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Cancer cell plasticity during tumor progression, metastasis and response to therapy

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Cell plasticity represents the ability of cells to be reprogrammed and to change their fate and identity, enabling homeostasis restoration and tissue regeneration following damage. Cell plasticity also contributes to pathological conditions, such as cancer, enabling cells to acquire new phenotypic and functional features by transiting across distinct cell states that contribute to tumor initiation, progression, metastasis and resistance to therapy. Here, we review the intrinsic and extrinsic mechanisms driving cell plasticity that promote tumor growth and proliferation as well as metastasis and drug tolerance. Finally, we discuss how cell plasticity could be exploited for anti-cancer therapy.

Although lineage specification and differentiation were long assumed to be unidirectional and irreversible, cell identity is currently recognized to be less rigid and more plastic than previously thought. Cell plasticity refers to the reprogramming of a cell toward a different fate in response to intrinsic or extrinsic factors[1,2]. Stem cells are plastic and have the capacity to self-renew and differentiate into one or more cell lineages. The capacity of terminally differentiated cells, such as fibroblasts, to be reprogrammed back to a pluripotent state shows that plasticity is not only a stem cell feature[3,4]. Cells can display plasticity through dedifferentiation (the reversion of a differentiated cell into an undifferentiated state within the same lineage), transdifferentiation (the conversion of a differentiated cell into another differentiated cell lineage, forming the basis of metaplasia)[5] (Fig. 1a) and epithelial-to-mesenchymal transition (EMT), a process through which epithelial cells lose epithelial characteristics, such as cell–cell junctions and polarity, and acquire a mesenchymal phenotype[6].

Plasticity is essential to restore homeostasis after tissue damage, inflammation or senescence but can also contribute to tumorigenesis. During cancer progression, tumor cells can switch between cell states, a process primarily mediated by cell plasticity, to overcome selective pressures. Thus, cell plasticity largely fuels intratumor heterogeneity[2,7,8] (as well as other sources such as DNA mutations[9,10]) and fitness, increasing the adaptability of tumor cells[9], and contributes substantially to tumor growth, metastasis and resistance to therapy.

Cell plasticity from homeostasis to tumorigenesis

Under physiological conditions in adult tissues, replenishment of differentiated cells is ensured by multipotent or lineage-restricted stem cells. During wound healing and tissue regeneration, the latter can become plastic and expand their differentiation potential to replace other cell types and promote tissue repair[8].

The intestinal epithelium is one of the most rapidly self-renewing tissues in mammals. Lgr5 marks the stem cells in the small intestine and colon[11] that initiate the formation of crypt–villus self-organizing mouse organoids[12]. Intestinal crypts contain stem cells and transit-amplifying progenitors that can revert to a multipotent state under regenerative conditions[13]. Following Lgr5$^+$ stem cell lineage ablation in mice, committed *Bmi1*-expressing cells can sustain homeostasis and replenish the pool of Lgr5$^+$ stem cells[14]. Even more differentiated Alpi$^+$ enterocyte progenitors can revert into Lgr5$^+$ cells[15]. Following damage, committed precursors, such as secretory Dll1$^+$ progenitors or Paneth cells, which are derived from Lgr5$^+$ cells, can revert to the latter to replenish the stem cell pool and enable regeneration in mice[16,17] (Fig. 1b).

In response to ionizing irradiation in the mouse intestine, YAP, the transcriptional activator of the Hippo pathway, promotes cell survival and a regenerative state required for tumor formation[18]. Colon regeneration following dextran sulfate sodium-induced colitis in mouse models activates the YAP–TAZ pathway to reprogram adult cells into a fetal-like state required for regeneration[19]. Parasitic helminth infection in mice suppresses the normal adult stem cell program and promotes

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a similar state[20]. The YAP1-dependent stem cell state has been associated with intestinal regeneration also by single-cell transcriptomics[21]. However, YAP has also been proposed to antagonize stemness during regeneration and act as a tumor suppressor in a mouse model of colorectal cancer, possibly reflecting differences in the models employed[22]. In intestinal tumors, different populations have been identified resembling Lgr5$^+$ crypt–base columnar stem cells and Lgr5$^-$ regenerative stem cells expressing the fetal-like state, the respective abundance of which is regulated by intrinsic and extrinsic stimuli[23].

The skin epidermis is composed of a pilosebaceous unit containing one hair follicle, its associated sebaceous gland and surrounding interfollicular epidermis[8]. During homeostasis, these different regions are maintained by their own pool of unipotent stem cells. During wound healing, different interfollicular epidermis stem and progenitor cells are recruited. Hair follicle and infundibulum stem cells migrate upward toward the interfollicular epidermis, are progressively reprogrammed into interfollicular epidermis stem cells, proliferate and contribute to skin repair[8,24–26]. The niche is important for this reprogramming: when mouse hair follicle stem cells are ablated, the empty niche can recruit more committed cells that revert to a stem cell-like state and stably replenish the stem cell pool[27] (Fig. 1c).

Many glandular epithelia are composed of an inner luminal layer surrounded by an outer layer of myoepithelial and/or basal cells and develop from multipotent progenitors, which are progressively replaced by unipotent stem cells during adult tissue homeostasis[8]. When taken out of their natural environment in the absence of luminal cells, basal stem cells exhibit greater differentiation potential, giving rise to luminal cells, and generate functional mammary glands in mice[28–30] (Fig. 1d). In the prostate, the existence of multipotent basal progenitors during postnatal development contrasts with the distinct pools of unipotent basal and luminal stem cells that mediate adult regeneration[31–33]. Luminal cell depletion by infection, E-cadherin knockout or genetic ablation can stimulate basal cell multipotency in glandular epithelia to replenish luminal cells[34–36].

The ability of differentiated cells to revert to a stem cell-like state has major implications for tumorigenesis, with some oncogenic drivers influencing plasticity during tumor initiation. Tumor suppressors such as TP53, RB1 and PTEN regulate developmental differentiation programs, and, when dysregulated, are associated with cancer[5]. In glandular epithelia, unipotent basal and luminal stem cells can reacquire multipotency during tumor initiation. During mouse prostate tumor initiation, *Pten* deletion in basal cells promotes basal-to-luminal transdifferentiation[33,37] (Fig. 1e). Combined *Trp53* and *Rb1* loss-of-function mutations promote transdifferentiation from adenocarcinoma to neuroendocrine carcinoma in mouse prostate cancer[38,39]. Similarly, in the mouse mammary gland, breast cancer 1, early onset (BRCA1) inactivation in luminal progenitors leads to basal-like breast cancer, displaying heterogeneous expression of basal and luminal markers[40]. Oncogenic phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (*Pik3ca*)^{H1047R} expression induces multipotency in mammary gland lineage-restricted progenitors early during tumor initiation, setting the basis for intratumor heterogeneity[41,42] (Fig. 1f).

Inflammation also regulates plasticity during regeneration and tumor initiation[43]. In the mouse small intestine, inflammation is followed by a loss of Lgr5$^+$ stem cells, thereby inducing Paneth cells to re-enter the cell cycle, acquire stem cell-like properties and contribute to tissue regeneration[44]. In the absence of inflammation, only intestinal stem cells can induce tumor formation following *Apc* deletion. Co-deletion of *Apc* and *Nfkbia* which activates nuclear factor-κB (NF-κB) signaling, induces tumor formation by non-stem cells, showing that inflammatory signals can expand their tumor-initiating capacities[45]. In the mouse prostate gland, bacterial infection-induced inflammation promotes basal-to-luminal transdifferentiation and accelerates tumor initiation from basal cells[34]. Inflammation promotes cell plasticity in the pancreas by triggering acinar-to-ductal metaplasia[46]. When oncogenic *Kras* is expressed in the presence of inflammation, metaplasia progresses to neoplasia[47,48]. Tissue regeneration in the presence of oncogenic *Kras* induces a unique chromatin state essential for tumor formation[49]. In *Nr5a2*$^{+/-}$ mice, a transcriptional switch from differentiation to inflammation mediated by the AP-1 transcription factor (heterodimer composed of members of the Jun, Fos, ATF and JDP families) potentially explains why mutations around the human *NR5A2* gene promote pancreatic cancer[50].

Tumor growth and proliferation

Tumors are composed by tumor cells of different states, accomplishing distinct functions. In this section, we discuss the extensively studied concept that tumor growth is sustained by cancer stem cells (CSCs).

CSCs and intrinsic regulation of proliferative states

CSCs express a stem cell-like program, are able to self-renew, sustain tumor growth and give rise to tumor cells with more restricted proliferative potential[51]. For example, colorectal CSCs express a gene signature reminiscent of normal intestinal stem cells[52,53].

Whereas the xenotransplantation assay was the main method initially used to define CSCs, other approaches including lineage tracing, barcoding and lineage ablation were developed[54] (Box 1 and Fig. 2a). These efforts showed that CSCs might not be a unique population but might instead represent several subpopulations. In a strict hierarchical organization, CSCs would give rise to subpopulations with more limited growth and differentiation potential, which could never revert to a CSC state[55,56]. However, evidence suggests that both CSCs and non-CSCs are plastic and might undergo phenotypic transitions under certain conditions (for example, therapy)[54]. For example, *JARID1B* (also known as *KDM5B*) expression is essential for continuous tumor growth in melanoma, with this phenotype being dynamic (JARID1B$^-$ cells can become JARID1B$^+$ cells and vice versa), suggesting that melanoma maintenance is a dynamic process mediated by a temporarily distinct subpopulation[57]. Differentiated colon cancer cells can revert to a CSC state to compensate for CSC loss and replenish the CSC population[58,59]. Genetic ablation of Lgr5$^+$ CSCs in xenografted mouse colorectal cancer organoids restricts tumor growth without leading to regression. Tumors are then maintained by proliferative Lgr5$^-$ cells that replenish the CSC pool. Lgr5$^+$ CSCs reappear when ablation is discontinued, leading to rapid tumor regrowth and indicating plasticity of more differentiated tumor cells following CSC ablation[58]. This finding is supported by patient-derived organoids. Following LGR5$^+$ CSC ablation in xenografted human colorectal cancer organoids, LGR5$^-$ cells replenish the LGR5$^+$ CSC pool,

Fig. 1 | Cell plasticity during homeostasis, regeneration and tumorigenesis. **a**, Stem cell differentiation, dedifferentiation and transdifferentiation occurring during cell plasticity. **b**, Lgr5$^+$ intestinal stem cells self-renew and give rise to distinct intestinal lineages during homeostasis. Following stem cell lineage ablation, more committed progenitors can replenish the pool of stem cells, enabling epithelium regeneration. **c**, During homeostasis, the different epidermal compartments are sustained by distinct pools of unipotent stem cells, whereas, during wound healing, interfollicular epidermis stem cells contribute to skin repair, but also stem cells from the infundibulum and bulge can migrate upward, proliferate and be reprogrammed into interfollicular epidermis stem cells to contribute to regeneration. **d**, Under homeostatic conditions, basal and luminal cells in the mammary gland are unipotent. Following transplantation into the mammary fat pad, basal cells become multipotent and can give rise to luminal cells, enabling the generation of a functional mammary gland. **e**, *Pten* deletion in basal cells of the prostate gland promotes basal-to-luminal transdifferentiation and leads to tumor initiation. **f**, *Pik3ca*^{H1047R} expression in basal cells in the mammary gland leads to transdifferentiation into luminal cells, while its expression in luminal cells enables transdifferentiation into basal cells. Both basal and luminal cells expressing *Pik3ca*^{H1047R} can initiate tumorigenesis. IFE, interfollicular epidermis; SC, stem cell.

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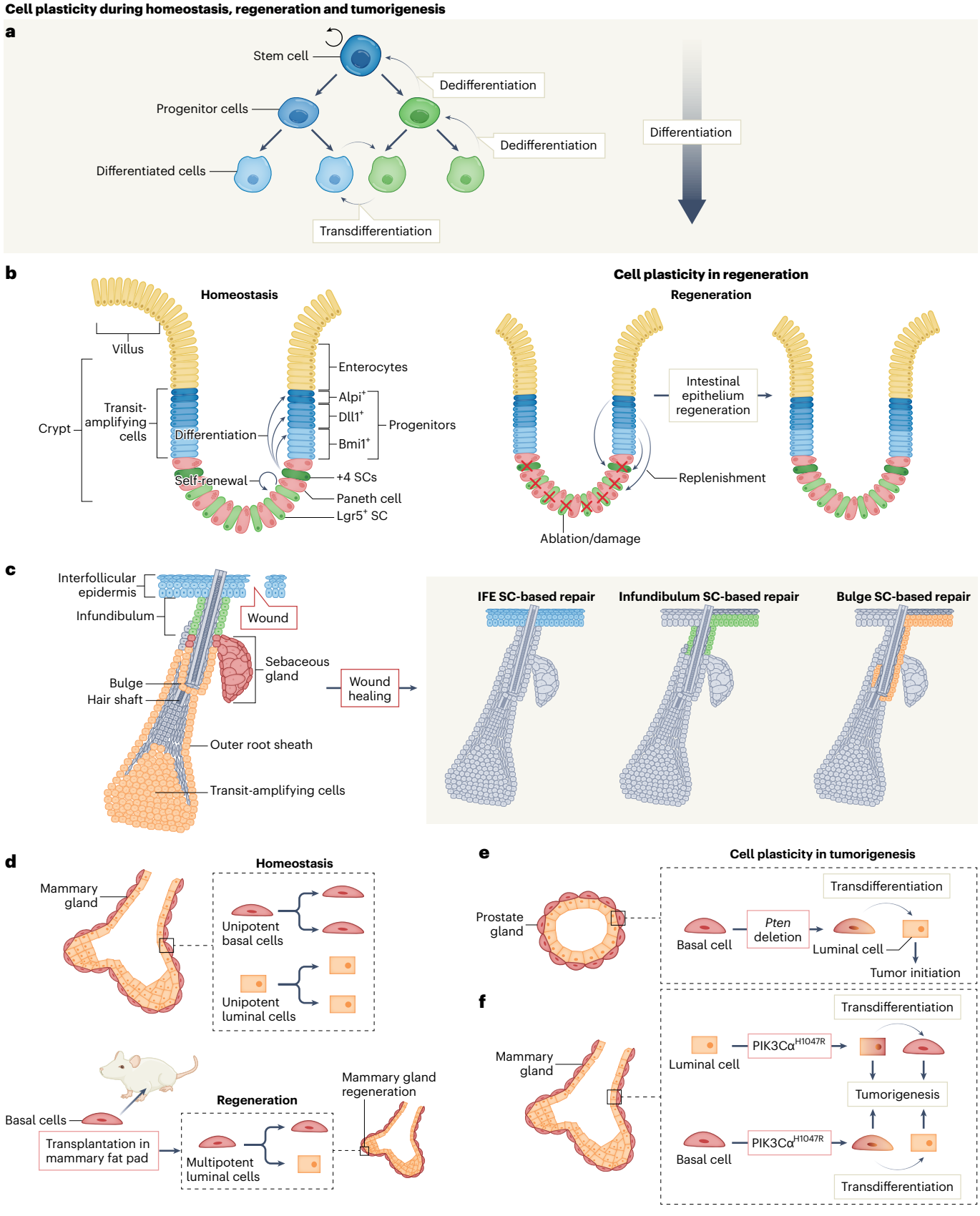
mediating tumor relapse[59] and suggesting that therapies targeting CSCs without preventing cell plasticity would be insufficient.

Clonal analysis combined with lineage tracing helped define the evolutionary dynamics of tumor growth, supporting, in some cases, a neutral drift of tumor evolution with the emergence of subclones. In mouse skin tumors, neutral competition of tumor cells in benign papilloma indicates that tumor growth is mediated by stochastic cell fate decisions, reminiscent of the clonal dynamics of normal stem cells[60,61],



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BOX 1

Functional strategies to identify CSCs

In classical xenotransplantation experiments, the capacity of a subpopulation to initiate a tumor following transplantation into immunodeficient mice over serial passages is interpreted as evidence of CSC presence[54,271] (Fig. 2a). These studies identified $CD34^+CD38^+$ CSCs in acute myeloid leukemia[272], $CD44^+CD24^{-/lo}$ CSCs in breast cancer[273], epithelial cell-adhesion molecule $(EpCAM)^{hi}CD44^+$ CSCs in colorectal cancer[274] and $CD133^+$ CSCs in brain[275], pancreas[276] and colon tumors[277–279].

Xenotransplantation experiments enable the study of the tumor-propagating capacity of a specific tumor subpopulation in patient-derived samples. However, this technique has inherent technical and biological limitations, such as the lack of native architecture and stroma[54,271]. Xenotransplantation might not consider clonal cooperation or competition and can present clonal selection, leading to the formation of dominant clones with low frequency in the primary tumor, and different degrees of mouse immunodeficiency might lead to variable results[280]. Xenotransplantation reveals the potential of certain subpopulations to form tumors, which might not be representative of the fate of tumor cells within their native microenvironment.

Lineage tracing is the gold-standard method for defining cell fate in vivo and has been used to study CSCs within their native microenvironment and the hierarchical organization of tumor growth[62,281] (Fig. 2a). Conventional lineage tracing was largely restricted to genetic mouse models, but CRISPR–Cas9 gene-editing technology enables us to perform lineage tracing in patient-derived tumor organoids, as shown by colorectal cancer studies[59,282]. Emerging lineage-tracing approaches combined with single-cell sequencing rely on naturally occurring molecular barcodes, such as somatic nuclear mutations and copy number variations to conduct longitudinal studies along disease progression[283]. Mitochondrial DNA mutations can also be used as phylogenetic barcodes to study clonal dynamics[284].

Laser-induced or genetically induced lineage ablation is another powerful approach to assess the importance of a subpopulation for tumor growth, maintaining the natural microenvironment of the tumor[54,271]. In tumors maintained by CSCs, CSC ablation will result in tumor regression, such as it occurs when ablating Nes^+ cells in mouse glioblastoma[285], $Sox2^+$ cells in mouse skin squamous cell carcinoma[286], $Dclk1^+$ cells in mouse intestinal tumors[287] or $LGR5^+$ cells in human colorectal cancer[59] (Fig. 2a).

further suggesting that tumor heterogeneity can sometimes be explained by neutral drift rather than selective pressures[62,63]. Barcoding human glioblastoma cells shows that clonal dynamics during tumor growth are consistent with neutral evolution fueled by glioblastoma stem cells[64]. The notion that tumors can evolve through neutral drift implies that non-genetic cancer cell plasticity, rather than the sole process of genetic selection driven by selective pressures and gain of fitness, contributes to tumor growth and adaptation in some cancers.

Proliferative states have been reported by single-cell transcriptomics in multiple cancer types, including mouse hepatocellular carcinoma[65] and human breast cancer[66], oligodendroglioma[67], glioblastoma[68,69] and lung cancer[70], supporting the idea that tumors present proliferative states corresponding to cells that fuel tumor growth and likely reflect CSCs.

The CSC niche

The niche describes the microenvironment that sustains renewal and restricts premature differentiation of the stem cell pool[71]. The CSC niche is composed of heterogeneous and interacting cell populations and plays a major role in tumorigenesis, being essential for CSC regulation and promoting cancer cell plasticity (Fig. 2b)[7]. Lineage tracing in human colon cancer xenografts reveals that functional colorectal CSCs that give rise to dominant clones driving tumor expansion predominantly reside at the leading edge, close to cancer-associated fibroblasts (CAFs), which produce osteopontin, a factor that drives in situ clonogenicity[72]. Similarly, osteopontin arising from the vascular niche enhances CSC phenotypes and promotes tumor growth in mouse glioma[73]. In physiological situations, stem cells or their differentiated progeny can participate in niche formation[74,75]. In cancer, some tumor subpopulations can contribute to niche formation by a Wnt-dependent mechanism[76].

The vascular niche refers to a specialized highly vascularized region composed of endothelial cells, pericytes, smooth muscle cells and immune cells, which creates a tumor-permissive microenvironment by influencing stemness, chemoresistance, invasion and metastasis[77]. Endothelial cells maintain stemness in CSCs by secreting Wnt and Notch ligands and direct cell–cell interactions, as shown in human pancreatic ductal adenocarcinoma organoids and breast cancer mouse models[78,79]. Endothelial cells also increase invasiveness and proliferation through interleukin (IL)-8 (ref. 80) and IL-6 secretion in skin squamous cell carcinoma[81] (Fig. 2b). In melanoma, the CSC pool localizes near the vasculature and endothelial cells stimulate tumor cell dedifferentiation, promoting growth through Notch 3-dependent cell–cell communication[82]. CSCs can induce vascular niche formation through vascular endothelial growth factor (VEGF) secretion, which subsequently regulates CSC renewal. VEGF secretion by CSCs promotes stemness in a cell-autonomous manner by an autocrine FLT1–NRP1 signaling loop in mouse skin cancer[83,84].

Apart from attracting and reprogramming endothelial cells during tumorigenesis, CSCs can transdifferentiate into endothelial-like cells through vascular mimicry. Low oxygen levels within the tumor might promote stemness and the acquisition of endothelial features by CSCs[85]. Human glioblastoma CSCs cultured under endothelial conditions can differentiate into endothelial cells, with a substantial proportion of them arising from tumor cell differentiation following xenotransplantation[86]. Transdifferentiation of tumor cells into endothelial cells has been shown in different human and murine cancers[87,88], but its biological relevance remains unclear. In mouse breast cancer, vascular mimicry occurs in a tumor subpopulation secreting the inhibitors Serpine2 and SLP1 independently of endothelial-mediated neovascularization and is thus resistant to classical anti-angiogenic therapy[85,89].

CAFs participate in CSC maintenance through cytokine secretion, including HGF, IGF2, TGFβ1, IL-6 and multiple CC chemokine ligands, and matrix remodeling through matrix metalloproteinase secretion and deposition of collagen and hyaluronan[90,91] (Fig. 2b). Only specific fibroblast subsets can promote tumor stemness. In patients with breast and lung cancer, a fibroblast subpopulation expressing CD10 and G protein-coupled receptor C5L2 (GPR77) promotes stemness through IL-6 and IL-8 secretion, localizes near CSCs and is characterized by sustained NF-κB pathway activation, dependent on GPR77-induced p65 phosphorylation. Anti-GPR77 treatment reduces tumor growth in patient-derived xenografts[92]. In mouse hepatocellular carcinoma, HGF secretion by myofibroblasts regulates CSC plasticity through c-Met–FRA1–HEY1 signaling[93]. Additionally, HGF promotes resistance to BRAF inhibitors in mouse and human melanoma and lung cancer[94,95]. In colon cancer, HGF-producing myofibroblasts activate Wnt, stimulate CSC features at tumor edges and promote invasion, suggesting that CSC identity is partly regulated by the microenvironment[96]. Tumor cell-intrinsic Wnt signaling can regulate fibroblast plasticity and induce a myofibroblast phenotype that promotes tumor growth and inhibits

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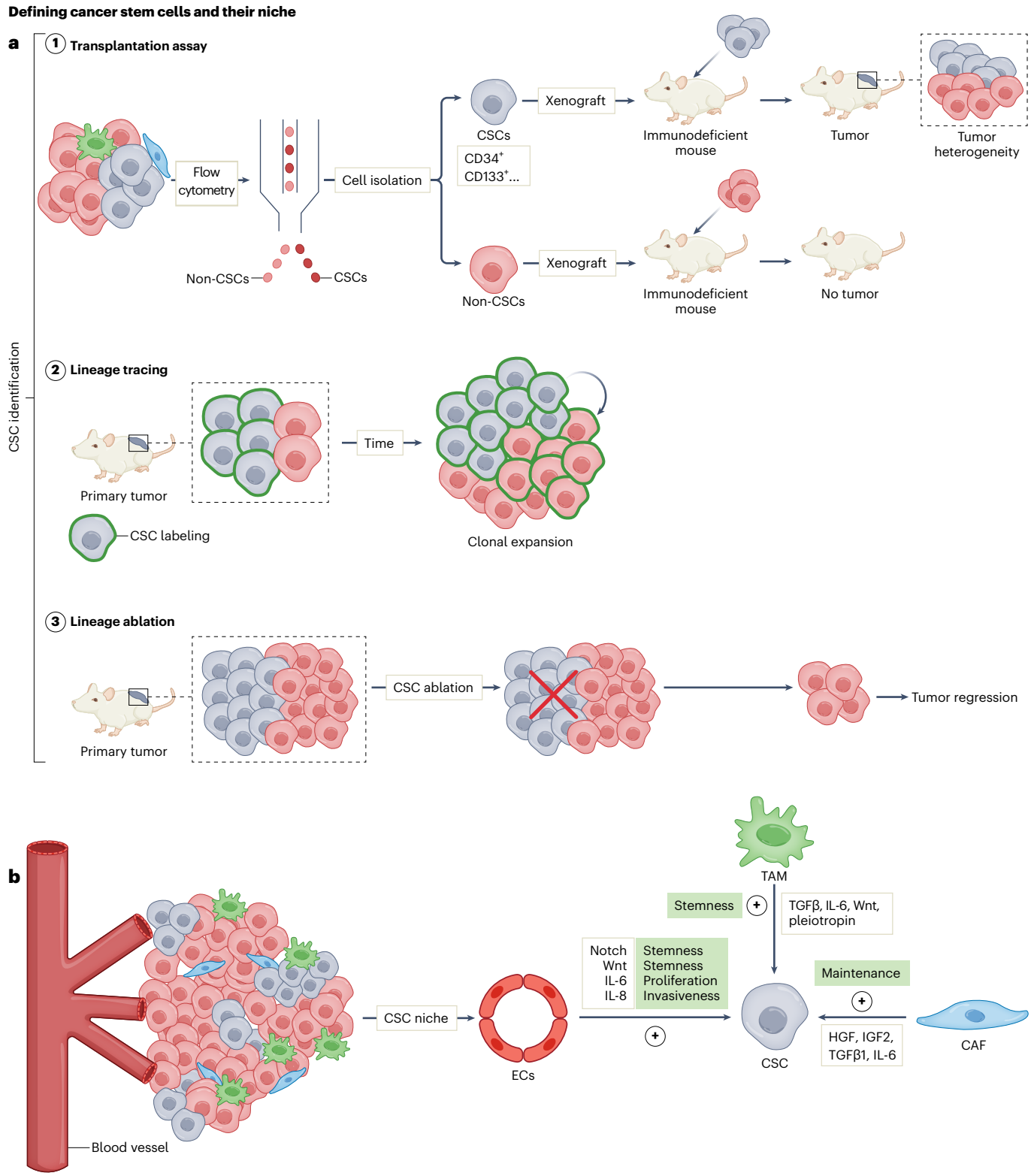

Fig. 2 | Defining CSCs and their niche. a, Functional strategies to identify CSCs include (1) transplantation assays (tumor subpopulations isolated by fluorescence-activated cell sorting are transplanted into immunodeficient mice. If CSCs are grafted, a tumor will appear and will recapitulate tumor heterogeneity, while non-CSCs will be less efficient to propagate the tumor following transplantation), (2) lineage tracing of CSCs (which allows to follow their fate during tumor progression and to assess clonal expansion) and (3) lineage ablation (which allows the elimination of a specific subpopulation. If CSCs are eliminated, the remaining subpopulations will not be able to sustain tumor growth, and tumor regression will occur). **b**, Cross-talk between CSCs and their microenvironment is essential to sustain tumor growth. CSCs are supported by a niche composed of CAFs, endothelial cells and immune cells, which extrinsically promote tumor stemness. EC, endothelial cell; TAM, tumor-associated macrophage.

EMT[97]. However, CAFs are a heterogeneous population and specific subtypes present antitumoral properties. In a murine model of metastatic colorectal cancer, myofibroblasts exert tumor-restraining functions through bone morphogenetic protein (BMP)4 secretion, which inhibits stemness in intestinal stem cells. Myofibroblast depletion results in an increased CSC pool[98]. CAF plasticity has been also suggested to occur in human solid tumors[99].

Immune cells are key components of the CSC niche[71]. Depletion of tumor-associated macrophages or inflammatory monocytes by inhibiting the myeloid cell receptors CCR2 or CSF1R decreases CSC features in pancreatic cancer[100]. CSC and macrophage communication occurs through direct interaction, as in breast cancer, where the macrophage-created CSC niche fuels EMT, inducing ephrin type A receptor 4 (EphA4) expression in CSCs, which in turn promotes cytokine secretion and sustains CSC stemness[101]. Cytokine secretion by macrophages (for example, TGFβ, IL-6, Wnt ligands and pleiotropin) promotes stemness in tumor cells, primarily through signal transducer and activator of transcription (STAT)3 signaling[102,103] (Fig. 2b).

CSC localization inside tumors is key for their functional properties. Gradients of cytokines, availability of nutrients and cell–cell interactions differ if cells are close to the tumor migration front, blood vessels or in the necrotic hypoxic tumor core. Hypoxic regions are associated with acidity and necrosis, promoting tumor aggressiveness, with hypoxia being an inducer of stemness[56] through hypoxia-induced factors 1 and 2 (HIF1 and HIF2), which are expressed in acute and long-term hypoxia, respectively[104]. Transplantation of breast cancer cell lines in a hypoxic mouse model increases the CSC population within hypoxic regions, which remains stable across serial transplantation and is maintained by the phosphoinositide 3-kinase (PI3K)–AKT pathway[105]. In human pancreatic cancer, hypoxia-mediated production of L-2-hydroxyglutarate through lactate dehydrogenase A (LDHA) activation results in histone H3 hypermethylation and increased stemness by altering transcription of differentiation genes and inducing CD133 and SRY-box transcription factor 2 (SOX2)[106].

Plasticity along the metastatic cascade

Metastasis occurs through a multistep cascade, which includes the detachment of cancer cells from the primary tumor, local invasion into the surrounding tissue, intravasation into the blood or lymphatic vessels, extravasation, colonization of a secondary organ and growth of a secondary tumor. Growing evidence indicates that only certain subpopulations of tumor cells, termed metastasis-initiating cells (MICs), are able to form metastases[107]. In contrast to tumor initiation, which is linked to mutations in cancer drivers, no metastasis-specific mutations have been identified[108,109], although certain mutations might predispose to metastasis[110,111]. MICs are highly plastic, displaying different degrees of stemness, EMT and metabolic plasticity along the entire metastatic cascade (Fig. 3).

Intrinsic regulation of cancer cell plasticity

Metastasis initiation. The importance of EMT for metastasis was first demonstrated by seminal work showing that Twist1 was essential for metastasis in breast cancer cell lines[112]. The deletion of genes encoding other EMT transcription factors also impairs metastasis, as shown with *Zeb1* deletion in pancreatic cancer models[113].

EMT can be triggered by different transcription factors, with SNAI1, SNAI2, Twist1, ZEB1 and ZEB2 being considered core EMT transcription factors that can induce the classic EMT program and are often coexpressed. Their redundancy and compensatory mechanisms might explain why the loss of one is not always sufficient to block metastasis. Nevertheless, these factors can have non-redundant functions involving stemness and survival, and, aside from these core factors, a growing number of factors can induce EMT, such as FOXC2, SOX4 and PRRX1 (ref. 113).

EMT was long considered a binary switch, but recent studies have demonstrated that EMT tumor cells present intermediate, partial or hybrid states that can transit from one to another while coexpressing epithelial and mesenchymal markers. In mouse skin squamous cell carcinoma and mammary tumors, distinct EMT subpopulations exhibit different plasticity and invasive and metastatic potential. Early hybrid EMT includes the most metastatic states, while late EMT states are the most invasive[114,115]. Early and late EMT are relatively stable in comparison to other intermediate states, which are highly plastic[116,117]. Single-cell transcriptomics has identified hybrid EMT states in mouse skin squamous cell carcinoma and mammary tumors[114] and in human nasopharyngeal carcinoma[118], glioblastoma[68], melanoma[119] and head and neck squamous cell carcinoma[120]. Hybrid EMT has been associated with poor patient outcome in 32 cancer types[121]. Partial EMT states are located at the tumor leading edge in human oral squamous cell carcinoma, suggesting an association with local invasion[120].

EMT promotes stemness, allowing MICs to give rise to secondary tumors[122–125] (Fig. 3). Lineage tracing has identified MICs within primary tumors and tracked tumor cells undergoing partial (expressing N-cadherin) and complete (expressing vimentin) EMT in mammary tumors[126,127]. N-cadherin, but not vimentin, labels MICs, supporting the notion that partial EMT is required for metastasis initiation[126,127]. An inducible CRISPR–Cas9-based lineage reporter approach combined with single-cell transcriptomics confirmed the high metastatic potential of hybrid EMT states in a pancreatic cancer mouse model[128]. In several human cancers, L1 cell-adhesion molecule (L1CAM) is expressed by MICs and enhances metastatic spreading, extravasation and outgrowth[129]. L1CAM$^+$ MICs emerge after the loss of epithelial integrity in a subset of cells mimicking the intestinal repair program[130,131].

During tumorigenesis, the metabolic phenotype of cancer cells can be modified depending on nutrient availability, proliferative rate and tumor mutational burden. The metastatic cascade imposes important adaptations for metastatic cells to overcome nutrient variations and oxidative stress[132]. MICs often present increased anaerobic glycolysis (also known as the Warburg effect)[133]. The dysregulation of oxidative phosphorylation is associated with poor prognosis and correlated with EMT in multiple cancers[134]. In human oral squamous cell carcinoma, tumor cells with low levels of mitochondrial tRNA^{Met} with the m^{5}C modification at position 34, which promotes translation of mitochondrial genes, are unable to transit from glycolysis to oxidative phosphorylation, displaying impaired metastatic capacity[135]. Lactate and pyruvate metabolism can induce signaling pathways that promote migration and invasion[136]. Moreover, a metabolic switch in the primary tumor can induce a pro-metastatic cancer cell phenotype. In breast cancer, downregulation of phosphoglycerate dehydrogenase and activation of the hexosamine–sialic acid pathway potentiates metastatic dissemination through a proliferative-to-invasive phenotypic switch[137].

Fig. 3 | Cell plasticity along the metastatic cascade. Tumor cells can acquire metastasis-initiating properties through the induction of EMT by intrinsic and extrinsic stimuli. EMT allows MICs to detach from the primary tumor, and the vascular niche facilitates MIC intravasation into the bloodstream, where single or clustered CTCs exhibit high plasticity and hybrid EMT. Interaction of CTCs with platelets and macrophages can promote plasticity, while platelet coating protects CTCs from the shredding force. The secondary organ is prepared by the primary tumor through the secretion of extracellular vesicles and soluble factors, which create a permissive microenvironment. Colonizing the metastatic site involves reversion of tumor cells to the epithelial state in response to signals coming from the metastatic niche. Following seeding, tumor cells can enter dormancy, which confers upon them immune evasion traits and resistance to therapy, or proliferate and give rise to macroscopic metastases. CDH1, cadherin 1; ECM, extracellular matrix; MSC, mesenchymal stem cell; TC, tumor cell; UPR, unfolded protein response.

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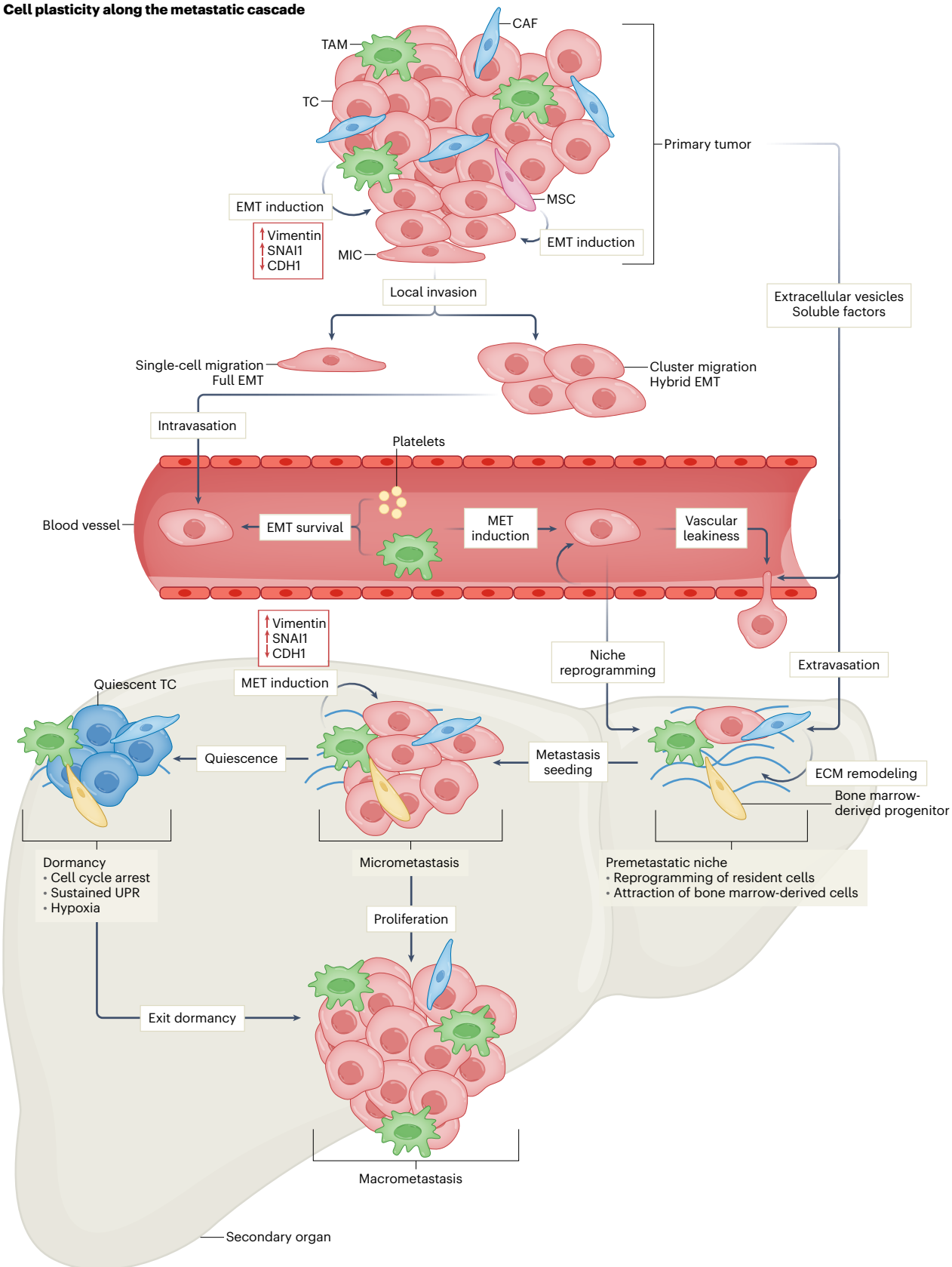
Whereas metastatic dissemination was considered a late event during tumor progression, increasing evidence suggests that it can occur relatively early during tumorigenesis[138]. In a breast cancer mouse model, metastatic spread occurs at the early stage of tumor formation, driven by progesterone and human epidermal growth factor receptor 2 (HER2) signaling. First, progesterone signaling promotes migration and dissemination, and, at later stages, increased cell density down-regulates the progesterone receptor, switching migration toward



proliferation[139]. Cell plasticity regulated by the transcription factor ZP281 induces a mesenchymal-like state that promotes early dissemination and dormancy in early metastatic lesions by preventing the switch to an epithelial-like proliferative state[140].

Local invasion and dissemination of tumor cells. Tumor cells in a full EMT state invade their surrounding tissue as mesenchymal single cells, whereas hybrid EMT states promote collective migration, with tumor cells at the leading edge presenting a more pronounced EMT phenotype than that of follower cells[141] (Fig. 3). Hybrid EMT cells migrating collectively are associated with plasticity, stemness, invasion and increased metastatic ability[114,127]. Next, tumor cells intravasate blood vessels as circulating tumor cells (CTCs), with some of these surviving to extravasate into a secondary organ, in which they will either proliferate to enable metastatic outgrowth or undergo dormancy[142] (Fig. 3). Xenografts revealed MIC markers among human luminal breast cancer CTCs that give rise to bone, lung and liver metastases. MIC-containing CTC subpopulations express EpCAM, CD44, CD47 and Met[143].

Whereas most CTCs are single cells in circulation, a less-prevalent fraction is shed and travels in clusters, showing increased metastatic potential and associating with poor outcomes[144–146]. Both single and clustered CTCs exhibit shifts in epithelial and mesenchymal marker expression, displaying plasticity during tumor progression. Whereas epithelial cells that lose adhesion-dependent survival signals and intravasate into blood vessels normally undergo anoikis, EMT enables single tumor cells to change their fate toward a mesenchymal phenotype, in which adherence-independent survival signals prevent cell death[144,147]. Rare primary tumor cells simultaneously express mesenchymal and epithelial markers, whereas CTC clusters in patients with breast cancer are positive for mesenchymal markers and weakly positive for epithelial markers, supporting a role of EMT in CTC dissemination[148]. CTCs detected in the blood of mice with skin squamous cell carcinoma are $EpCAM^-$ and enriched in hybrid EMT states, demonstrating that cells with hybrid phenotypes exhibit increased colonization potential and intravasate more efficiently[114,149]. Hybrid EMT has been detected in CTCs from patients with non-small cell lung cancer[150] and prostate[151], colorectal[152], pancreatic[153], breast, liver, gastric and nasopharyngeal cancers[115]. The sodium channel NALCN regulates CTC dissemination, with its loss of function in a mouse model increasing the proportion of CTCs and blood trafficking of normal unmutated cells[154].

Plasticity within distinct CTC phenotypes has been shown to contribute to cancer progression and chemoresistance. Analysis of CTCs from women with ER^+HER2^- breast tumors reveals that 84% of CTCs acquire HER2 expression without genetic amplification. Cultured $HER2^+$ and $HER2^-$ CTCs interconvert spontaneously, with oxidative stress and chemotherapy enhancing transition toward the $HER2^-$ phenotype, whereas the $HER2^+$ state is the most proliferative[155]. While in circulation, the oxidative stress of CTCs increases, and, to prevent reactive oxygen species (ROS)-mediated cell death, tumor cells increase antioxidant production[156]. In melanoma patient-derived xenografts and mouse models, metastatic cells increasingly depend on nicotinamide adenine dinucleotide phosphate (NADPH)-generating enzymes from the folate pathway to regenerate glutathione and withstand oxidative stress[157]. Efficiently, metastatic cells increase lactate uptake through upregulation of monocarboxylate transporter 1 (MCT1), preventing oxidative stress[158]. Metabolic changes depend on the path by which tumor cells reach the secondary organ. In melanoma, CTCs migrating through blood vessels are subjected to higher oxidative stress and ferroptosis than CTCs in lymphatic vessels and become dependent on the ferroptosis inhibitor GPX4 to survive, whereas CTCs migrating through lymphatic vessels rely on antioxidant-like oleic acid and glutathione[159]. CTC clustering protects from ROS production through HIF1α induction and mitophagy, switching energy production toward glycolysis. Blocking metabolic rewiring following CTC clustering inhibits metastasis[160].

Metastatic colonization. EMT reversion by mesenchymal-to-epithelial transition (MET) can promote metastasis (Fig. 3). Loss of E-cadherin increases invasiveness, but its expression protects cells from oxidative stress during dissemination and seeding, promoting metastatic colonization[161]. Tumor cells can form heterotypic junctions using E-cadherin and N-cadherin expressed by stromal cells in the metastatic niche, promoting survival and growth[162]. Some MICs display hybrid EMT, maintaining E-cadherin expression and mesenchymal traits[163].

Whereas metastasis is associated with EMT in mouse skin squamous cell carcinoma, most metastases do not display EMT features, suggesting that MET can be important for colonization[149]. Evidence shows that metastases can reacquire an epithelial phenotype, but whether this is a cause or consequence of the metastatic cascade remains unknown[164]. Several studies highlight the need of downregulating EMT factors for metastasis formation. Twist1-mediated EMT in squamous cell carcinoma promotes invasion and CTC circulation, whereas Twist1 downregulation promotes metastatic colonization[165]. PRRX1 promotes EMT and invasion in pancreatic ductal adenocarcinoma but needs to be repressed for metastatic colonization[166]. The action of PRRX1 was later shown to be mediated by two distinct isoforms: PRRX1b promoting EMT, invasion and migration and PRRX1a stimulating liver metastatic outgrowth, tumor differentiation and MET. Thus, metastatic dissemination needs a switch from PRRX1b at the first step of the metastatic cascade to PRRX1a at its end[167].

MICs can arise from CSCs or be generated by the dedifferentiation of non-CSCs. In mouse models of colorectal cancer, disseminated cells do not express the stem cell marker Lgr5. However, a fraction of the disseminated cells re-express *Lgr5* during macro-metastasis formation[168], which explains why $Lgr5^+$ lineage ablation inhibits liver metastasis formation in colorectal cancer[58]. Recently, metastatic recurrence in colorectal cancer has been shown to arise from residual epithelial membrane protein 1 (*Emp1*)-expressing cells, a subset of $Lgr5^-$ tumor cells endowed with migratory properties. The ablation of $Emp1^+$ cells in vivo during primary colorectal cancer growth prevents metastatic dissemination, whereas ablation after primary tumor resection does not affect metastatic progression. Therefore, $Emp1^+$ cells can be considered the cell of origin of metastasis in colorectal cancer, whereas the $Lgr5^+$ stem cell and proliferation programs are necessary for metastatic outgrowth, demonstrating the importance of cell plasticity in metastasis formation[169]. Additionally, the organotropism of metastatic cells is partially dictated by the conjunction of their metabolic needs and the nutrients available in the secondary organ. Metastatic breast cancer cells preferentially metastasize to the lung because they use local pyruvate to boost collagen hydroxylation, leading to the establishment of a metastatic niche[170].

Extrinsic regulation of cancer cell plasticity

Metastasis initiation and the tumor niche. The niche is crucial for EMT induction and metastasis initiation (Fig. 3). Fibroblasts support tumor cells by secreting extracellular matrix and matrix metalloproteinases, promoting migration, invasion and angiogenesis and favoring tumor cell plasticity. TGFβ secretion by tumor cells is essential for fibroblast recruitment and activation during the first steps of tumorigenesis. Activated fibroblasts then activate autocrine and paracrine secretion of TGFβ, inducing EMT in tumor cells and promoting immune escape[171,172] (Fig. 4). Co-transplantation experiments of CSCs and fibroblasts with high TGFβ expression show increased lung metastasis in a TGFβ-dependent manner in squamous cell carcinoma[173]. Fibroblasts can indirectly induce EMT by promoting increased extracellular matrix stiffness, leading to mechanotransduction signals[174,175] (Fig. 4).

The abundance of blood vessels within the vascular niche of the primary tumor increases the bloodstream accessibility of tumor cells. Stromal and tumor cells secrete cytokines and chemokines to recruit immunosuppressive and pro-tumoral macrophages and

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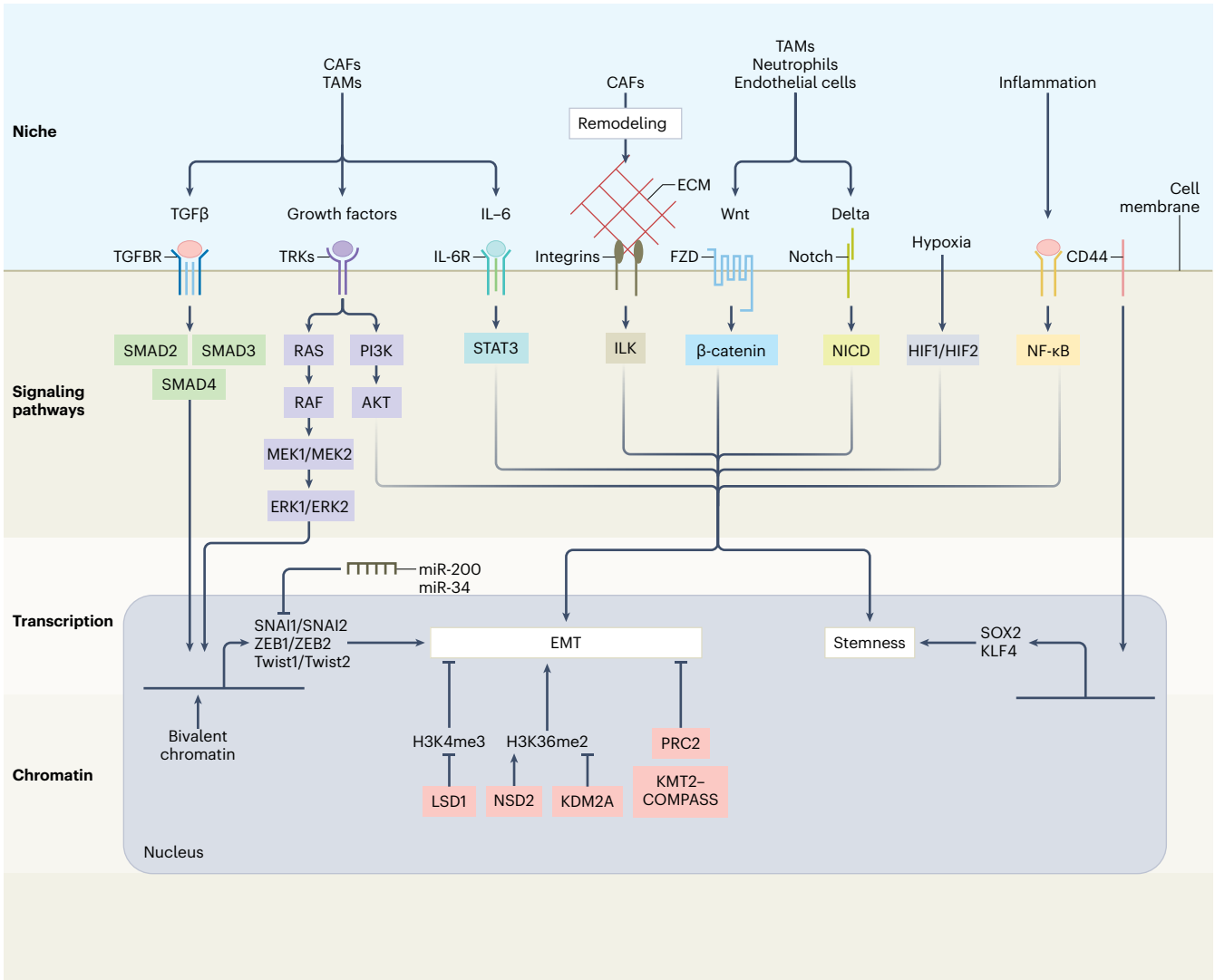

Fig. 4 | Molecular mechanisms regulating cancer cell plasticity. Cancer cell plasticity is regulated extracellularly, by signals coming from the microenvironment, and intrinsically, through signaling pathways, transcriptional programs and chromatin remodeling. TGFβ and RAS–MAPK pathways can act jointly to induce EMT. CD44 and Wnt regulate stemness, while Notch, JAK–STAT and integrins act on stemness and EMT in a context-dependent manner. Hypoxia induces stemness, while NF-κB is involved in plasticity by its role in inflammation. These pathways activate transcriptional programs regulated by key transcription factors involved in EMT (for example, SNAI1, SNAI2, ZEB1, ZEB2, Twist1, Twist2) and stemness (for example, SOX2, KLF4). Their action can be modulated by negative feedback loops involving microRNA (for example, ZEB–miR-200 and SNAI1–miR-34) and depends on the chromatin landscape. LSD1 can remove the transcriptionally active H3K4 trimethylation (H3K4me3) histone mark and collaborate with SNAI1 to silence epithelial genes. Nuclear receptor-binding SET domain protein 2 (NSD2) and lysine demethylase 2A (KDM2A) exhibit antagonist actions, as writer and eraser of histone 3 lysine 36 dimethylation (H3K36me2), a histone mark increased during EMT. Polycomb repressive complex 2 (PRC2) and type 2 lysine methyltransferase (KMT2)–complex of proteins associated with Set1 (COMPASS) are critical to regulate the epithelial state. FZD, frizzled; IL-6R, IL-6 receptor; ILK, integrin-linked kinase; NICD, Notch intracellular domain; TGFBR, transforming growth factor receptor; TRK, tyrosine receptor kinase.

tumor-associated neutrophils that promote invasiveness by secreting EGF and modulating the extracellular matrix through cathepsins and matrix metalloproteinase 9 and can increase MIC survival[176] (Fig. 3). Mesenchymal stem-like cells in tumor niches arise from the bone marrow and other perivascular regions (for example, adipose tissue) and interact with tumor and stromal cells to promote vascularization, immune modulation and extracellular matrix remodeling[177]. They can induce EMT through exosome communication, TGFβ secretion and extracellular matrix remodeling, especially through hyaluronan secretion, activating CD44 and upregulating lysyl oxidase and Twist1 in breast cancer cells[178,179] (Fig. 3). Macrophages also influence EMT and tumor cell plasticity. In glioblastoma, macrophages induce EMT through oncostatin M secretion, activating the STAT3 pathway in tumor cells[180] (Fig. 4). In both mouse and human non-small cell lung cancer, resident macrophages promote EMT and invasion during early metastatic dissemination and protect tumor cells from immune destruction by inducing a regulatory T cell response[181] (Fig. 3). In skin cancer, macrophage infiltration increases in hybrid or full EMT tumor areas, as compared to epithelial regions. Macrophage depletion increases epithelial states and decreases EMT, showing the importance of macrophage–tumor cell communication in regulating EMT[114].

Dissemination of tumor cells and cross-talk with the tumor microenvironment. Tumor cells survive in the bloodstream by being coated with platelets and interacting with white blood cells, fibroblasts,

macrophages and endothelial cells[147]. Cross-talk between tumor cells and macrophages is required for CTC-mediated colorectal cancer metastasis and promotes EMT-related plasticity[182] (Fig. 3). Neutrophil–tumor cell clusters seem to be more metastatic than tumor cell clusters alone, due to increased neutrophil-mediated cell cycle progression in tumor cells[183]. Interaction with platelets provides resistance to the bloodstream shredding force and induces EMT through TGFβ and NF-κB pathway activation[184] (Fig. 4).

Metastatic niche. The metastatic niche is the specific microenvironment generated by stromal cells, the extracellular matrix and diffusing signals that stimulate metastasis formation. Perivascular niches create excellent metastatic niches. Although cross-talk between the metastatic perivascular niche and tumor cells is not fully understood, several mechanisms have been identified. In breast-to-lung cancer metastasis, tumor cells secrete tenascin C, which activates macrophages through Toll-like receptor 4. Macrophages activate endothelial cells through tumor necrosis factor-α and nitric oxide secretion, supporting metastasis formation[185]. Therapy might favor metastatic niche formation. Lung radiotherapy can create a pro-metastatic microenvironment through neutrophil activation, which then activates Notch signaling, inducing tumor stemness and enhancing metastasis[186] (Fig. 4). The metastatic niche promotes metastatic outgrowth but can favor further dissemination. For instance, the bone microenvironment promotes multi-organ metastases through epigenetic reprogramming of tumor cells, mediated by enhanced activity of the methyltransferase EZH2, promoting disseminated tumor cell stemness in the bone[187].

The mechanisms of MET induction in MICs are not fully understood but involve signals from the metastatic niche. E-selectin secretion in the metastatic niche induces a specific form of MET in the bone through Wnt pathway activation[188]. Secretion of the cytokine LIF by bone mesenchymal stem cells induces MET through the activation of LIF receptor, the kinase ERK and STAT3 in early disseminated CSCs[189]. In liver metastasis from colon cancer, MET can be induced through inhibition of the Src kinase and epidermal growth factor receptor (EGFR) pathways[190]. In lung metastasis, versican secretion by bone marrow-derived myeloid progenitors recruited to the lung inhibits SMAD2 phosphorylation and SNAI1 expression in MICs, resulting in MET and increased proliferation[191]. In breast cancer-derived lung metastasis, MET can be induced by fibroblasts through TGFβ pathway inhibition and BMP activation[192] (Fig. 3). Fibroblast activation occurs through MIC-secreted thrombospondin 2, which depends on MIC mesenchymal features, showing that MET is not required in the first step of colonization but needs to be induced through microenvironment reprogramming[192]. MET induction can occur through protein kinase A activation in human breast cancer but blocks tumor-initiating properties and decreases metastasis by promoting differentiation[193].

Increasing evidence suggests that tumor cells prepare their niche before colonization. Premetastatic niche conditioning involves vascular leakiness, reprogramming of resident cells and attraction of bone marrow-derived cells[194] (Fig. 3). Some mechanisms are induced by disseminated cells at the metastatic site, but distant reprogramming by the primary tumor through secretion of soluble molecules and exosomes also occurs. MiR-25-3p-containing exosomes secreted by colorectal cancer can induce angiogenesis and vascular leakiness through inhibition of the transcription factors KLF2 and KLF4 in endothelial cells. In vivo treatment with these exosomes leads to increased vascular permeability in lung and liver, whereas depleting miR-25-3p reduces metastasis in both organs[195]. A phenotypic switch in pericytes and vascular smooth muscle cells of the premetastatic niche toward a more undifferentiated state is mediated by increased KLF4 expression due to tumor-derived factors and exosomes. Reprogrammed perivascular cells exhibit increased proliferation and expression of extracellular matrix components, creating a permissive soil for metastasis[196].

Tumor dormancy

Disseminated cells can enter dormancy at the metastatic site (Fig. 3). This growth arrest occurs by a balance between proliferation and apoptosis due to poor vascularization, immune destruction, lack of nutrients and growth factors or through inhibitory signals from the microenvironment (for example, TGFβ)[197–199]. Dormant cells are characterized by activated survival pathways, cell cycle arrest and sustained unfolded protein response and hypoxia[200] (Fig. 3). Quiescence allows cells to evade immune responses and chemotherapy, remaining undetectable by imaging techniques but being responsible for relapse even years after clinical remission[200].

Mechanisms by which tumor cells enter and exit dormancy are not fully understood (Fig. 3). Dormant cells display plasticity to transit between states, but whether EMT or MET promote reactivation and awakening from dormancy remains unclear. EMT induced by inflammation in a ZEB1-dependent manner awakens dormant tumor cells in xenografting experiments[124,201]. However, in breast cancer, TGFβ exhibits cytostatic effects, impairs the cell cycle and promotes dormancy, whereas the TGFβ antagonist Coco promotes reactivation of dormant cells in the lung[199,202]. Additionally, mesenchymal CSCs need to undergo MET and express E-cadherin to enable contact between tumor cells and promote survival and proliferation[203].

Dormancy is tightly controlled by the microenvironment. Secretion of collagen III by tumor cells at the metastatic site favors dormancy, whereas disruption of the collagen III-enriched matrix induces awakening and proliferation of dormant cells through discoidin domain receptor tyrosine kinase 1-mediated STAT1 signaling[204]. In the lung, inflammation induces the formation of neutrophil extracellular traps, which favor the awakening of tumor cells through laminin cleavage and integrin $\alpha_3\beta_1$ activation[205]. Cancer cells can be primed by the primary tumor to become dormant. In breast cancer and head and neck squamous cell carcinoma, tumor cells exposed to hypoxia are prone to becoming dormant[206]. Modifications of the microenvironment during aging also play a role in entering or exiting dormancy. Age-related changes in fibroblasts have been linked to increased metastasis in melanoma. Aged dermal fibroblasts show increased secretion of the Wnt antagonist sFRP2, which induces resistance to the ROS-mediated DNA damage response in melanoma cells, conferring resistance to therapy and increased metastasis. Aged fibroblasts in the lung secrete more sFRP1 and block Wnt5a-mediated induction of dormancy, stimulating metastatic growth[207,208]. Age-related changes affecting the microenvironment might explain the resurgence of metastatic lesions years after treatment.

Fig. 5 | Genetically induced drug resistance and non-genetic drug tolerance in anti-cancer therapy. a,**b**, Pre-existing (**a**) or acquired (**b**) mutations can confer intrinsic genetic drug resistance, by which mutated tumor cells can display a clonal selection, survive and proliferate under a particular therapeutic regimen. **c**, Non-genetic drug tolerance can occur through transcriptional selection of primed cells that acquire a DTP dormant state during therapy and can lead to tumor relapse after therapy. **d**, Non-genetic drug tolerance can occur through an adaptation to the therapeutic pressure, by which plastic tumor cells acquire a DTP quiescent state following therapy and can lead to tumor relapse after therapy. **e**, Targeting the signaling pathways activated in the DTP state enables its eradication. The DTP state induced upon treatment with inhibitors of BRAF and MEK (BRAFi, MEKi) in melanoma relies on FAK signaling, and the transcriptional program of this state is largely driven by the nuclear receptor RXR. Consistently, the DTP state can be targeted by FAK inhibition and RXR antagonism (FAKi, RXRi). However, de novo mutations could still lead to genetic resistance and tumor relapse[221,222]. RAR, retinoic acid receptor; SC, stem cell.

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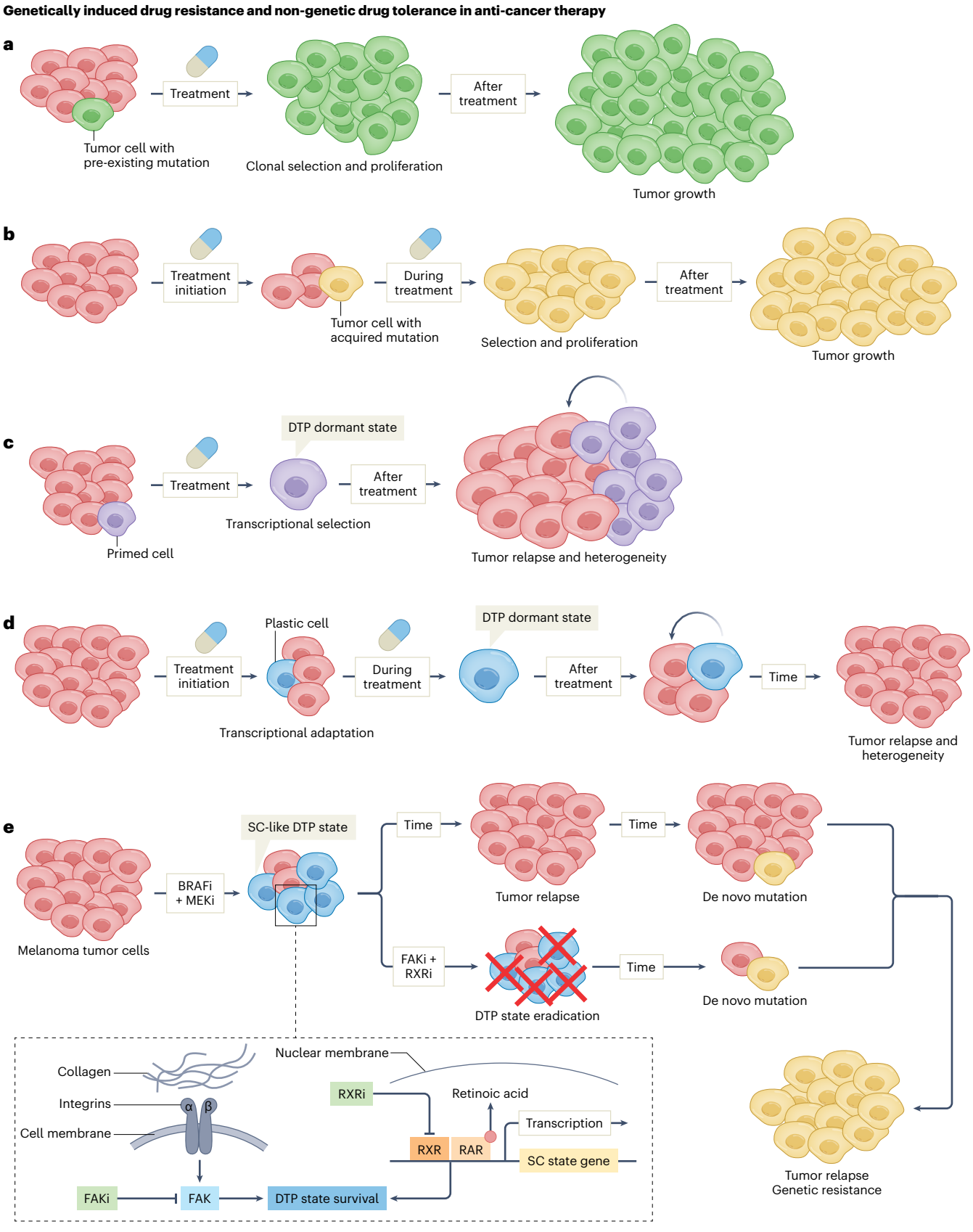
Cell plasticity and cancer therapy

Drug tolerance constitutes a major obstacle for therapy. In the following section, we discuss the roles of plasticity in therapy resistance.

Drug-tolerance mechanisms

Although therapeutic resistance was thought to be exclusively a consequence of genetic alterations in tumor cells (Fig. 5a,b), accumulating evidence suggests that drug-tolerant states exist in the absence of



mutations. Drug-tolerant persistent (DTP) cells display four hallmarks: slow proliferation, metabolic flexibility, adaptation to the microenvironment and phenotypic plasticity. The major difference between mutations conferring resistance and DTP states is the absence of reversibility or plasticity in mutations, whereas DTP cells survive but do not proliferate under treatment and their progeny remains sensitive to treatment after drug withdrawal[209,210].

Primed DTP cells might exist before treatment, with expression of a particular transcriptional program providing them with intrinsic tolerance to a drug and leading to their selection under treatment (Fig. 5c). In other cases, DTP cells become induced upon treatment, as tumor cells adapt to therapeutic pressures and activate a transcriptional program that provides a selective advantage to escape[209,210] (Fig. 5d). The acquired DTP state exploits plasticity, as tumor cells undergo a phenotypic switch and adopt a reversible quiescent state to survive. The DTP state can manifest as transient or stable. Transient DTP cells regenerate the initial tumor heterogeneity after drug withdrawal, with the tumor remaining sensitive to therapy. By contrast, in a stable tolerance situation, the tumor adapts to therapy, becoming insensitive to it. The therapy-evasive traits of DTP cells are mediated by epigenetic, transcriptional and translational regulatory processes and complex interactions between tumor cells and within their microenvironment[10,209,210]. Tumor cells employ a developmentally conserved mechanism similar to diapause to drive the DTP state, as observed in organoids, patient-derived xenografts and patient samples[211,212].

EMT promotes drug-tolerant states, and EMT tumor cells are highly resistant to anti-cancer therapy[209]. A recent study has demonstrated that RhoJ, a small GTPase, controls the resistance of EMT tumor cells to a wide range of chemotherapeutic agents by promoting DNA repair through the regulation of nuclear actin[213]. Primed DTP cells have been described in melanoma and breast cancer. In vitro studies in *BRAF*-mutant melanoma identify a DTP state upon BRAF inhibition that arises through a multistep process[214]. Before therapy, rare subpopulations display a transient primed state with high expression of resistance markers (for example, EGFR), with this state becoming stable through epigenetic reprogramming following treatment. Genetic factors such as those encoded by *SOX10* and *MITF* affect fate decisions, revealing a plasticity model of resistance to BRAF inhibition that pushes cells toward differentiation[214,215]. Single-cell sequencing of triple-negative breast cancers treated with chemotherapy shows resistant genotypes to be pre-existing but also reveals the existence of a small fraction of primed DTPs, whereas chemotherapy induces an acquired DTP state through transcriptional reprogramming[216].

Emerging evidence indicates that tolerance can be acquired by switching to a phenotypically distinct DTP state. In prostate cancer, DTP cell plasticity is promoted by combined loss-of-function mutations in *TP53*, *RB1* or *PTEN*[39]. Both mouse and human models demonstrate that tumors develop resistance to androgen-deprivation therapy with enzalutamide by a phenotypic shift from androgen receptor-dependent luminal epithelial cells to androgen receptor-independent basal-like cells, enabled by the loss of TP53 and RB1 functions and mediated by increased *SOX2* and *EZH2* expression[39,217]. Single-cell transcriptomics of patient samples with prostate cancer reveals that resistant adenocarcinoma cells upregulate EMT and TGFβ signaling gene programs, whereas small cell carcinoma exhibits higher activity of proteins encoded by *NANOG*, *SOX2* and *EZH2* (ref. 218). Mouse and human organoids and genetically engineered mouse models of prostate cancer show the emergence of a DTP state in an epithelial population by JAK–STAT signaling following androgen receptor inhibition[219,220].

In *BRAF*-mutant melanoma patient-derived xenografts, dedifferentiation into a reversible neural crest stem cell-like state driven by retinoid X receptor (RXR) γ (RXRG) and FAK signaling contributes to the development of resistance to inhibitors of the kinases RAF and MEK[221,222] (Fig. 5e). In basal cell carcinoma, Hedgehog pathway inhibition by vismodegib leads to differentiation toward squamous and sebaceous identities, but some tumor cells enter a quiescent *Lgr5*-expressing state characterized by Wnt signaling[223,224]. In patients with resistant non-small cell lung cancer with *EGFR* mutations, transformation to small cell lung cancer is observed histologically following EGFR inhibition. DTP cells present retinoblastoma protein loss and transdifferentiate into a different epigenetic state that does not require EGFR signaling[225]. Single-cell transcriptomics of non-small cell lung cancer patient biopsies before and after targeted therapy reveals the existence of a slowly proliferating population with alveolar traits[226]. Induction of a slow-cycling DTP state seems to be a common survival mechanism. Despite most cells remaining quiescent, recent work in lung cancer reveals DTP lineages that can maintain their proliferative capacity in the presence of drugs[227].

Epigenetic reprogramming mechanisms also drive DTP state plasticity in vitro and in vivo. A DTP state maintained by an altered chromatin state that requires the histone demethylase KDM5A (JARID1) was identified in *EGFR*-mutant non-small cell lung cancer following tyrosine kinase-inhibitor treatment[228,229]. Upon receptor tyrosine kinase inhibition, glioblastoma stem cells transit to a DTP state characterized by upregulation of neurodevelopmental programs, dependency on Notch signaling, redistribution of repressive histone methylation and dependency on histone demethylases KDM6A and KDM6B[230]. In breast basal-like cancer, the DTP state upon treatment with MEK and/or PI3K–mammalian target of rapamycin (mTOR) inhibitors is EMT related and driven by changes in bromodomain-containing protein 4 (BRD4), the lysine demethylase KDM5B and EZH2 (ref. 231). Following γ-secretase inhibition in T cell acute lymphoblastic leukemia, pre-existing DTP cells adopt an altered chromatin state and are dependent on BRD4 (ref. 232).

The importance of EMT in therapy resistance has been shown in different contexts[6,113]. Snail determines the response to mTOR kinase inhibitors by transcriptional repression of the repressor 4E-BP1 in human breast, colon and lung cancer cell lines[233]. A mesenchymal undifferentiated DTP state that often expresses ZEB1 and depends on a druggable lipid peroxidase pathway that protects against ferroptosis has been observed in human tumors and cell lines under multiple treatment modalities across cancer lineages[234].

Wnt signaling is the major oncogenic driver of colorectal cancer. Whereas, in most cases, constitutive activation is mediated by mutations in downstream pathway components, such as APC or β-catenin, a fraction of colorectal cancers is mediated by a fusion protein between the Wnt co-receptors RSPO3 and PTPRK[235], which renders tumor cells sensitive to Wnt signaling inhibition. A blocking antibody against RSPO3 inhibits tumor growth and induces the switch from a stemness state toward a differentiated state[236]. YAP signaling can promote Wnt independence in these tumors by lineage reversion to a fetal-like state[237]. In colorectal cancer patient-derived xenografts, minimal residual disease following EGFR blockade is associated with acquisition of a DTP state that displays a Paneth cell-like phenotype characterized by high Wnt signaling and regulated by YAP inactivation[238]. Colorectal cancer patient-derived organoids show that chemotherapy induces quiescence in *TP53*-wild-type tumor cells, linked to acquisition of the fetal-like state, with the RNA-binding protein MEX3A marking a latent $Lgr5^+$ DTP state, which persists by downregulating Wnt after chemotherapy and adopts a transient state reminiscent of YAP^+ intestinal progenitors[239,240]. $Lgr5^+$ CSCs that display a dormant behavior express p27 (encoded by *CDKN1B*). $Lgr5^+p27^+$ cells wake from dormancy through focal adhesion kinase (FAK)–YAP activation[241].

Elimination of drug-tolerant cells

Multiple plasticity mechanisms can promote DTP state acquisition. Although some mechanisms could be specific to tumors, altering cell fate decisions by targeting hallmarks of DTP cells across cancers, including slow proliferation, signaling pathway activation, adapted metabolism and microenvironment regulators, could help eliminate minimal residual disease and avoid relapse[209,210].

A first approach to eradicate DTP cells relies on targeting their slow proliferation by incorporating epigenetic modulators into existing therapies. Disrupting the repressed chromatin state that maintains resistance to EGFR tyrosine kinase-inhibitors in non-small cell lung cancer by histone deacetylase (HDAC) inhibition or by IGF1 receptor inhibition is lethal to DTP cells in vitro[228,229]. Several clinical studies examined the combination of an HDAC inhibitor with a tyrosine kinase-inhibitor, which appears to be well tolerated and presents clinical benefits in non-small cell lung cancer progression (NCT01302808)[242]. Similarly, co-treatment with the PI3K–mTOR inhibitor BEZ235 and the bromodomain and extra-terminal domain (BET)–BRD4 inhibitor JQ1 in basal-like breast cancer prevents chromatin remodeling, inhibiting acquisition of the DTP state and resulting in cell death in vitro and xenograft regression in vivo[231]. JQ1 induces DTP cell apoptosis in vitro in T cell acute lymphoblastic leukemia following γ-secretase inhibition, whereas combined therapy with JQ1 is effective in vivo[232].

Targeting signaling pathways activated in tumor cells could eliminate DTP cells. The stem cell-like state acquired following RAF–MEK inhibition in melanoma can be targeted by a combination of FAK inhibition and RXR antagonism[221,222]. Although eliminating the DTP subpopulation is sufficient to avoid non-genetic tolerance, resistance can occur through the acquisition of de novo mutations[221,222] (Fig. 5e). In basal cell carcinoma, targeting the Wnt and Hedgehog pathways together leads to DTP state eradication in vivo[223,224]. Inhibition of JAK–STAT signaling in mouse and human prostate organoids re-sensitizes tumors to androgen receptor-targeted therapy[219]. Targeting YAP–TAZ might prevent or reverse Wnt-inhibitor resistance in intestinal cancer and eliminate quiescent cells in colorectal cancer[237,239,241]. TGFβ inhibition increases squamous cell carcinoma susceptibility to chemotherapy, preventing entry into a quiescent state[243]. Blocking TGFβ signaling reduces stemness and attenuates metastasis upon chemotherapy in breast cancer[244]. In EMT cells, the DTP state depends on GPX4, the loss of which results in ferroptotic death in vitro and prevents relapse in vivo[234,245].

Targeting microenvironment regulators could contribute to eliminating DTP cells. The microenvironment elicits innate resistance to RAF inhibitors through the expression of HGF, while dual inhibition of BRAF and the HGF receptor Met prevents drug resistance in *BRAF*-mutant melanoma[246]. Chemotherapy induces c-Jun N-terminal kinase (JNK) pathway activation in patients with breast cancer, enhancing expression of extracellular matrix and stem cell niche components osteopontin, SPP1 and TNC and conferring chemoresistance. JNK or SPP1 inhibition sensitizes mouse tumors and metastases to chemotherapy[247]. Inflammatory fibroblasts control the response to therapy in rectal cancer[248]. IL-1-dependent signaling elevates DNA damage in inflammatory fibroblasts, promoting senescence and resulting in therapy resistance, which could be overcome by IL-1 receptor (IL-1R) inhibition, leading to a clinical trial testing the combination of chemoradiotherapy with an IL-1R antagonist in rectal cancer (NCT04942626)[248].

The highly dynamic, heterogeneous and plastic properties of the DTP state are a major challenge. Transcriptional profiling by single-cell sequencing to measure phenotypic changes along clinical evolution could enable individualized therapies to overcome drug tolerance.

Targeting cell plasticity

Strategies to inhibit CSC self-renewing capacities or to promote their differentiation can lead to CSC exhaustion and tumor regression. Anti-CSC therapy was first shown for acute promyelocytic leukemia, with all-*trans* retinoic acid promoting leukemic cell differentiation into terminally differentiated myeloid cells[249]. Today, the combination of retinoic acid, arsenic trioxide and/or chemotherapy cures more than 90% of patients with this type of leukemia[249].

Lysine demethylase 1A (LSD1) is required to sustain the tumorigenic program of CSCs in several cancer types and is important for maintaining plasticity and proliferation in Merkel cell carcinoma in vivo[250]. Histone 3 lysine 4 (H3K4) methylation is required for retinoic acid-driven differentiation, but this methylation mark is lost in acute myeloid leukemia due to LSD1 overexpression. A phase I trial (NCT02273102) recently demonstrated that responsiveness to retinoic acid can be potentiated by LSD1 inhibition[251]. Epigenetic therapy also relies on HDAC and JAK–STAT inhibitors. The JAK1–JAK2 inhibitor ruxolitinib and the HDAC inhibitor belinostat independently enhance dependence on B cell lymphoma 2 (BCL-2) for survival, sensitizing leukemic cells to the BCL-2 inhibitor venetoclax[252]. Other epigenetic drugs include DNA methyltransferase inhibitors (for example, azacitidine and decitabine, approved for myelodysplastic syndromes) and EZH2 and BET inhibitors, which are being tested in clinical studies of hematologic malignancies[253]. A better understanding of sensitive tumor cells and the effect of epigenetic inhibitors on normal cells would improve the rationale of using epigenetic therapy to target plasticity and avoid toxic side effects.

Markers defining the stemness tumor state have been considered unlikely candidates for antibody therapy, as they are expressed by healthy stem cells. Accordingly, an antibody–drug conjugate directed against $CD33^+$ CSCs in acute myeloid leukemia received approval of the Food and Drug Administration but was withdrawn due to toxicity[54]. A bivalent antibody against EGFR and LGR5 inhibits EGFR in CSCs, suppressing tumor growth in epithelial tumors and blocking metastasis initiation[254].

An alternative approach relies on inhibiting CSC signaling pathways. In preclinical glioblastoma studies, combined therapy with a Notch–γ-secretase inhibitor, radiotherapy and temozolomide reduces stemness markers and tumor growth while prolonging survival[255]. Notch inhibition has been assessed in clinical trials for more malignancies, such as breast and lung cancer, failing to meet expectations due to dose-limiting gastrointestinal toxicity[256,257]. Most signaling pathways involved in plasticity are key developmental pathways, the targeting of which commonly leads to off-tumor toxicities because of effects on normal cells. Resistance to therapy targeting CSCs due to plasticity of non-CSCs, which can replenish the CSC pool, limits its efficacy[54,258]. Combined treatment with molecules preventing plasticity of non-CSCs would be required for successful clinical outcomes. Dormancy remains a major challenge for therapy, and awakening this subpopulation to increase its susceptibility to chemotherapy (for example, by activating the interferon α pathway) is being considered[259]. Maintaining the quiescent state to prevent metastatic outgrowth is an alternative, although it would require lifelong treatment.

Intratumor heterogeneity and cell plasticity also pose persisting challenges. Impairing plasticity as a therapeutic approach to limit the degree of heterogeneity and restrain the capacity of tumor cells to resist therapy seems promising, as blocking the mechanisms inducing plasticity in DTP cells might lead to therapeutic benefits. However, these mechanisms might differ among tumors and multiple adaptation mechanisms may act redundantly to sustain the DTP state. Further efforts would be needed to develop clinically relevant treatments targeting plasticity in solid cancers[260].

As tumor cell plasticity is often mediated by the microenvironment, targeting it to sensitize tumor cells might be a promising therapeutic approach. Wnt16B could become an attractive target for increasing responsiveness to chemotherapy in prostate cancer, as Wnt16B expression in the microenvironment attenuates the effects of chemotherapy in vivo[261].

Immune escape

Cell plasticity and stemness play an important role in immune evasion. CSCs appear to be the first tumor subpopulation to escape immune surveillance, due to their slow-cycling traits and their ability to downregulate the expression of antigen-presenting machinery[262]. In squamous cell carcinoma, CSCs responding to TGFβ resist immunotherapy based on adoptive cytotoxic T cell transfer. These CSCs express the immune marker CD80 and inhibit cytotoxic activity of

T cells by exhaustion, following cytotoxic T lymphocyte-associated protein 4 (CTLA4) engagement. Immunotherapy blocking CTLA4 or TGFβ1 sensitizes CSCs to adoptive cytotoxic T cell transfer in mouse and human tumors[263].

Metastatic cells escape immune surveillance through quiescence. Metastases from breast cancer expressing SOX2 and SOX9 and displaying CSC features can escape natural killer cell-mediated clearance by entering a slow-cycling state through downregulation of Wnt signaling in vivo[264]. EMT induction in tumor cells has been associated with immune evasion and resistance to cytotoxic T cells and natural killer cells[265]. Mechanisms driving resistance are not fully understood but include perturbation of the immune synapse, induction of autophagy and programmed cell death ligand 1 (PD-L1) expression[266,267].

Combined therapy to reduce the immunosuppressive microenvironment and cell plasticity by targeting cytokines, such as TGFβ, has the potential to increase the efficacy of immune checkpoint blockade. The presence of TGFβ in the microenvironment blocks the acquisition of the $CD4^+$ type 1 helper T cell phenotype[268]. Moreover, TGFβ signaling in fibroblasts restricts the localization of $CD8^+$ T cells in the peritumoral stroma rich in fibroblasts and collagen, whereas TGFβ inhibition allows T cell infiltration into the tumor[268,269]. However, a bifunctional antibody targeting both TGFβ ligand and PD-L1 has recently failed in a clinical trial for metastatic colorectal cancer (NCT03436563), and substantial tumor progression in the first four patients led to premature discontinuation of the study[270].

Preclinical findings in mice would need to be highly reproducible and rigorously validated with human biospecimens to be considered for patient selection criteria in clinical trials. Improving the drug-optimization and lead-selection process would improve the success of a given drug candidate targeting plasticity.

Concluding remarks

This Review presents the importance of cell plasticity in cancer initiation and progression, metastasis and resistance to therapy. Distinct modes of plasticity are involved in maintaining tumor growth through proliferative states and CSCs, which are also essential in the metastatic cascade. Plasticity also allows tumor cells to evade selective pressures and overcome therapy. A better understanding of tumor cell-intrinsic and -extrinsic mechanisms that regulate plasticity could open the road to new therapeutic strategies and improve patient survival in the near future.

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Competing interests

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