

## REVIEW

# Tumor dormancy: EMT beyond invasion and metastasis

Patrick Aouad<sup>1,2,3</sup>  | Hazel M. Quinn<sup>1</sup>  | Adeline Berger<sup>2</sup> | Cathrin Brisken<sup>1,4</sup>

<sup>1</sup>ISREC—Swiss Institute for Experimental Cancer Research, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

<sup>2</sup>Department of Ophthalmology, University of Lausanne, Jules-Gonin Eye Hospital, Fondation Asile des Aveugles, Lausanne, Switzerland

<sup>3</sup>Department of Discovery Oncology, Genentech, South San Francisco, California, USA

<sup>4</sup>The Breast Cancer Now Toby Robins Breast Cancer Research Centre, The Institute of Cancer Research, London, UK

## Correspondence

Hazel M. Quinn and Cathrin Brisken, ISREC—Swiss Institute for Experimental Cancer Research, École Polytechnique Fédérale de Lausanne (EPFL), SV2.832 Station 19, CH-1015 Lausanne, Switzerland.  
Email: [hazel.quinn@epfl.ch](mailto:hazel.quinn@epfl.ch) and [cathrin.brisken@epfl.ch](mailto:cathrin.brisken@epfl.ch)

## Funding information

Deutsche Forschungsgemeinschaft; H2020 Marie Skłodowska-Curie Actions; Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung

## Summary

More than two-thirds of cancer-related deaths are attributable to metastases. In some tumor types metastasis can occur up to 20 years after diagnosis and successful treatment of the primary tumor, a phenomenon termed late recurrence. Metastases arise from disseminated tumor cells (DTCs) that leave the primary tumor early on in tumor development, either as single cells or clusters, adapt to new environments, and reduce or shut down their proliferation entering a state of dormancy for prolonged periods of time. Dormancy has been difficult to track clinically and study experimentally. Recent advances in technology and disease modeling have provided new insights into the molecular mechanisms orchestrating dormancy and the switch to a proliferative state. A new role for epithelial-mesenchymal transition (EMT) in inducing plasticity and maintaining a dormant state in several cancer models has been revealed. In this review, we summarize the major findings linking EMT to dormancy control and highlight the importance of pre-clinical models and tumor/tissue context when designing studies. Understanding of the cellular and molecular mechanisms controlling dormant DTCs is pivotal in developing new therapeutic agents that prevent distant recurrence by maintaining a dormant state.

## KEYWORDS

cell cycle, disseminated tumor cells, dormancy, epithelial-mesenchymal plasticity, mesenchymal-epithelial transition

## 1 | INTRODUCTION

Metastasis accounts for at least two-thirds of all cancer-related deaths (Dillekås et al., 2019). This process entails the escape of tumor cells from their primary site and subsequent growth in distant organs with distinct microenvironments (Steeg, 2006). Treated patients lacking clinically detectable metastases are generally considered disease-free (Riggio et al., 2021). However, the observation of distant recurrence after tumor excision and successful treatment demonstrates the existence of disseminated tumor cells (DTCs) and/or micro-metastatic lesions in different organs (Klein, 2020). These

“invisible metastases” escape clinical diagnosis due to the inability of non-invasive imaging modalities to detect lesions smaller than 0.2 mm (Zhang, 2017). Unfortunately, although the presence of circulating tumor cells and DTCs in sentinel lymph nodes and more rarely, bone marrow aspirates is prognostic, it fails to predict whether tumors will recur in distant organs (Braun et al., 2005; Bundred, 2001; Cristofanilli et al., 2004; Diel et al., 1996).

Dormancy is observed in multiple tumor types such as breast, melanoma, and prostate cancer (Aguirre-Ghiso, 2007; Crowley & Seigler, 1990), representing an important challenge and an unmet clinical need. It is attributed to DTCs seeding and remaining dormant or quiescent at secondary sites for many years (metastatic dormancy) (Klein, 2011). This concept is supported by the clinical observation

Patrick Aouad and Hazel M. Quinn contributed equally to this work.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2023 The Authors. *genesis* published by Wiley Periodicals LLC.

that cancer transmission can occur after organ transplantation from donors with a history of melanoma, breast, and prostate cancers (Buell et al., 2004; Crowley & Seigler, 1990; Matser et al., 2018; Strauss & Thomas, 2010). In immunosuppressed recipients who received organs from a donor with occult breast cancer (BC), dormant metastatic lesions erupted between 16 months and 6 years. The transmissible disease demonstrates the existence of individual DTCs, and their ability to remain dormant (growth-arrested or low-proliferative) in distant organs and develop overt metastatic disease.

Dormancy has long evaded experimental investigation due to our inability to capture single dormant DTCs or micro-metastatic clusters from patients. However, advances in single-cell sequencing technologies have paved the way for a better understanding of these rare cell populations (Werner-Klein et al., 2020) allowing extensive investigation into the underlying molecular mechanisms regulating tumor cell dormancy.

Slowed proliferation, drug-resistance, stemness, quiescence, and reversible senescence have all been observed in dormant tumor cells (Francescangeli et al., 2023). They are best described as a population of growth-arrested cells with the unique capability of resuming proliferation upon either withdrawal of growth-restrictive factors or reception of activating signals from the microenvironment (Figure 1). A multitude of tumor-type-dependent molecular pathways and factors have been identified as dormancy regulators (Francescangeli et al., 2023). Surprisingly, contrary to the traditional view of metastatic progression, epithelial-mesenchymal transition (EMT) has been shown to modulate dormancy-associated metastasis. EMT is a complex biological process by which polarized epithelial cells lose their apical-basal polarity and acquire a spindle-shaped, fibroblast-like morphology (Thiery et al., 2009). In the context of dormancy, EMT renders cells invisible by entering a growth-arrested state and activating survival mechanisms. Here, we will explore the pre-clinical evidence linking EMT to dormancy of DTCs.

## 2 | EMT: PLEIOTROPISM AND CONTEXT-DEPENDENT ROLES IN TUMORIGENESIS

EMT is classified into three distinct types based on its biological context: physiologically during embryonic development (type I), in response to tissue injury (type II), and pathologically during cancer invasion and metastasis (type III; Brabletz et al., 2018; Kalluri & Weinberg, 2009; Yang et al., 2020). Type III EMT has long been associated with disease progression and metastasis, being a central part of the metastatic cascade. However, in recent years, the current consensus on the role of EMT in disease progression has been challenged, highlighting the pleiotropism and cancer/subtype-specific roles of EMT in cancer.

### 2.1 | Differential roles of EMT in invasion and metastasis

After the seminal discovery demonstrating that loss of an epithelial phenotype induces invasion of carcinoma cells, it was postulated that

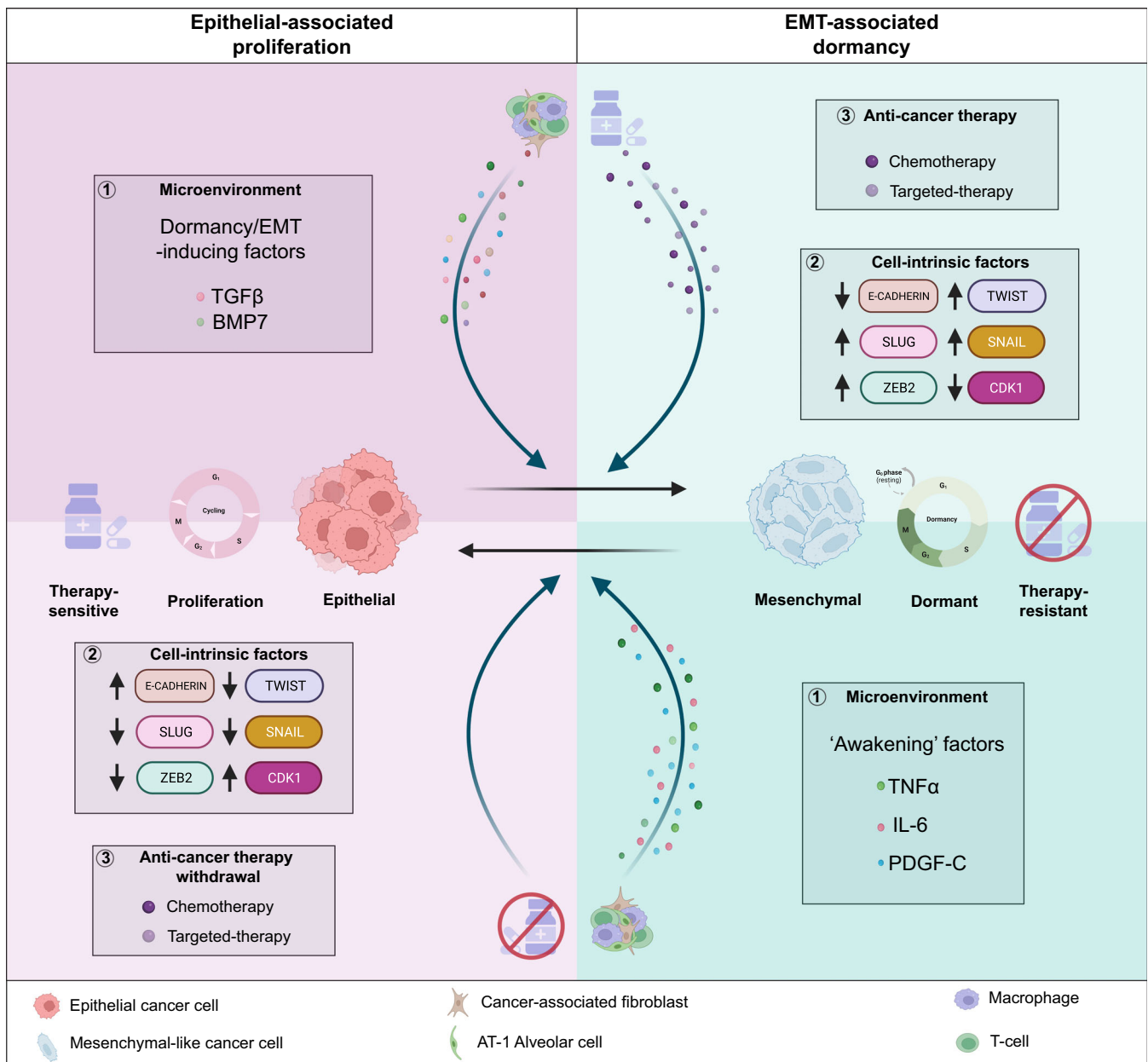
the breakdown of adhesion junctions was a prerequisite for invasion and metastasis of tumors (Behrens et al., 1989; Weidner et al., 1990). Later defined as EMT (Hay, 1995; Hay & Zuk, 1995), this process was further shown to be required for tumor cell invasion and metastasis (Wei et al., 2015; Yang et al., 2004). In recent years, different tumor models have revealed the distinct roles of EMT and mesenchymal-epithelial transition (MET) in tumor initiation, invasion, and metastatic progression. For instance, under physiological conditions, the ovarian epithelium from which cancers arise is negative for the adherens junction protein, E-cadherin. However, E-cadherin was shown to be expressed in tumor cells during tumor development and maintained throughout metastasis albeit at lower levels in some cases (Sundfeldt et al., 1997). In contrast, mouse mammary carcinomas arising from luminal E-cadherin-positive cells were shown to depend on the acquisition of a more mesenchymal state (Quinn et al., 2021). These and many other conflicting reports suggest that the role of EMT in tumor development depends on the cellular context and phenotype of the tumor-initiating cell.

In different breast, pancreatic, prostate, and squamous carcinoma models, an EMT state has been shown to be required for tumor cell dissemination to distant sites, yet the re-acquisition of epithelial features through MET appears to be required for metastatic outgrowth in various settings (Ocaña et al., 2012). On the one hand, the widely used *MMTV-PyMT* mammary carcinoma model required *Snail* and *Zeb1* for tumor formation and metastasis (Ni et al., 2016; Tran et al., 2014; Jiang et al., 2020), and deletion of N-cadherin, an adhesion protein expressed in cells which have undergone EMT, significantly reduced metastasis (Li et al., 2020). On the other hand in *MMTV-HER2/Neu-driven* mammary carcinomas, early tumor cell dissemination was shown to rely on a Wnt-driven stem cell/EMT transcriptional program (Harper et al., 2016; Hosseini et al., 2016). Overall, these findings suggest that the specific tumor drivers may impact on the elicited EMT program and beg the question as to why EMT is required for driving invasion and metastasis in some tumors while impinging on tumor progression in others.

### 2.2 | Tumor subtype-specific roles of EMT

Data from different preclinical models, such as genetically engineered mouse models (GEMMs), allografts, and xenografts, do not reach an overarching consensus regarding the contribution of EMT to tumor invasion and metastasis. Indeed, different cancer subtypes may have different requirements for EMT. Below, we pinpoint the subtype-specific requirements of EMT as it applies to BC and highlight the importance of the models used.

BC is a heterogeneous disease encompassing four major subtypes. Hormone receptor-positive BCs (Luminal A and B) are the most frequent and account for more than 70% of cases, the triple-negative (TN) subtype accounts for 10%–15% of all cases, while 15%–20% are diagnosed as HER2+ (Perou et al., 2000). Dormancy is a prominent clinical phenomenon in ER+ BCs with recurrence up to decades after the initial diagnosis. In contrast, TN BCs exhibit a low incidence of recurrence beyond 5 years (Jatoi et al., 2011). Most preclinical studies



**FIGURE 1** Graphical scheme depicting intrinsic and extrinsic factors contributing to EMT-associated dormancy. (1) Microenvironmental-secreted factors including TGFβ and BMP7 induce mesenchymal/dormancy while TNFα, IL-6, and PDGF-C result in the reversal of mesenchymal/dormant DTCs into an epithelial/proliferative cell state. (2) Cell-intrinsic factors such as EMT-associated gene expression changes in mesenchymal/dormant vs. epithelial/proliferative DTC cell states. (3) Effect of anti-cancer therapy treatment or withdrawal on the epithelial/proliferative and mesenchymal/dormant cell states. AT-1, Alveolar Type 1; BMP7, bone morphogenetic protein 7; CDK1, cyclin dependent kinase 1; E-cadherin, epithelial cadherin; EMT, epithelial-mesenchymal transition; IL-6, interleukin-6; PDGF-C, platelet-derived growth factor C; SNAIL, snail family transcriptional repressor 1; SLUG, snail family transcriptional repressor 2; TGFβ, transforming growth factor beta; TNFα, tumor necrosis factor alpha; ZEB2, zinc finger E-box binding homeobox 2. Figure created with Biorender.com.

on metastasis and dormancy have been conducted using GEMMs which largely develop estrogen receptor-negative (ER<sup>-</sup>) tumors. In addition, injection of cell line models directly into the bloodstream is used to circumvent the accelerated growth rate that precludes metastasis generated by subcutaneously engrafted tumor cells and therefore fails to recapitulate the clinical progression of the disease (Roarty & Echeverria, 2021).

We recently showed that intraductal inoculation of hormone receptor-positive BC cell lines and patient-derived BC cells improves take rates and enables in vivo growth of these cells without the need to supplement 17-β-estradiol to the host. By this approach, the clinical manifestation of the disease from an in situ to invasive disease and metastasis to distant organs is recapitulated (Fiche et al., 2019; Sflomos et al., 2016, 2021). Importantly, the intraductal

microenvironment allows the engrafted cells to maintain their luminal epithelial phenotype whereas BC cells grafted to the mammary fat pad undergo SLUG-dependent basal differentiation in response to TGF $\beta$  from the stromal environment (Sflomos et al., 2016). We speculate that the physical confinement of intraductally injected cells in the milk duct lumen shields them from stromal factors like TGF $\beta$  and enables them to establish physiologically relevant cell–cell interactions as well as contacts with the basement membrane. The striking impact of the engraftment site on ER+ BC cells (Sflomos et al., 2016) highlights the vital contribution of the microenvironment for tumor phenotype and growth (Sflomos et al., 2016; Sflomos & Briskin, 2017).

In this model, both intraductally xenografted TN and hormone receptor-positive BC cells disseminate early but their metastatic progression is distinct. Both invasion and metastatic seeding of ER+ BCs are EMT-independent and require E-cadherin. The xenografted TN BC cells proliferated at the same rate in the primary tumor and lungs, while ER+ BCs lowered their proliferative indices at the distant site. Thus, specifically ER+ not TN BC cells enter dormancy at metastatic sites and do so by entering an EMT state (Aouad et al., 2022). It is highly likely that the discrepancies observed in the reports on EMT in BC are a result of the selection of pre-clinical models and the specific type of tumor under study.

### 3 | MECHANISTIC UNDERPINNINGS OF EMT IN REGULATING DORMANCY

While EMT plays a crucial role in regulating therapy-induced and metastatic dormancy across multiple tumor types, the mechanisms regulating EMT-dependent dormancy are largely context-dependent. The slowed proliferation, increased survival, and therapy resistance observed as a result of EMT induction appear to be driven by a multitude of microenvironmental, systemic, and tumor cell-intrinsic mechanisms (Figure 1; Endo & Inoue, 2019).

#### 3.1 | The role of EMT in cell cycle regulation during tissue homeostasis and cancer

The regenerative capacity of tissues relies on the maintenance of the adult stem cell pool. These cells conserve a quiescent/dormant-like state and divide asymmetrically to maintain the regenerative capacity of tissues (Knoblich, 2008) and EMT has a role to play in this. Indeed, TGF $\beta$ 1 treatment was originally shown to induce EMT and cell cycle arrest in mouse hepatocytes, (Hocevar & Howe, 1998; Yang et al., 2006), and in mouse embryos, expression of the EMT transcriptional factors (TFs) *Snail* or *Slug* correlates with slow proliferation and resistance to apoptosis (Vega et al., 2004). More recently, *Zeb1* was shown to maintain basal cell fate and stem cell quiescence in the mouse mammary gland through an EMT-associated gene network (Han et al., 2022). In renal pathophysiology, the acquisition of a *Snai1* or *Twist-1*-mediated EMT state induced a G<sub>2</sub> cell cycle arrest in

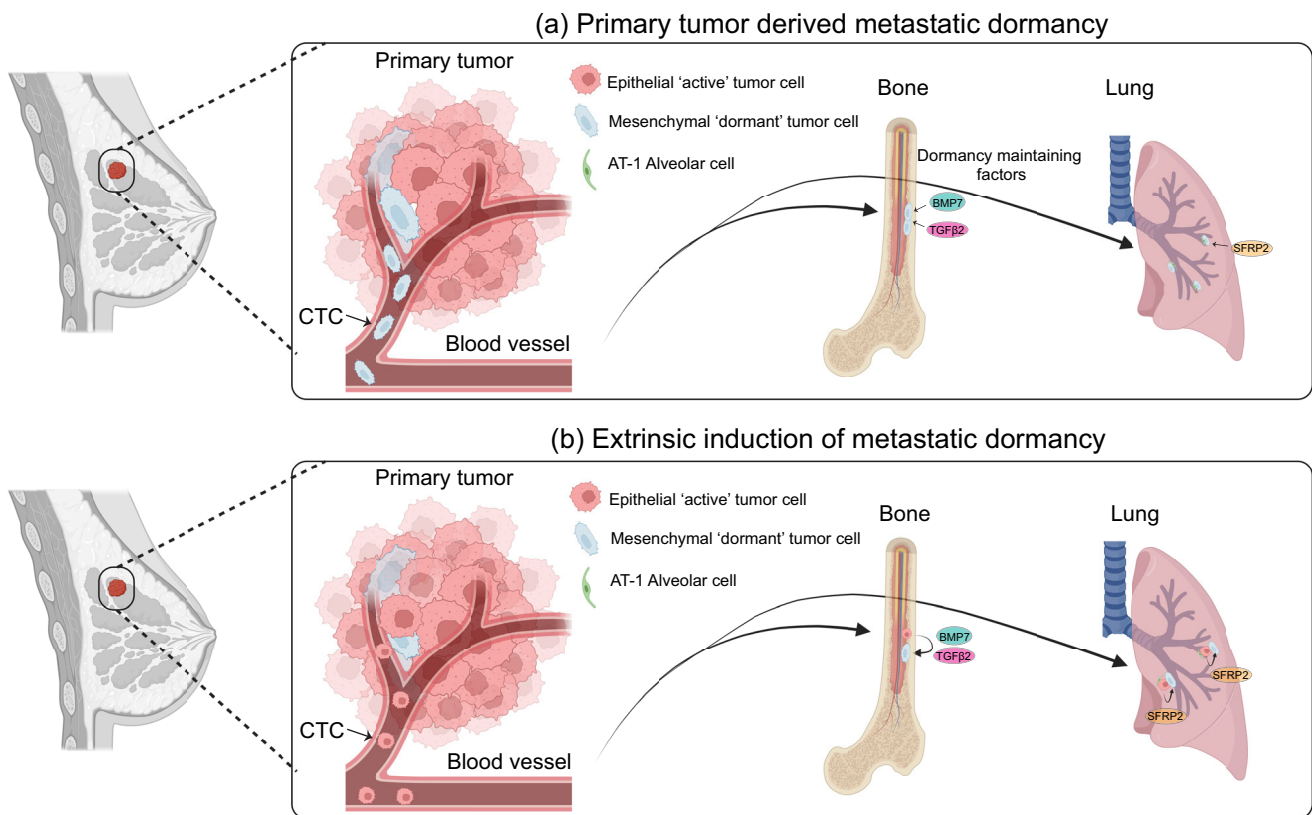
tubular epithelial cells (Lovisa et al., 2015). Evidentially, EMT plays an essential role in normal stem cell homeostasis by regulating stem cell cycle quiescence.

In cancer, normal developmental processes required for tissue homeostasis are hijacked by malignant cells, and processes required for adult stem cell quiescence are exploited in tumor cell dormancy. The EMT-TF *ZEB2*, for instance, induces G<sub>1</sub> cell cycle arrest in epidermoid carcinoma cells and mediates a mesenchymal-like quiescent phenotype in colorectal and non-small cell lung carcinomas (Mejlvang et al., 2007; Cuccu et al., 2022; Francescangeli et al., 2020). In the ER + BC cell line MCF7, *SNAIL* over-expression has been shown to regulate proteins involved in G<sub>0</sub>/G<sub>1</sub> cell cycle arrest such as CDK1 (de Souza Palma et al., 2016). Similarly, constitutive expression of *Snai1* inhibits the growth of xenografted prostate cancer cells, PC3, at the primary site (Cellà-Terrassa et al., 2012). In the slow-growing lobular breast carcinoma cells, SUM44, loss of E-cadherin upregulates *Id2*, which in turn inhibits cell cycle progression (Rätze et al., 2022; Figure 1 (2)). Thus, a plethora of mechanisms are involved in cell cycle regulation of stem and cancer cells, thereby inducing a dormancy phenotype.

#### 3.2 | EMT-dependent survival and therapy resistance

Dormancy enables tumor cells to survive in new microenvironments. The EMT state has been associated with cell survival and dormancy in various contexts. Tumor cells in an EMT state exhibit enhanced resistance to apoptosis and chemotherapy (Debaugnies et al., 2023). Induction of the EMT program in response to anti-cancer therapies has also been shown to lead to cell quiescence and ultimately therapy resistance (Kurppa et al., 2020).

The acquisition of EMT-associated stem cell-like properties by tumor cells also plays a crucial role in their survival in unfamiliar microenvironments, such as those encountered during metastasis (Dave et al., 2012; Eyler & Rich, 2008). In line with this scenario, single-cell RNA sequencing of DTCs from TN BC xenografts found in low-burden distant organs expressed high levels of stem cell/EMT-associated genes in contrast to cells in organs with high metastatic burden which displayed the epithelial traits of the primary tumor (Lawson et al., 2015). Similarly, the latent lung adenocarcinoma and HER2+ BC cell lines, H2087 and HCC1954, were shown to have SOX-dependent stem cell-like characteristics in distinct organs (Malladi et al., 2016). The clinical relevance of these findings is supported by transcriptome and genomic profiling of individual cytokeratin- and EPCAM-positive DTCs from the bone marrow of BC patients (Klein et al., 2002). Early bone marrow DTCs were shown to be in a stem-like state regulated by IL-6 trans-signaling via gp130 in response to human mesenchymal stem cells (Werner-Klein et al., 2020). Recently, in treatment-naïve squamous cell carcinoma tumors, EMT+ cells were found to harbor resistance to chemotherapy. Further investigation found that RhoJ, a member of the Rho



**FIGURE 2** Graphical scheme depicting potential origins of dormant DTCs in distant organs. (a) Pre-existing dormant cancer cells derived from the primary tumor seed at distant sites and are maintained in a dormant cell state by the metastatic niche. (b) Proliferative/epithelial primary tumor cells seed at distant organs where they are induced into a dormant cell state by metastatic niche factors such as BMP7 and TGF $\beta$ . AT-1, Alveolar type 1; BMP7, bone morphogenetic protein 7; CTC, circulating tumor cell; TGF $\beta$ 2, transforming growth factor beta 2; SFRP2, secreted frizzled related protein 2. Figure created with [Biorender.com](https://www.biorender.com).

family of small GTPases is overexpressed in cells that have undergone EMT and modulates resistance to chemotherapy (Debaugnies et al., 2023). These findings further support the concept that disseminated mesenchymal- or stem-like cells survive otherwise hostile environments because of their intrinsic EMT-associated dormant cell state (Figure 1 (2)).

Exposure to anti-cancer therapies can induce EMT and thereby confer drug resistance through acquiring a dormant cell state (Figure 1 (3)). Residual BC tissues isolated from patients who underwent endocrine or chemotherapy were found to display characteristics of both mesenchymal (EMT) and tumor-initiating cells (stemness) (Creighton et al., 2009), highlighting the clinical relevance of the aforementioned preclinical findings. Similarly, in leukemia and NSCLC patients and cell lines, targeted- and chemo-therapy respectively induced a stemness/EMT-dependent reversible senescence phenotype (Kurppa et al., 2020; Milanovic et al., 2018). These findings indicate that the persistence of residual tumor cells following treatment may be attributed to the acquisition of stem cell-like characteristics and EMT properties. These cellular changes could potentially contribute to disease recurrence and the development of therapy resistance, resembling mechanisms observed during dormancy.

#### 4 | ORIGINS AND ESCAPE OF DORMANCY

The EMT state is not always favorable for metastatic progression and metastatic outgrowth requires MET in several tumor types (Celià-Terrassa et al., 2012; Ocaña et al., 2012; Tsai et al., 2012). Historically, EMT and MET were viewed as binary states. It is now well-established that multiple EMT states lie on a continuous spectrum defined by plastic epithelial and mesenchymal transcriptional programs (Jordan et al., 2011). Cells fluctuate between these dynamic states and it is this very plasticity, also known as epithelial-mesenchymal plasticity, that enables dormancy induction and consequentially the survival of cancer cells in different environments or evasion from anti-cancer therapy (Bornes et al., 2021). Dormancy was initially thought to be a pre-existing state in the primary tumor determined by a population of cancer cells displaying quiescence and therapy resistance often referred to as cancer stem cells. In recent years, increasing evidence suggests that extrinsic factors such as those mediated by immune cells and fibroblasts induce EMT plasticity and dormancy entry/exit (Linde et al., 2016; Obenauf & Massagué, 2015). It now remains debated whether EMT-dependent dormancy is a pre-existing state originating from the primary tumor or an acquired cell state induced by extrinsic factors, possibly at the distant site (Figure 2).



#### 4.1 | Dormancy as a pre-existing or induced cellular state

Several studies point to pre-existing dormant cells in the primary tumor being the origin of tumor and metastatic dormancy (Figure 2a). Serial transplantation and lineage tracing of primary human colorectal cancer xenografts identified a rare population of latent/dormant cells in treatment naïve tumors that only expanded upon chemotherapy treatment leading to the dominance of a therapy-tolerant ZEB2+ cancer cell population (Francescangeli et al., 2020; Kreso et al., 2013). Likewise, in PDX models of acute lymphoblastic leukemia, a rare population of non-proliferative cells was identified and found to survive chemotherapy resembling minimal residual disease in patients (Ebinger et al., 2016). In the metastatic setting, single-cell RNA sequencing of matched primary tumor and DTCs from the GEMM of HER2+ BC identified the presence of a subpopulation of primary tumor cells sharing mesenchymal-enriched gene signatures with their respective DTCs. Here, it was shown that expression of FGF2 in the primary tumor induces the dormancy regulator ZFP281 leading to dissemination (Nobre et al., 2022).

In the ER+ BC model, intraductal MCF7 xenografts, although a small cluster of primary tumor cells shared the same EMT-dependent dormancy signatures found in DTCs, induction of EMT in this and other ER+ models failed to increase the number of metastatic lesions, suggesting that dormancy may be induced at the metastatic site (Figure 2b; Aouad et al., 2022).

In recent years, a number of extrinsic, dormancy-inducing factors have been identified (Figure 2b). In BC, the hypoxic environment of the bone marrow has been shown to induce the epigenetic regulation of dormancy in DTCs (Ferrer et al., 2020). Injection of dormant-like prostate cancer cell lines into the bones of athymic nude mice revealed that these cells release the secreted protein acidic and cysteine-rich and stimulate the secretion of BMP7, a member of the TGF $\beta$  superfamily, from bone marrow stromal cells, resulting in a sustained dormant state (Sharma et al., 2016). Similarly, osteoblast secretion of TGF $\beta$ 2 induced dormancy of prostate cancer cells in SCID mice (Yu-Lee et al., 2018), while intracardiac injection of the prostate cancer cell line, PC-3, leads to osteoblast secretion of Wnt5a induced DTC dormancy (Ren et al., 2019). In addition to secreted factors, direct cell-DTC interactions were shown to be essential for dormancy induction. In the metastatic lung niche, the interaction of the syngeneic D2.0R mammary carcinoma cells with host lung AT1 alveolar cells triggered dormancy-associated gene expression mediated by SFRP2 (Montagner et al., 2020). Interactions between osteogenic cells and the ER+ BC cell lines, MCF-7 and ZR-751, in the bone metastatic niche, led to ER loss and the generation of a hybrid EMT state (Bado et al., 2021). Overall, these studies suggest that dormancy may be a pre-existing state in some tumors while induced at distant sites by extrinsic factors or by instructive niches in others.

#### 4.2 | Emerging from dormancy

Dormancy escape can result in tumor growth and progression (Klein, 2020; Sosa et al., 2014). Therefore understanding the factors

that control tumor dormancy exit is an important area of research. The metastatic niche, including nutrient availability and stromal cells (Joyce & Pollard, 2009) has been extensively revealed as a key player in the regulation of EMT-dependent dormancy. In the MMTV-HER2/Neu mouse mammary carcinoma model, single-cell RNA-sequencing of early DTCs revealed increased expression of EMT-TFs controlled by ZFP281 (Nobre et al., 2022). This TF locked DTCs in a quiescent state, and only by repressing it, could metastatic outgrowth and expression of epithelial features such as E-cadherin occur (Nobre et al., 2022). Similarly, it was shown that the dormant murine mammary cell line, D2.0R, expresses high levels of EMT-TFs (Montagner et al., 2020). Epithelial reversion through E-cadherin restoration in dormant DTCs promoted cell cycle entry and overcame EMT/dormancy in a MET fashion (Aouad et al., 2022). In line with these findings, overexpression of E-cadherin was recently shown to promote proliferation and macro-metastasis formation of ER+ BCs via the MEK/ERK signaling pathway (Russo et al., 2022). These studies also suggest that although the EMT state may lock DTCs into dormancy, this can be reversed by shifting cells to a more epithelial cell state, highlighting the plastic nature of DTCs.

#### 4.3 | Microenvironmental control of dormancy and awakening

Microenvironmental factors have also been shown to play a significant role in awakening from dormancy. Escape from metastatic dormancy was shown to be promoted by inflammatory signals creating a pro-tumorigenic microenvironment that supports the growth and survival of DTCs. Factors, such as cytokines and chemokines, can activate signaling pathways that promote DTC survival and proliferation. In BC models, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6) have been shown to promote metastasis dormancy exit through activation of NF- $\kappa$ B and JAK/STAT3 signaling, respectively (Aouad et al., 2022; Korkaya et al., 2012; Yu et al., 2017). In addition to cytokines, a number of inflammatory cells and secreted molecules are known to contribute to a pro-tumorigenic microenvironment and likely affect dormancy. In the pre-metastatic lung of the MMTV-PyMT BC model, myeloid progenitor-secreted versican induces decreases p-SMAD2 levels resulting in MET and accelerated metastasis (Gao et al., 2012). Myeloid cells themselves have also been shown to be activated by tumor-secreted versican, leading to the production of TNF $\alpha$  and enhanced cancer cell survival. In this context, TNF $\alpha$  also recruits leukocytes to create a pro-inflammatory pre-metastatic niche (Kim et al., 2009). In the E0771 syngeneic mouse model of BC, macrophages lie in close proximity to NR2F1+ dormant tumor cells and induce a dormant cell state resulting in increased extravasation and survival (Borriello et al., 2022). Natural killer (NK) cells within the liver metastatic niche were also found to sustain the 4T07 BC dormancy phenotype. NK cell depletion using an anti-asialo-GM1 antibody induced exit from dormancy and metastatic outgrowth (Correia et al., 2021). Such crosstalk between inflammation, tumor cells, and their microenvironments likely impinges on DTC growth and survival in a context-specific manner (Boire et al., 2019).

A striking example of the context-dependency of dormancy was recently demonstrated using syngeneic ER<sup>+</sup> mouse mammary carcinoma cell line models that were injected into hosts of different ages. PDGF-C within the lung microenvironment of aging mice induced dormancy exit and promoted macro-metastatic disease, while DTCs remained dormant in young hosts (Turrell et al., 2023). In head and neck squamous cell carcinoma patients, NR2F1 was found to be a critical node in the induction of DTC dormancy which was downregulated in proliferating cancer cells (Sosa et al., 2015). In an effort to therapeutically induce dormancy, an NR2F1 agonist was identified and shown to prevent overt metastases of lung DTCs by inducing cancer dormancy (Khalil et al., 2022). Considering the distinct mechanisms regulating dormancy, pinpointing the precise extrinsic factors and contexts using a tumor type- and metastatic niche-specific approach in clinically relevant models is a pre-requisite for the development of dormancy-targeted therapies.

## 5 | CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Most of our understanding on tumor dormancy stems from pre-clinical studies which have helped identify molecular mechanisms, potential biomarkers, and possible targets. It is now evident that EMT plays a key role in determining whether cells remain dormant or become active (Aouad et al., 2021; Kurppa et al., 2020; Nobre et al., 2022). Understanding the mechanisms controlling dormancy exit in DTCs is pivotal. By targeting specific mechanisms or extrinsic factors that permit dormancy exit, it may be possible to prevent the reactivation of dormant cells and improve patient outcomes as was shown for the dormancy-inducing NR2F1 agonist, C26 (Khalil et al., 2022). In light of this proof of principle study on exploiting dormancy as an anti-cancer therapy, further elucidation of the tumor-specific cell states regulating dormancy will permit further fine-tuning of therapeutic strategies pushing them closer to the clinic.

Recent advances in single-cell sequencing technology have enabled researchers to study the gene expression profiles of individual DTCs and their interactions with the microenvironment (Aouad et al., 2022; Janghorban et al., 2022; Lawson et al., 2015; Nobre et al., 2022). These studies have revealed that DTCs can undergo dynamic changes in gene expression displaying EMT-dependent phenotypes in response to microenvironmental cues (Linde et al., 2016; Obenauf & Massagué, 2015). Most studies on metastatic dormancy to date have been based on over-expression and repression of EMT-related factors. These approaches failed to capture the true plasticity of DTCs. The fast-paced development of sensitive single-cell OMICS, spatial transcriptomics, 3D whole organ imaging, and lineage tracing technologies, will provide novel tools to unravel (1) the contribution of the dormancy permissive metastatic niche, (2) from when and where dormant DTCs arise, and (3) what are the molecular mechanisms regulating tumor cell dormancy. The combination of these technologies will guide us to unravel the secrets of dormancy regulation and provide a rationale for the development of dormancy-inducing

therapies. We may soon enter a new era of anti-cancer therapies with the ability to prevent late recurrence or fine-tune cancers into a chronic but controlled disease.

## AUTHOR CONTRIBUTIONS

*Conceptualization:* Patrick Aouad, Cathrin Brisken. *Writing:* Patrick Aouad, Hazel M. Quinn, Cathrin Brisken. *Writing—review and editing:* Patrick Aouad, Hazel M. Quinn, Adeline Berger, Cathrin Brisken. All authors have read and agreed to the published version of the manuscript.

## ACKNOWLEDGMENTS

The authors would like to thank George Sflomos for careful reading of the review. Patrick Aouad was supported by SNF 310030\_179163/1. Exploring key steps of the metastatic cascade in ER<sup>+</sup> BC in vivo. Hazel M. Quinn is supported by Deutsche Krebshilfe. Cathrin Brisken has support from H2020-MSCA-ITN (ITN-2019-859860-CANCER-PREV). Graphical figures were created with [BioRender.com](https://BioRender.com). Open access funding provided by Ecole Polytechnique Federale de Lausanne.

## ORCID

Patrick Aouad  <https://orcid.org/0000-0001-6568-2151>

Hazel M. Quinn  <https://orcid.org/0000-0002-1336-5709>

## REFERENCES

- Aguirre-Ghiso, J. A. (2007). Models, mechanisms, and clinical evidence for cancer dormancy. *Nature Reviews. Cancer*, 7(11), 834–846. <https://doi.org/10.1038/nrc2256>
- Aouad, P., Zhang, Y., de Martino, F., Stibolt, C., Ali, S., Ambrosini, G., Mani, S. A., Maggs, K., Quinn, H. M., Sflomos, G., & Brisken, C. (2022). Epithelial-mesenchymal plasticity determines estrogen receptor positive breast cancer dormancy and epithelial reconversion drives recurrence. *Nature Communications*, 13(1), 4975. <https://doi.org/10.1038/s41467-022-32523-6>
- Aouad, P., Zhang, Y., Stibolt, C., Mani, S. A., Sflomos, G., & Brisken, C. (2021). Epithelial-mesenchymal plasticity determines estrogen receptor positive (ER<sup>+</sup>) breast cancer dormancy and reacquisition of an epithelial state drives awakening (p. 2021.07.22.453458). *bioRxiv*. <https://doi.org/10.1101/2021.07.22.453458>
- Bado, I. L., Zhang, W., Hu, J., Xu, Z., Wang, H., Sarkar, P., Li, L., Wan, Y.-W., Liu, J., Wu, W., Lo, H. C., Kim, I. S., Singh, S., Janghorban, M., Muscarella, A. M., Goldstein, A., Singh, P., Jeong, H.-H., Liu, C., ... Zhang, X. H.-F. (2021). The bone microenvironment increases phenotypic plasticity of ER<sup>+</sup> breast cancer cells. *Developmental Cell*, 56(8), 1100–1117.e9. <https://doi.org/10.1016/j.devcel.2021.03.008>
- Behrens, J., Mareel, M. M., van Roy, F. M., & Birchmeier, W. (1989). Dissecting tumor cell invasion: Epithelial cells acquire invasive properties after the loss of uvomorulin-mediated cell-cell adhesion. *The Journal of Cell Biology*, 108(6), 2435–2447. <https://doi.org/10.1083/jcb.108.6.2435>
- Boire, A., Coffelt, S. B., Quezada, S. A., Vander Heiden, M. G., & Weeraratna, A. T. (2019). Tumour dormancy and reawakening: Opportunities and challenges. *Trends in Cancer*, 5(12), 762–765. <https://doi.org/10.1016/j.trecan.2019.10.010>
- Bornes, L., Belthier, G., & van Rheenen, J. (2021). Epithelial-to-mesenchymal transition in the light of plasticity and hybrid E/M states. *Journal of Clinical Medicine*, 10(11), 2403. <https://doi.org/10.3390/jcm10112403>

- Borriello, L., Coste, A., Traub, B., Sharma, V. P., Karagiannis, G. S., Lin, Y., Wang, Y., Ye, X., Duran, C. L., Chen, X., Friedman, M., Sosa, M. S., Sun, D., Dalla, E., Singh, D. K., Oktay, M. H., Aguirre-Ghiso, J. A., Condeelis, J. S., & Entenberg, D. (2022). Primary tumor associated macrophages activate programs of invasion and dormancy in disseminating tumor cells. *Nature Communications*, 13(1), 626. <https://doi.org/10.1038/s41467-022-28076-3>
- Brabletz, T., Kalluri, R., Nieto, M. A., & Weinberg, R. A. (2018). EMT in cancer. *Nature Reviews Cancer*, 18(2), 128–134. <https://doi.org/10.1038/nrc.2017.118>
- Braun, S., Vogl, F. D., Naume, B., Janni, W., Osborne, M. P., Coombes, R. C., Schlimok, G., Diel, I. J., Gerber, B., Gebauer, G., Pierga, J.-Y., Marth, C., Oruzio, D., Wiedswang, G., Solomayer, E.-F., Kundt, G., Strobl, B., Fehm, T., Wong, G. Y. C., ... Pantel, K. (2005). A pooled analysis of bone marrow micrometastasis in breast cancer. *New England Journal of Medicine*, 353(8), 793–802. <https://doi.org/10.1056/NEJMoa050434>
- Buell, J. F., Beebe, T. M., Trofe, J., Gross, T. G., Alloway, R. R., Hanaway, M. J., & Woodle, E. S. (2004). Donor transmitted malignancies. *Annals of Transplantation*, 9(1), 53–56.
- Bundred, N. J. (2001). Prognostic and predictive factors in breast cancer. *Cancer Treatment Reviews*, 27(3), 137–142. <https://doi.org/10.1053/ctrv.2000.0207>
- Cuccu, A., Francescangeli, F., De Angelis, M. L., Bruselles, A., Giuliani, A., & Zeuner, A. (2022). Analysis of dormancy-associated transcriptional networks reveals a shared quiescence signature in lung and colorectal cancer. *International Journal of Molecular Sciences*, 23(17), 9869. <https://doi.org/10.3390/ijms23179869>
- Celià-Terrassa, T., Meca-Cortés, Ó., Mateo, F., Martínez de Paz, A., Rubio, N., Arnal-Estapé, A., Ell, B. J., Bermudo, R., Díaz, A., Guerra-Rebollo, M., Lozano, J. J., Estarás, C., Ulloa, C., Alvarez-Simón, D., Milà, J., Vilella, R., Paciucci, R., Martínez-Balbás, M., García de Herreros, A., ... Thomson, T. M. (2012). Epithelial-mesenchymal transition can suppress major attributes of human epithelial tumor-initiating cells. *The Journal of Clinical Investigation*, 122(5), 1849–1868. <https://doi.org/10.1172/JCI59218>
- Correia, A. L., Guimaraes, J. C., Auf der Maur, P., de Silva, D., Trefny, M. P., Okamoto, R., Bruno, S., Schmidt, A., Mertz, K., Volkmann, K., Terracciano, L., Zippelius, A., Vetter, M., Kurzeder, C., Weber, W. P., & Bentires-Alj, M. (2021). Hepatic stellate cells suppress NK cell-sustained breast cancer dormancy. *Nature*, 594(7864), 566–571. <https://doi.org/10.1038/s41586-021-03614-z>
- Creighton, C. J., Li, X., Landis, M., Dixon, J. M., Neumeister, V. M., Sjolund, A., Rimm, D. L., Wong, H., Rodriguez, A., Herschkowitz, J. I., Fan, C., Zhang, X., He, X., Pavlick, A., Gutierrez, M. C., Renshaw, L., Larionov, A. A., Faratian, D., Hilsenbeck, S. G., ... Chang, J. C. (2009). Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. *Proceedings of the National Academy of Sciences of the United States of America*, 106(33), 13820–13825. <https://doi.org/10.1073/pnas.0905718106>
- 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. M. M., & Hayes, D. F. (2004). Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *New England Journal of Medicine*, 351(8), 781–791. <https://doi.org/10.1056/NEJMoa040766>
- Crowley, N. J., & Seigler, H. F. (1990). Late recurrence of malignant melanoma. Analysis of 168 patients. *Annals of Surgery*, 212(2), 173–177.
- Dave, B., Mittal, V., Tan, N. M., & Chang, J. C. (2012). Epithelial-mesenchymal transition, cancer stem cells and treatment resistance. *Breast Cancer Research: BCR*, 14(1), 202. <https://doi.org/10.1186/bcr2938>
- de Souza Palma, C., Grassi, M. L., Thomé, C. H., Ferreira, G. A., Albuquerque, D., Pinto, M. T., Melo, F. U. F., Kashima, S., Covas, D. T., Pitteri, S. J., & Faça, V. M. (2016). Proteomic analysis of epithelial to mesenchymal transition (EMT) reveals cross-talk between SNAIL and HDAC1 proteins in breast cancer cells\*. *Molecular & Cellular Proteomics*, 15(3), 906–917. <https://doi.org/10.1074/mcp.M115.052910>
- Debaugnies, M., Rodríguez-Acebes, S., Blondeau, J., Parent, M.-A., Zocco, M., Song, Y., de Maertelaer, V., Moers, V., Latil, M., Dubois, C., Coulonval, K., Impens, F., van Haver, D., Dufour, S., Uemura, A., Sotiropoulou, P. A., Méndez, J., & Blanpain, C. (2023). RHOJ controls EMT-associated resistance to chemotherapy. *Nature*, 1–8, 168–175. <https://doi.org/10.1038/s41586-023-05838-7>
- Diel, I. J., Kaufmann, M., Costa, S. D., Holle, R., von Minckwitz, G., Solomayer, E. F., Kaul, S., & Bastert, G. (1996). Micrometastatic breast cancer cells in bone marrow at primary surgery: Prognostic value in comparison with nodal status. *Journal of the National Cancer Institute*, 88(22), 1652–1658. <https://doi.org/10.1093/jnci/88.22.1652>
- Dillekås, H., Rogers, M. S., & Straume, O. (2019). Are 90% of deaths from cancer caused by metastases? *Cancer Medicine*, 8(12), 5574–5576. <https://doi.org/10.1002/cam4.2474>
- Ebinger, S., Özdemir, E. Z., Ziegenhain, C., Tiedt, S., Castro Alves, C., Grunert, M., Dworzak, M., Lutz, C., Turati, V. A., Enver, T., Horny, H.-P., Sotlar, K., Parekh, S., Spiekermann, K., Hiddemann, W., Schepers, A., Polzer, B., Kirsch, S., Hoffmann, M., ... Jeremias, I. (2016). Characterization of rare, dormant, and therapy-resistant cells in acute lymphoblastic leukemia. *Cancer Cell*, 30(6), 849–862. <https://doi.org/10.1016/j.ccell.2016.11.002>
- Endo, H., & Inoue, M. (2019). Dormancy in cancer. *Cancer Science*, 110(2), 474–480. <https://doi.org/10.1111/cas.13917>
- Eyler, C. E., & Rich, J. N. (2008). Survival of the fittest: Cancer stem cells in therapeutic resistance and angiogenesis. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, 26(17), 2839–2845. <https://doi.org/10.1200/JCO.2007.15.1829>
- Ferrer, A., Roser, C. T., El-Far, M. H., Savanur, V. H., Eljarrah, A., Gergues, M., Kra, J. A., Etchegaray, J.-P., & Rameshwar, P. (2020). Hypoxia-mediated changes in bone marrow microenvironment in breast cancer dormancy. *Cancer Letters*, 488, 9–17. <https://doi.org/10.1016/j.canlet.2020.05.026>
- Fiche, M., Scabia, V., Aouad, P., Battista, L., Treboux, A., Stravodimou, A., Zaman, K., RLS, Dormoy, V., Ayyanan, A., Sflomos, G., & Brisken, C. (2019). Intraductal patient-derived xenografts of estrogen receptor  $\alpha$ -positive breast cancer recapitulate the histopathological spectrum and metastatic potential of human lesions. *The Journal of Pathology*, 247(3), 287–292. <https://doi.org/10.1002/path.5200>
- Francescangeli, F., Contavalli, P., De Angelis, M. L., Careccia, S., Signorelli, M., Haas, T. L., Salaris, F., Baiocchi, M., Boe, A., Giuliani, A., Tcheremenskaia, O., Pagliuca, A., Guardiola, O., Minchiotti, G., Colace, L., Ciardi, A., D'Andrea, V., la Torre, F., Medema, J., ... Zeuner, A. (2020). A pre-existing population of ZEB2+ quiescent cells with stemness and mesenchymal features dictate chemoresistance in colorectal cancer. *Journal of Experimental & Clinical Cancer Research: CR*, 39(1), 2. <https://doi.org/10.1186/s13046-019-1505-4>
- Francescangeli, F., de Angelis, M. L., Rossi, R., Cuccu, A., Giuliani, A., de Maria, R., & Zeuner, A. (2023). Dormancy, stemness, and therapy resistance: Interconnected players in cancer evolution. *Cancer and Metastasis Reviews*, 42, 197–215. <https://doi.org/10.1007/s10555-023-10092-4>
- Gao, D., Joshi, N., Choi, H., Ryu, S., Hahn, M., Catena, R., Sadik, H., Argani, P., Wagner, P., Vahdat, L. T., Port, J. L., Stiles, B., Sukumar, S., Altorki, N. K., Raffi, S., & Mittal, V. (2012). Myeloid progenitor cells in the premetastatic lung promote metastases by inducing mesenchymal to epithelial transition. *Cancer Research*, 72(6), 1384–1394. <https://doi.org/10.1158/0008-5472.CAN-11-2905>
- Han, Y., Villarreal-Ponce, A., Gutierrez, G., Nguyen, Q., Sun, P., Wu, T., Sui, B., Berx, G., Brabletz, T., Kessenbrock, K., Zeng, Y. A., Watanabe, K., & Dai, X. (2022). Coordinate control of basal epithelial cell fate and stem cell maintenance by core EMT transcription factor



- Zeb1. *Cell Reports*, 38(2), 110240. <https://doi.org/10.1016/j.celrep.2021.110240>
- Harper, K. L., Sosa, M. S., Entenberg, D., Hosseini, H., Cheung, J. F., Nobre, R., Avivar-Valderas, A., Nagi, C., Girmius, N., Davis, R. J., Farias, E. F., Condeelis, J., Klein, C. A., & Aguirre-Ghiso, J. A. (2016). Mechanism of early dissemination and metastasis in Her2+ mammary cancer. *Nature*, 540(7634), 588–592. <https://doi.org/10.1038/nature20609>
- Hay, E. D. (1995). An overview of epithelio-mesenchymal transformation. *Acta Anatomica*, 154(1), 8–20. <https://doi.org/10.1159/000147748>
- Hay, E. D., & Zuk, A. (1995). Transformations between epithelium and mesenchyme: Normal, pathological, and experimentally induced. *American Journal of Kidney Diseases: The Official Journal of the National Kidney Foundation*, 26(4), 678–690. [https://doi.org/10.1016/0272-6386\(95\)90610-x](https://doi.org/10.1016/0272-6386(95)90610-x)
- Hocevar, B. A., & Howe, P. H. (1998). Mechanisms of TGF-beta-induced cell cycle arrest. *Mineral and Electrolyte Metabolism*, 24(2–3), 131–135. <https://doi.org/10.1159/000057360>
- Hosseini, H., Obradović, M. M. S., Hoffmann, M., Harper, K. L., Sosa, M. S., Werner-Klein, M., Nanduri, L. K., Werno, C., Ehrl, C., Maneck, M., Patwary, N., Haunschild, G., Gužvić, M., Reimelt, C., Grauvogel, M., Eichner, N., Weber, F., Hartkopf, A. D., Taran, F.-A., ... Klein, C. A. (2016). Early dissemination seeds metastasis in breast cancer. *Nature*, 540(7634), 552–558. <https://doi.org/10.1038/nature20785>
- Janghorban, M., Yang, Y., Zhao, N., Hamor, C., Nguyen, T. M., Zhang, X. H.-F., & Rosen, J. M. (2022). Single-cell analysis unveils the role of the tumor immune microenvironment and notch signaling in dormant minimal residual disease. *Cancer Research*, 82(5), 885–899. <https://doi.org/10.1158/0008-5472.CAN-21-1230>
- Jatoi, I., Anderson, W. F., Jeong, J.-H., & Redmond, C. K. (2011). Breast cancer adjuvant therapy: Time to consider its time-dependent effects. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, 29(17), 2301–2304. <https://doi.org/10.1200/JCO.2010.32.3550>
- Jiang, H., Zhou, C., Zhang, Z., Wang, Q., Wei, H., Shi, W., Li, J., Wang, Z., Ou, Y., Wang, W., Wang, H., Zhang, Q., Sun, W., Sun, P., & Yang, S. (2020). Jagged1-Notch1-deployed tumor perivascular niche promotes breast cancer stem cell phenotype through Zeb1. *Nature Communications*, 11(1), 5129. <https://doi.org/10.1038/s41467-020-18860-4>
- Jordan, N. V., Johnson, G. L., & Abell, A. N. (2011). Tracking the intermediate stages of epithelial-mesenchymal transition in epithelial stem cells and cancer. *Cell Cycle*, 10(17), 2865–2873. <https://doi.org/10.4161/cc.10.17.17188>
- Joyce, J. A., & Pollard, J. W. (2009). Microenvironmental regulation of metastasis. *Nature Reviews Cancer*, 9(4), 239–252. <https://doi.org/10.1038/nrc2618>
- Kalluri, R., & Weinberg, R. A. (2009). The basics of epithelial-mesenchymal transition. *The Journal of Clinical Investigation*, 119(6), 1420–1428. <https://doi.org/10.1172/JCI39104>
- Khalil, B. D., Sanchez, R., Rahman, T., Rodriguez-Tirado, C., Moritsch, S., Martinez, A. R., Miles, B., Farias, E., Mezei, M., Nobre, A. R., Singh, D., Kale, N., Sproll, K. C., Sosa, M. S., & Aguirre-Ghiso, J. A. (2022). An NR2F1-specific agonist suppresses metastasis by inducing cancer cell dormancy. *The Journal of Experimental Medicine*, 219(1), e20210836. <https://doi.org/10.1084/jem.20210836>
- Kim, S., Takahashi, H., Lin, W.-W., Descargues, P., Grivennikov, S., Kim, Y., Luo, J.-L., & Karin, M. (2009). Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis. *Nature*, 457(7225), 102–106. <https://doi.org/10.1038/nature07623>
- Klein, C. A. (2011). Framework models of tumor dormancy from patient-derived observations. *Current Opinion in Genetics & Development*, 21(1), 42–49. <https://doi.org/10.1016/j.gde.2010.10.011>
- Klein, C. A. (2020). Cancer progression and the invisible phase of metastatic colonization. *Nature Reviews Cancer*, 20, 681–694. <https://doi.org/10.1038/s41568-020-00300-6>
- Klein, C. A., Seidl, S., Petat-Dutter, K., Offner, S., Geigl, J. B., Schmidt-Kittler, O., Wendler, N., Passlick, B., Huber, R. M., Schlimok, G., Baeuerle, P. A., & Riethmüller, G. (2002). Combined transcriptome and genome analysis of single micrometastatic cells. *Nature Biotechnology*, 20(4), 387–392. <https://doi.org/10.1038/nbt0402-387>
- Knoblich, J. A. (2008). Mechanisms of asymmetric stem cell division. *Cell*, 132(4), 583–597. <https://doi.org/10.1016/j.cell.2008.02.007>
- Korkaya, H., Kim, G.-I., Davis, A., Malik, F., Henry, N. L., Ithimakin, S., Quraishi, A. A., Tawakkol, N., D'Angelo, R., Paulson, A. K., Chung, S., Luther, T., Paholak, H. J., Liu, S., Hassan, K. A., Zen, Q., Clouthier, S. G., & Wicha, M. S. (2012). Activation of an IL6 inflammatory loop mediates trastuzumab resistance in HER2+ breast cancer by expanding the cancer stem cell population. *Molecular Cell*, 47(4), 570–584. <https://doi.org/10.1016/j.molcel.2012.06.014>
- Kreso, A., O'Brien, C. A., van Galen, P., Gan, O. I., Notta, F., Brown, A. M. K., Ng, K., Ma, J., Wienholds, E., Dunant, C., Pollett, A., Gallinger, S., McPherson, J., Mullighan, C. G., Shibata, D., & Dick, J. E. (2013). Variable clonal repopulation dynamics influence chemotherapy response in colorectal cancer. *Science*, 339(6119), 543–548. <https://doi.org/10.1126/science.1227670>
- Kurppa, K. J., Liu, Y., To, C., Zhang, T., Fan, M., Vajdi, A., Knelson, E. H., Xie, Y., Lim, K., Cejas, P., Portell, A., Lizotte, P. H., Ficarro, S. B., Li, S., Chen, T., Haikala, H. M., Wang, H., Bahcall, M., Gao, Y., ... Jänne, P. A. (2020). Treatment-induced tumor dormancy through YAP-mediated transcriptional reprogramming of the apoptotic pathway. *Cancer Cell*, 37(1), 104–122.e12. <https://doi.org/10.1016/j.ccell.2019.12.006>
- Lawson, D. A., Bhakta, N. R., Kessenbrock, K., Prummel, K. D., Yu, Y., Takai, K., Zhou, A., Eyob, H., Balakrishnan, S., Wang, C. Y., Yaswen, P., Goga, A., & Werb, Z. (2015). Single-cell analysis reveals a stem-cell program in human metastatic breast cancer cells. *Nature*, 526(7571), 131–135. <https://doi.org/10.1038/nature15260>
- Li, Y., Lv, Z., Zhang, S., Wang, Z., He, L., Tang, M., Pu, W., Zhao, H., Zhang, Z., Shi, Q., Cai, D., Wu, M., Hu, G., Lui, K. O., Feng, J., Nieto, M. A., & Zhou, B. (2020). Genetic fate mapping of transient cell fate reveals N-cadherin activity and function in tumor metastasis. *Developmental Cell*, 54(5), 593–607.e5. <https://doi.org/10.1016/j.devcel.2020.06.021>
- Linde, N., Fluegen, G., & Aguirre-Ghiso, J. A. (2016). The relationship between dormant cancer cells and their microenvironment. *Advances in Cancer Research*, 132, 45–71. <https://doi.org/10.1016/bs.acr.2016.07.002>
- Lovisa, S., LeBleu, V. S., Tampe, B., Sugimoto, H., Vадnagara, K., Carstens, J. L., Wu, C.-C., Hagos, Y., Burckhardt, B. C., Pentcheva-Hoang, T., Nischal, H., Allison, J. P., Zeisberg, M., & Kalluri, R. (2015). Epithelial-to-mesenchymal transition induces cell cycle arrest and parenchymal damage in renal fibrosis. *Nature Medicine*, 21(9), 998–1009. <https://doi.org/10.1038/nm.3902>
- Malladi, S., Macalino, D. G., 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(1), 45–60. <https://doi.org/10.1016/j.cell.2016.02.025>
- Matser, Y. A. H., Terpstra, M. L., Nadalin, S., Nossent, G. D., de Boer, J., van Bemmel, B. C., van Eeden, S., Budde, K., Brakemeier, S., & Bemelman, F. J. (2018). Transmission of breast cancer by a single multiorgan donor to 4 transplant recipients. *American Journal of Transplantation*, 18(7), 1810–1814. <https://doi.org/10.1111/ajt.14766>
- Mejlvang, J., Krijajevska, M., Vandewalle, C., Chernova, T., Sayan, A. E., Bex, G., Mellon, J. K., & Tulchinsky, E. (2007). Direct repression of cyclin D1 by SIP1 attenuates cell cycle progression in cells undergoing an epithelial mesenchymal transition. *Molecular Biology of the Cell*, 18(11), 4615–4624. <https://doi.org/10.1091/mbc.E07-05-0406>
- Milanovic, M., Fan, D. N. Y., Belenki, D., Däbritz, J. H. M., Zhao, Z., Yu, Y., Dörr, J. R., Dimitrova, L., Lenze, D., Monteiro Barbosa, I. A., Mendoza-Parra, M. A., Kanashova, T., Metzner, M., Pardon, K., Reimann, M., Trumpp, A., Dörken, B., Zuber, J., Gronemeyer, H., ... Schmitt, C. A.

- (2018). Senescence-associated reprogramming promotes cancer stemness. *Nature*, 553(7686), 96–100. <https://doi.org/10.1038/nature25167>
- Montagner, M., Bhome, R., Hooper, S., Chakravarty, P., Qin, X., Sufi, J., Bhargava, A., Ratcliffe, C. D. H., Naito, Y., Pocaterra, A., Tape, C. J., & Sahai, E. (2020). Crosstalk with lung epithelial cells regulates Sfrp2-mediated latency in breast cancer dissemination. *Nature Cell Biology*, 22(3), 289–296. <https://doi.org/10.1038/s41556-020-0474-3>
- Ni, T., Li, X.-Y., Lu, N., An, T., Liu, Z.-P., Fu, R., Lv, W.-C., Zhang, Y.-W., Xu, X.-J., Grant Rowe, R., Lin, Y.-S., Scherer, A., Feinberg, T., Zheng, X.-Q., Chen, B.-A., Liu, X. S., Guo, Q.-L., Wu, Z.-Q., & Weiss, S. J. (2016). Snail1-dependent p53 repression regulates expansion and activity of tumour-initiating cells in breast cancer. *Nature Cell Biology*, 18(11), 1221–1232. <https://doi.org/10.1038/ncb3425>
- Nobre, A. R., Dalla, E., Yang, J., Huang, X., Wullkopf, L., Risson, E., Razghandi, P., Anton, M. L., Zheng, W., Seoane, J. A., Curtis, C., Kenigsberg, E., Wang, J., & Aguirre-Ghiso, J. A. (2022). ZFP281 drives a mesenchymal-like dormancy program in early disseminated breast cancer cells that prevents metastatic outgrowth in the lung. *Nature Cancer*, 3(10), 1165–1180. <https://doi.org/10.1038/s43018-022-00424-8>
- Obenauf, A. C., & Massagué, J. (2015). Surviving at a distance: Organ specific metastasis. *Trends in Cancer*, 1(1), 76–91. <https://doi.org/10.1016/j.trecan.2015.07.009>
- Ocaña, O. H., Córcoles, R., Fabra, A., Moreno-Bueno, G., Acloque, H., Vega, S., Barrallo-Gimeno, A., Cano, A., & Nieto, M. A. (2012). Metastatic colonization requires the repression of the epithelial-mesenchymal transition inducer Prrx1. *Cancer Cell*, 22(6), 709–724. <https://doi.org/10.1016/j.ccr.2012.10.012>
- Perou, C. M., Sørlie, T., Eisen, M. B., van de Rijn, M., Jeffrey, S. S., Ress, C. A., Pollack, J. R., Ross, D. T., Johnsen, H., Akslen, L. A., Fluge, Ø., Pergammenschikov, A., Williams, C., Zhu, S. X., Lønning, P. E., Børresen-Dale, A. L., Brown, P. O., & Botstein, D. (2000). Molecular portraits of human breast tumours. *Nature*, 406(6797), 747–752. <https://doi.org/10.1038/35021093>
- Quinn, H. M., Vogel, R., Popp, O., Mertins, P., Lan, L., Messerschmidt, C., Landshammer, A., Lisek, K., Château-Joubert, S., Marangoni, E., Koren, E., Fuchs, Y., & Birchmeier, W. (2021). YAP and  $\beta$ -catenin cooperate to drive oncogenesis in basal breast cancer. *Cancer Research*, 81(8), 2116–2127. <https://doi.org/10.1158/0008-5472.can-20-2801>
- Rätze, M. A. K., Koorman, T., Sijnesael, T., Basse-Archibong, B., van de Ven, R., Enserink, L., Visser, D., Jaksani, S., Viciano, I., Bakker, E. R. M., Richard, F., Tutt, A., O'Leary, L., Fitzpatrick, A., Roca-Cusachs, P., van Diest, P. J., Desmedt, C., Daniel, J. M., Isacke, C. M., & Derksen, P. W. B. (2022). Loss of E-cadherin leads to Id2-dependent inhibition of cell cycle progression in metastatic lobular breast cancer. *Oncogene*, 41(21), 2932–2944. <https://doi.org/10.1038/s41388-022-02314-w>
- Ren, D., Dai, Y., Yang, Q., Zhang, X., Guo, W., Ye, L., Huang, S., Chen, X., Lai, Y., Du, H., Lin, C., Peng, X., & Song, L. (2019). Wnt5a induces and maintains prostate cancer cells dormancy in bone. *The Journal of Experimental Medicine*, 216(2), 428–449. <https://doi.org/10.1084/jem.20180661>
- Riggio, A. I., Varley, K. E., & Welm, A. L. (2021). The lingering mysteries of metastatic recurrence in breast cancer. *British Journal of Cancer*, 124(1), 13–26. <https://doi.org/10.1038/s41416-020-01161-4>
- Roarty, K., & Echeverria, G. V. (2021). Laboratory models for investigating breast cancer therapy resistance and metastasis. *Frontiers in Oncology*, 11, 645698. <https://doi.org/10.3389/fonc.2021.645698>
- Russo, G. C., Crawford, A. J., Clark, D., Cui, J., Carney, R., Karl, M. N., Su, B., Starich, B., Lih, T.-S., Kamat, P., Zhang, Q., Wu, P.-H., Lee, M.-H., Leong, H. S., Rebecca, V. W., Zhang, H., & Wirtz, D. (2022). E-cadherin interacts with EGFR resulting in hyper-activation of ERK in multiple models of breast cancer (p. 2020.11.04.368746). *bioRxiv* <https://doi.org/10.1101/2020.11.04.368746>
- Sflomos, G., Battista, L., Aouad, P., de Martino, F., Scabia, V., Stravodimou, A., Ayyanan, A., Ifticene-Treboux, A., RLS, Bucher, P., Fiche, M., Ambrosini, G., & Brisken, C. (2021). Intraductal xenografts show lobular carcinoma cells rely on their own extracellular matrix and LOXL1. *EMBO Molecular Medicine*, 13(3), e13180. <https://doi.org/10.15252/emmm.202013180>
- Sflomos, G., & Brisken, C. (2017). Breast cancer microenvironment and the metastatic process. In U. Veronesi, A. Goldhirsch, P. Veronesi, O. D. Gentilini, & M. C. Leonardi (Eds.), *Breast cancer* (pp. 39–48). Springer International Publishing. [https://doi.org/10.1007/978-3-319-48848-6\\_5](https://doi.org/10.1007/978-3-319-48848-6_5)
- Sflomos, G., Dormoy, V., Metsalu, T., Jeitziner, R., Battista, L., Scabia, V., Raffoul, W., Delaloye, J.-F., Treboux, A., Fiche, M., Vilo, J., Ayyanan, A., & Brisken, C. (2016). A preclinical model for ER $\alpha$ -positive breast cancer points to the epithelial microenvironment as determinant of luminal phenotype and hormone response. *Cancer Cell*, 29(3), 407–422. <https://doi.org/10.1016/j.ccell.2016.02.002>
- Sharma, S., Xing, F., Liu, Y., Wu, K., Said, N., Pochampally, R., Shiozawa, Y., Lin, H.-K., Balaji, K. C., & Watabe, K. (2016). Secreted protein acidic and Rich in cysteine (SPARC) mediates metastatic dormancy of prostate cancer in bone. *The Journal of Biological Chemistry*, 291(37), 19351–19363. <https://doi.org/10.1074/jbc.M116.737379>
- Sosa, M. S., Bragado, P., & Aguirre-Ghiso, J. A. (2014). Mechanisms of disseminated cancer cell dormancy: An awakening field. *Nature Reviews. Cancer*, 14(9), 611–622. <https://doi.org/10.1038/nrc3793>
- Sosa, M. S., Parikh, F., Maia, A. G., Estrada, Y., Bosch, A., Bragado, P., Ekin, E., George, A., Zheng, Y., Lam, H.-M., Morrissey, C., Chung, C.-Y., Farias, E. F., Bernstein, E., & Aguirre-Ghiso, J. A. (2015). NR2F1 controls tumour cell dormancy via SOX9- and RAR $\beta$ -driven quiescence programmes. *Nature Communications*, 6, 6170. <https://doi.org/10.1038/ncomms7170>
- Steege, P. S. (2006). Tumor metastasis: Mechanistic insights and clinical challenges. *Nature Medicine*, 12(8), 895–904. <https://doi.org/10.1038/nm1469>
- Strauss, D. C., & Thomas, J. M. (2010). Transmission of donor melanoma by organ transplantation. *The Lancet Oncology*, 11(8), 790–796. [https://doi.org/10.1016/S1470-2045\(10\)70024-3](https://doi.org/10.1016/S1470-2045(10)70024-3)
- Sundfeldt, K., Piontekewitz, Y., Ivarsson, K., Nilsson, O., Hellberg, P., Brännström, M., Janson, P. O., Enerback, S., & Hedin, L. (1997). E-cadherin expression in human epithelial ovarian cancer and normal ovary. *International Journal of Cancer*, 74(3), 275–280. [https://doi.org/10.1002/\(sici\)1097-0215\(19970620\)74:3<275::aid-ijc7>3.0.co;2-w](https://doi.org/10.1002/(sici)1097-0215(19970620)74:3<275::aid-ijc7>3.0.co;2-w)
- Thiery, J. P., Acloque, H., Huang, R. Y. J., & Nieto, M. A. (2009). Epithelial-mesenchymal transitions in development and disease. *Cell*, 139(5), 871–890. <https://doi.org/10.1016/j.cell.2009.11.007>
- Tran, H. D., Luitel, K., Kim, M., Zhang, K., Longmore, G. D., & Tran, D. D. (2014). Transient SNAIL1 expression is necessary for metastatic competence in breast cancer. *Cancer Research*, 74(21), 6330–6340. <https://doi.org/10.1158/0008-5472.CAN-14-0923>
- Tsai, J. H., Donaher, J. L., Murphy, D. A., Chau, S., & Yang, J. (2012). Spatio-temporal regulation of epithelial-mesenchymal transition is essential for squamous cell carcinoma metastasis. *Cancer Cell*, 22(6), 725–736. <https://doi.org/10.1016/j.ccr.2012.09.022>
- Turrell, F. K., Orha, R., Guppy, N. J., Gillespie, A., Guelbert, M., Starling, C., Haider, S., & Isacke, C. M. (2023). Age-associated microenvironmental changes highlight the role of PDGF-C in ER+ breast cancer metastatic relapse. *Nature Cancer*, 4(4), 468–484. <https://doi.org/10.1038/s43018-023-00525-y>
- Vega, S., Morales, A. V., Ocaña, O. H., Valdés, F., Fabregat, I., & Nieto, M. A. (2004). Snail blocks the cell cycle and confers resistance to cell death. *Genes & Development*, 18(10), 1131–1143. <https://doi.org/10.1101/gad.294104>

- Wei, S. C., Fattet, L., Tsai, J. H., Guo, Y., Pai, V. H., Majeski, H. E., Chen, A. C., Sah, R. L., Taylor, S. S., Engler, A. J., & Yang, J. (2015). Matrix stiffness drives epithelial-mesenchymal transition and tumour metastasis through a TWIST1-G3BP2 mechanotransduction pathway. *Nature Cell Biology*, 17(5), 678–688. <https://doi.org/10.1038/ncb3157>
- Weidner, K. M., Behrens, J., Vandekerckhove, J., & Birchmeier, W. (1990). Scatter factor: Molecular characteristics and effect on the invasiveness of epithelial cells. *The Journal of Cell Biology*, 111(5 Pt 1), 2097–2108. <https://doi.org/10.1083/jcb.111.5.2097>
- Werner-Klein, M., Grujovic, A., Irlbeck, C., Obradović, M., Hoffmann, M., Koerkel-Qu, H., Lu, X., Treitschke, S., Köstler, C., Botteron, C., Weidele, K., Werno, C., Polzer, B., Kirsch, S., Gužvić, M., Warfsmann, J., Honarnejad, K., Czyn, Z., Feliciello, G., ... Klein, C. A. (2020). Interleukin-6 trans-signaling is a candidate mechanism to drive progression of human DCCs during clinical latency. *Nature Communications*, 11(1), 4977. <https://doi.org/10.1038/s41467-020-18701-4>
- Yang, J., Antin, P., Berx, G., Blanpain, C., Brabletz, T., Bronner, M., Campbell, K., Cano, A., Casanova, J., Christofori, G., Dedhar, S., Derynck, R., Ford, H. L., Fuxe, J., García de Herreros, A., Goodall, G. J., Hadjantonakis, A.-K., Huang, R. Y. J., Kalcheim, C., ... Sheng, G. (2020). Guidelines and definitions for research on epithelial-mesenchymal transition. *Nature Reviews Molecular Cell Biology*, 21(6), 341–352. <https://doi.org/10.1038/s41580-020-0237-9>
- Yang, J., Mani, S. A., Donaher, J. L., Ramaswamy, S., Itzykson, R. A., Come, C., Savagner, P., Gitelman, I., Richardson, A., & Weinberg, R. A. (2004). Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell*, 117(7), 927–939. <https://doi.org/10.1016/j.cell.2004.06.006>
- Yang, Y., Pan, X., Lei, W., Wang, J., & Song, J. (2006). Transforming growth factor-beta1 induces epithelial-to-mesenchymal transition and apoptosis via a cell cycle-dependent mechanism. *Oncogene*, 25(55), 7235–7244. <https://doi.org/10.1038/sj.onc.1209712>
- Yu, P. F., Huang, Y., Han, Y. Y., Lin, L. Y., Sun, W. H., Rabson, A. B., Wang, Y., & Shi, Y. F. (2017). TNF $\alpha$ -activated mesenchymal stromal cells promote breast cancer metastasis by recruiting CXCR2+ neutrophils. *Oncogene*, 36(4), 482–490. <https://doi.org/10.1038/nc.2016.217>
- Yu-Lee, L.-Y., Yu, G., Lee, Y.-C., Lin, S.-C., Pan, J., Pan, T., Yu, K.-J., Liu, B., Creighton, C. J., Rodriguez-Canales, J., Villalobos, P. A., Wistuba, I. I., de Nadal, E., Posas, F., Gallick, G. E., & Lin, S.-H. (2018). Osteoblast-secreted factors mediate dormancy of metastatic prostate cancer in the bone via activation of the TGF $\beta$ RIII-p38MAPK-pS249/T252RB pathway. *Cancer Research*, 78(11), 2911–2924. <https://doi.org/10.1158/0008-5472.CAN-17-1051>
- Zhang, M. (2017). Early detection of multi-organ metastases. *Nature Biomedical Engineering*, 1(12), 934–936. <https://doi.org/10.1038/s41551-017-0173-y>

**How to cite this article:** Aouad, P., Quinn, H. M., Berger, A., & Briskin, C. (2023). Tumor dormancy: EMT beyond invasion and metastasis. *genesis*, e23552. <https://doi.org/10.1002/dvg.23552>