance in non-small cell lung cancer. Cancer Genom. Proteom. 9, 275-286.

Gupta G.P. and Massague J. (2006). Cancer metastasis: Building a framework. Cell 127, 679-695.

Gupta P.B., Onder T.T., Jiang G., Tao K., Kuperwasser C., Weinberg R.A. and Lander E.S. (2009). Identification of selective inhibitors of cancer stem cells by high-throughput screening. Cell 138, 645-659.

Han J., Hou W., Goldstein L.A., Lu C., Stolz D.B., Yin X.M. and Rabinowich H. (2008). Involvement of protective autophagy in trail resistance of apoptosis-defective tumor cells. J. Biol. Chem. 283, 19665-19677.

Haraguchi N., Ishii H., Mimori K., Tanaka F., Ohkuma M., Kim H.M., Akita H., Takiuchi D., Hatano H., Nagano H., Barnard G.F., Doki Y. and Mori M. (2010). Cd13 is a therapeutic target in human liver cancer stem cells. J. Clin. Invest. 120, 3326-3339.

Hermann P.C., Huber S.L., Herrler T., Aicher A., Ellwart J.W., Guba M., Bruns C.J. and Heeschen C. (2007). Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell 1, 313-323.

Hiraga T., Ito S. and Nakamura H. (2013). Cancer stem-like cell marker cd44 promotes bone metastases by enhancing tumorigenicity, cell motility, and hyaluronan production. Cancer Res. 73, 4112-4122.

Hurt E.M., Kawasaki B.T., Klarmann G.J., Thomas S.B. and Farrar W.L. (2008). Cd44+ cd24(-) prostate cells are early cancer progenitor/stem cells that provide a model for patients with poor prognosis. Br. J. Cancer 98, 756-765.

Hussein O. and Komarova S.V. (2011). Breast cancer at bone metastatic sites: Recent discoveries and treatment targets. J. Cell. Commun. Signal 5, 85-99.

Jamieson C.H., Ailles L.E., Dylla S.J., Muijtjens M., Jones C., Zehnder J.L., Gotlib J., Li K., Manz M.G., Keating A., Sawyers C.L. and Weissman I.L. (2004). Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis cml. New England J. Med. 351, 657-667.

Jin L., Hope K.J., Zhai Q., Smadja-Joffe F. and Dick J.E. (2006). Targeting of cd44 eradicates human acute myeloid leukemic stem cells. Nat. Med. 12, 1167-1174.

Jordan C.T. (2009). Cancer stem cells: Controversial or just misunderstood? Cell Stem Cell 4, 203-205.

Kang S.Y. and Watnick R.S. (2008). Regulation of tumor dormancy as a function of tumor-mediated paracrine regulation of stromal tsp-1 and vegf expression. APMIS 116, 638-647.

Kim J.K., Jung Y., Wang J., Joseph J., Mishra A., Hill E.E., Krebsbach P.H., Pienta K.J., Shiozawa Y. and Taichman R.S. (2013). Tbk1 regulates prostate cancer dormancy through mtor inhibition. Neoplasia 15, 1064-1074.

Konopleva M., Zhao S., Hu W., Jiang S., Snell V., Weidner D., Jackson C.E., Zhang X., Champlin R., Estey E., Reed J.C. and Andreeff M. (2002). The anti-apoptotic genes bcl-x(l) and bcl-2 are over-expressed and contribute to chemoresistance of non-proliferating leukaemic cd34+ cells. Br. J. Haematol. 118, 521-534.

Krivtsov A.V., Twomey D., Feng Z., Stubbs M.C., Wang Y., Faber J., Levine J.E., Wang J., Hahn W.C., Gilliland D.G., Golub T.R. and Armstrong S.A. (2006). Transformation from committed progenitor to leukaemia stem cell initiated by mll-af9. Nature 442, 818-822.

Krock B.L., Skuli N. and Simon M.C. (2011). Hypoxia-induced angiogenesis: Good and evil. Genes Cancer 2, 1117-1133.

Kumar D., Shankar S. and Srivastava R.K. (2013). Rottlerin-induced autophagy leads to the apoptosis in breast cancer stem cells: Molecular mechanisms. Mol. Cancer 12, 171.

Lagadinou E.D., Sach A., Callahan K., Rossi R.M., Neering S.J., Minhajuddin M., Ashton J.M., Pei S., Grose V., O'Dwyer K.M., Liesveld J.L., Brookes P.S., Becker M.W. and Jordan C.T. (2013). Bcl-2 inhibition targets oxidative phosphorylation and selectively eradicates quiescent human leukemia stem cells. Cell Stem Cell 12, 329-341.

Lapidot T., Sirard C., Vormoor J., Murdoch B., Hoang T., Caceres-Cortes J., Minden M., Paterson B., Caligiuri M.A. and Dick J.E. (1994). A cell initiating human acute myeloid leukaemia after transplantation into scid mice. Nature 367, 645-648.

Larzabal L., El-Nikhely N., Redrado M., Seeger W., Savai R. and Calvo A. (2013). Differential effects of drugs targeting cancer stem cell (csc) and non-csc populations on lung primary tumors and metastasis. PLoS One 8, e79798.

Lecharpentier A., Vielh P., Perez-Moreno P., Planchard D., Soria J.C. and Farace F. (2011). Detection of circulating tumour cells with a hybrid (epithelial/mesenchymal) phenotype in patients with metastatic non-small cell lung cancer. Br. J. Cancer 105, 1338-1341.

Leversha M.A., Han J., Asgari Z., Danila D.C., Lin O., Gonzalez-Espinoza R., Anand A., Lilja H., Heller G., Fleisher M. and Scher H.I. (2009). Fluorescence in situ hybridization analysis of circulating tumor cells in metastatic prostate cancer. Clin. Cancer Res. 15, 2091-2097.

Li C., Heidt D.G., Dalerba P., Burant C.F., Zhang L., Adsay V., Wicha M., Clarke M.F. and Simeone D.M. (2007). Identification of pancreatic cancer stem cells. Cancer Res. 67, 1030-1037.

Liu H., Patel M.R., Prescher J.A., Patsialou A., Qian D., Lin J., Wen S., Chang Y.F., Bachmann M.H., Shimono Y., Dalerba P., Adorno M., Lobo N., Bueno J., Dirbas F.M., Goswami S., Somlo G., Condeelis J., Contag C.H., Gambhir S.S. and Clarke M.F. (2010). Cancer stem

cells from human breast tumors are involved in spontaneous metastases in orthotopic mouse models. Proc. Natl. Acad. Sci. USA 107, 18115-18120.

Lu K.V., Chang J.P., Parachoniak C.A., Pandika M.M., Aghi M.K., Meyronet D., Isachenko N., Fouse S.D., Phillips J.J., Cheresh D.A., Park M. and Bergers G. (2012). Vegf inhibits tumor cell invasion and mesenchymal transition through a met/vegfr2 complex. Cancer Cell 22, 21-35.

Lu X., Mu E., Wei Y., Riethdorf S., Yang Q., Yuan M., Yan J., Hua Y., Tiede B.J., Haffty B.G., Pantel K., Massague J. and Kang Y. (2011). Vcam-1 promotes osteolytic expansion of indolent bone micrometastasis of breast cancer by engaging alpha4beta1-positive osteoclast progenitors. Cancer Cell 20, 701-714.

Ma I. and Allan A.L. (2011). The role of human aldehyde dehydrogenase in normal and cancer stem cells. Stem Cell Rev. 7, 292-306.

Mani S.A., Guo W., Liao M.J., Eaton E.N., Ayyanan A., Zhou A.Y., Brooks M., Reinhard F., Zhang C.C., Shipitsin M., Campbell L.L., Polyak K., Brisken C., Yang J. and Weinberg R.A. (2008). The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133, 704-715.

Marcato P., Dean C.A., Pan D., Araslanova R., Gillis M., Joshi M., Helyer L., Pan L., Leidal A., Gujar S., Giacomantonio C.A. and Lee P.W. (2011). Aldehyde dehydrogenase activity of breast cancer stem cells is primarily due to isoform aldh1a3 and its expression is predictive of metastasis. Stem Cells 29, 32-45.

Marlow R., Honeth G., Lombardi S., Cariati M., Hessey S., Pipili A., Mariotti V., Buchupalli B., Foster K., Bonnet D., Grigoriadis A., Rameshwar P., Purushotham A., Tutt A. and Dontu G. (2013). A novel model of dormancy for bone metastatic breast cancer cells. Cancer Res. 73, 6886-6899.

Martin-Padura I., Marighetti P., Agliano A., Colombo F., Larzabal L., Redrado M., Bleau A.M., Prior C., Bertolini F. and Calvo A. (2012). Residual dormant cancer stem-cell foci are responsible for tumor relapse after antiangiogenic metronomic therapy in hepatocellular carcinoma xenografts. Lab. Invest. 92, 952-966.

Meng S., Tripathy D., Frenkel E.P., Shete S., Naftalis E.Z., Huth J.F., Beitsch P.D., Leitch M., Hoover S., Euhus D., Haley B., Morrison L., Fleming T.P., Herlyn D., Terstappen L.W., Fehm T., Tucker T.F., Lane N., Wang J. and Uhr J.W. (2004). Circulating tumor cells in patients with breast cancer dormancy. Clin. Cancer Res. 10, 8152-8162.

Meng R.D., Shelton C.C., Li Y.M., Qin L.X., Notterman D., Paty P.B. and Schwartz G.K. (2009). Gamma-secretase inhibitors abrogate oxaliplatin-induced activation of the notch-1 signaling pathway in colon cancer cells resulting in enhanced chemosensitivity. Cancer Res. 69, 573-582.

Miyamoto D.T., Lee R.J., Stott S.L., Ting D.T., Wittner B.S., Ulman M., Smas M.E., Lord J.B., Brannigan B.W., Trautwein J., Bander N.H., Wu C.L., Sequist L.V., Smith M.R., Ramaswamy S., Toner M., Maheswaran S. and Haber D.A. (2012). Androgen receptor signaling in circulating tumor cells as a marker of hormonally responsive prostate cancer. Cancer Discovery 2, 995-1003.

Mu X., Isaac C., Greco N., Huard J. and Weiss K. (2013). Notch signaling is associated with aldh activity and an aggressive metastatic phenotype in murine osteosarcoma cells. Front. Oncol. 3, 143.

Mukhopadhyay S., Panda P.K., Sinha N., Das D.N. and Bhutia S.K. (2014). Autophagy and apoptosis: Where do they meet? Apoptosis 19, 555-566.

Naumov G.N., Akslen L.A. and Folkman J. (2006). Role of angiogenesis in human tumor dormancy: Animal models of the angiogenic switch. Cell Cycle 5, 1779-1787.

Naumov G.N., Townson J.L., MacDonald I.C., Wilson S.M., Bramwell V.H., Groom A.C. and Chambers A.F. (2003). Ineffectiveness of doxorubicin treatment on solitary dormant mammary carcinoma cells or late-developing metastases. Breast Cancer Res. Treat. 82, 199-206.

Nechemia-Arbely Y., Barkan D., Pizov G., Shriki A., Rose-John S., Galun E. and Axelrod J.H. (2008). Il-6/il-6r axis plays a critical role in acute kidney injury. J. Am. Soc. Nephrol. 19, 1106-1115.

Nguyen D.X., Bos P.D. and Massague J. (2009). Metastasis: From dissemination to organ-specific colonization. Nat. Rev. Cancer 9, 274-284.

Noda T., Nagano H., Takemasa I., Yoshioka S., Murakami M., Wada H., Kobayashi S., Marubashi S., Takeda Y., Dono K., Umeshita K., Matsuura N., Matsubara K., Doki Y., Mori M. and Monden M. (2009). Activation of wnt/beta-catenin signalling pathway induces chemoresistance to interferon-alpha/5-fluorouracil combination therapy for hepatocellular carcinoma. Br. J. Cancer 100, 1647-1658.

Nowell P.C. (1976). The clonal evolution of tumor cell populations. Science 194, 23-28.

Paez D., Labonte M.J., Bohanes P., Zhang W., Benhanim L., Ning Y., Wakatsuki T., Loupakis F. and Lenz H.J. (2012). Cancer dormancy: A model of early dissemination and late cancer recurrence. Clin. Cancer Res. 18, 645-653.

Paget S. (1989). The distribution of secondary growths in cancer of the breast. Cancer Metastasis Rev. 8, 98-101.

Pang R., Law W.L., Chu A.C., Poon J.T., Lam C.S., Chow A.K., Ng L., Cheung L.W., Lan X.R., Lan H.Y., Tan V.P., Yau T.C., Poon R.T. and Wong B.C. (2010). A subpopulation of cd26+ cancer stem cells with metastatic capacity in human colorectal cancer. Cell Stem Cell 6, 603-615.

Pantel K., Alix-Panabieres C. and Riethdorf S. (2009). Cancer micrometastases. Nat. Rev. Clin. Oncol. 6, 339-351.

Passegue E., Wagner E.F. and Weissman I.L. (2004). Junb deficiency leads to a myeloproliferative disorder arising from hematopoietic stem cells. Cell 119, 431-443.

Psaila B. and Lyden D. (2009). The metastatic niche: Adapting the foreign soil. Nat. Rev. Cancer 9, 285-293.

Rack B., Bock C., Andergassen U. and Doisneau-Sixou S. (2012). Hormone receptor status, erbb2 expression and cancer stem cell characteristics of circulating tumor cells in breast cancer patients. Histol. Histopathol. 27, 855-864.

Ricci-Vitiani L., Lombardi D.G., Pilozzi E., Biffoni M., Todaro M., Peschle C. and De Maria R. (2007). Identification and expansion of human colon-cancer-initiating cells. Nature 445, 111-115.

Roodman G.D. (2012). Genes associate with abnormal bone cell activity in bone metastasis. Cancer Metastasis Rev. 31, 569-578.

Schafer A., Teufel J., Ringel F., Bettstetter M., Hoepner I., Rasper M., Gempt J., Koeritzer J., Schmidt-Graf F., Meyer B., Beier C.P. and Schlegel J. (2012). Aldehyde dehydrogenase 1a1--a new mediator of resistance to temozolomide in glioblastoma. Neuro Oncol. 14, 1452-1464.

Scheel C., Eaton E.N., Li S.H., Chaffer C.L., Reinhardt F., Kah K.J., Bell G., Guo W., Rubin J., Richardson A.L. and Weinberg R.A. (2011). Paracrine and autocrine signals induce and maintain mesenchymal and stem cell states in the breast. Cell 145, 926-940.

Scheel C. and Weinberg R.A. (2012). Cancer stem cells and epithelial-mesenchymal transition: Concepts and molecular links. Semin Cancer Biol. 22, 396-403.

Scher H.I., Jia X., de Bono J.S., Fleisher M., Pienta K.J., Raghavan D. and Heller G. (2009). Circulating tumour cells as prognostic markers in progressive, castration-resistant prostate cancer: A reanalysis of immc38 trial data. Lancet Oncol. 10, 233-239.

Shibue T., Brooks M.W., Inan M.F., Reinhardt F. and Weinberg R.A. (2012). The outgrowth of micrometastases is enabled by the formation of filopodium-like protrusions. Cancer Discov. 2, 706-721.

Shuman Moss L.A., Jensen-Taubman S. and Stetler-Stevenson W.G. (2012). Matrix metalloproteinases: Changing roles in tumor progression and metastasis. Am. J. Pathol. 181, 1895-1899.

Singh S.K., Clarke I.D., Terasaki M., Bonn V.E., Hawkins C., Squire J. and Dirks P.B. (2003). Identification of a cancer stem cell in human brain tumors. Cancer Res. 63, 5821-5828.

Singh A. and Settleman J. (2010). Emt, cancer stem cells and drug resistance: An emerging axis of evil in the war on cancer. Oncogene 29, 4741-4751.

Sosa M.S., Bragado P., Debnath J. and Aguirre-Ghiso J.A. (2013). Regulation of tumor cell dormancy by tissue microenvironments and autophagy. Adv. Exp. Med. Biol. 734, 73-89.

Steeg P.S. (2006). Tumor metastasis: Mechanistic insights and clinical challenges. Nat. Med. 12, 895-904.

Sullivan J.P., Spinola M., Dodge M., Raso M.G., Behrens C., Gao B., Schuster K., Shao C., Larsen J.E., Sullivan L.A., Honorio S., Xie Y., Scaglioni P.P., DiMaio J.M., Gazdar A.F., Shay J.W., Wistuba, II and Minna J.D. (2010). Aldehyde dehydrogenase activity selects for lung adenocarcinoma stem cells dependent on notch signaling. Cancer Res. 70, 9937-9948.

Sun Y., Campisi J., Higano C., Beer T.M., Porter P., Coleman I., True L. and Nelson P.S. (2012). Treatment-induced damage to the tumor microenvironment promotes prostate cancer therapy resistance through wnt16b. Nat. Med. 18, 1359-1368.

Tanei T., Morimoto K., Shimazu K., Kim S.J., Tanji Y., Taguchi T., Tamaki Y. and Noguchi S. (2009). Association of breast cancer stem cells identified by aldehyde dehydrogenase 1 expression with resistance to sequential paclitaxel and epirubicin-based chemotherapy for breast cancers. Clin. Cancer Res. 15, 4234-4241.

Taussig D.C., Miraki-Moud F., Anjos-Afonso F., Pearce D.J., Allen K., Ridler C., Lillington D., Oakervee H., Cavenagh J., Agrawal S.G., Lister T.A., Gribben J.G. and Bonnet D. (2008). Anti-cd38 antibody-mediated clearance of human repopulating cells masks the heterogeneity of leukemia-initiating cells. Blood 112, 568-575.

Teicher B.A. and Fricker S.P. (2010). Cxcl12 (sdf-1)/cxcr4 pathway in cancer. Clin. Cancer Res. 16, 2927-2931.

Tirino V., Desiderio V., Paino F., De Rosa A., Papaccio F., La Noce M., Laino L., De Francesco F. and Papaccio G. (2013). Cancer stem cells in solid tumors: An overview and new approaches for their isolation and characterization. FASEB J. 27, 13-24.

Tlsty T.D. and Coussens L.M. (2006). Tumor stroma and regulation of cancer development. Annu. Rev. Pathol. 1, 119-150.

Tran D.D., Corsa C.A., Biswas H., Aft R.L. and Longmore G.D. (2011). Temporal and spatial cooperation of snail1 and twist1 during epithelial-mesenchymal transition predicts for human breast cancer recurrence. Mol. Cancer Res. 9, 1644-1657.

Ulasov I.V., Nandi S., Dey M., Sonabend A.M. and Lesniak M.S. (2011). Inhibition of sonic hedgehog and notch pathways enhances sensitivity of cd133(+) glioma stem cells to temozolomide therapy. Mol. Med. 17, 103-112.

Valastyan S. and Weinberg R.A. (2011). Tumor metastasis: Molecular insights and evolving paradigms. Cell 147, 275-292.

Vinogradov S. and Wei X. (2012). Cancer stem cells and drug resistance: The potential of nanomedicine. Nanomedicine (Lond) 7, 597-615.

Visus C., Wang Y., Lozano-Leon A., Ferris R.L., Silver S., Szczepanski M.J., Brand R.E., Ferrone C.R., Whiteside T.L., Ferrone S., DeLeo A.B. and Wang X. (2011). Targeting aldh(bright) human carcinoma-initiating cells with aldh1a1-specific cd8(+) t cells. Clin. Cancer Res. 17, 6174-6184.

White A.C., Khuu J.K., Dang C.Y., Hu J., Tran K.V., Liu A., Gomez S., Zhang Z., Yi R., Scumpia P., Grigorian M. and Lowry W.E. (2014). Stem cell quiescence acts as a tumour suppressor in squamous tumours. Nat. Cell Biol. 16, 99-107.

Xin H.W., Hari D.M., Mullinax J.E., Ambe C.M., Koizumi T., Ray S., Anderson A.J., Wiegand G.W., Garfield S.H., Thorgeirsson S.S. and Avital I. (2012). Tumor-initiating label-retaining cancer cells in human gastrointestinal cancers undergo asymmetric cell division. Stem Cells 30, 591-598.

Yang Z.J. and Wechsler-Reya R.J. (2007). Hit 'em where they live: Targeting the cancer stem cell niche. Cancer Cell 11, 3-5.

Yu M., Stott S., Toner M., Maheswaran S. and Haber D.A. (2011). Circulating tumor cells: Approaches to isolation and characterization. J. Cell Biol. 192, 373-382.

Yu M., Bardia A., Wittner B.S., Stott S.L., Smas M.E., Ting D.T., Isakoff S.J., Ciciliano J.C., Wells M.N., Shah A.M., Concannon K.F., Donaldson M.C., Sequist L.V., Brachtel E., Sgroi D., Baselga J., Ramaswamy S., Toner M., Haber D.A. and Maheswaran S. (2013). Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. Science 339, 580-584.

Zhang S., Balch C., Chan M.W., Lai H.C., Matei D., Schilder J.M., Yan P.S., Huang T.H. and Nephew K.P. (2008). Identification and characterization of ovarian cancer-initiating cells from primary human tumors. Cancer Res. 68, 4311-4320.

Zhang S.S., Han Z.P., Jing Y.Y., Tao S.F., Li T.J., Wang H., Wang Y., Li R., Yang Y., Zhao X., Xu X.D., Yu E.D., Rui Y.C., Liu H.J., Zhang L. and Wei L.X. (2012). Cd133(+)cxcr4(+) colon cancer cells exhibit metastatic potential and predict poor prognosis of patients. BMC Med. 10, 85.

Zhe X., Cher M.L. and Bonfil R.D. (2011). Circulating tumor cells: Finding the needle in the haystack. Am. J. Cancer Res. 1, 740-751.

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