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Mechanisms of disseminated cancer cell dormancy: an awakening field

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Abstract

Metastases arise from residual disseminated tumour cells (DTCs). This can happen years after primary tumour treatment because residual tumour cells can enter dormancy and evade therapies. As the biology of minimal residual disease seems to diverge from that of proliferative lesions, understanding the underpinnings of this new cancer biology is key to prevent metastasis. Analysis of approximately 7 years of literature reveals a growing focus on tumour and normal stem cell quiescence, extracellular and stromal microenvironments, autophagy and epigenetics as mechanisms that dictate tumour cell dormancy. In this Review, we attempt to integrate this information and highlight both the weaknesses and the strengths in the field to provide a framework to understand and target this crucial step in cancer progression.

The inability to treat metastasis, the major source of cancer-related deaths, is the most important challenge faced by modern oncologists[1,2]. Importantly, dissemination has already occurred in many patients at the time of diagnosis[1]. Adjuvant treatments are thought to prevent the development of local recurrences or metastasis by targeting residual disease. However, although some patients benefit temporarily from hormonal or targeted therapies[2], adjuvant treatments are not always effective. Why is this? The answer may lie in the fact that the biology of residual disseminated disease seems to be highly divergent from that of the primary tumour and/or overt metastasis[3]. This divergence includes the ability of the

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Competing interests statement
The authors declare competing interests: see Web version for details.

DATABASES
The Human Protein Atlas: http://www.proteinatlas.org/BMP4|BMPR2|Fibronectin|NR2F1|POSTN|TGFB1|TGFB2|TGFBR3

FURTHER INFORMATION
An *in vitro* system to study tumor dormancy and the switch to metastatic growth: http://www.jove.com/video/2914/an-vitro-system-to-study-tumor-dormancy-switch-to-metastatic
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disseminated disease to remain clinically asymptomatic[3,4] because disseminated tumour cells (DTCs) can enter dormancy and become refractory to targeted or conventional therapies[1,2,4] (BOX 1; FIG. 1). Unfortunately, our knowledge of the biology of dormant disseminated disease is cripplingly limited. Understanding dormancy is important because dormant cells may be the source of tumour recurrence. For example, ~62% of all deaths from breast cancer occur after the 5-year survival mark[1], suggesting that dormant DTCs may cause recurrence and that targeting dormant DTCs may be of great benefit to many patients.

Box 1

Early dissemination as a source of heterogeneity, dormant DTCs and pre-metastatic niches

Dormancy of disseminated tumour cells (DTCs) may not be a process exclusive to metastatic cells that arise from established primary tumours. This is because pre-invasive lesions also contain epithelial cells that can undergo epithelial–mesenchymal transition and disseminate; these cells are referred to as early DTCs. Such early DTCs can develop metastatic growth capacity that manifests after long periods of dormancy[1,66] (FIG. 1). Early dissemination, which has not been explored by many laboratories[65,66,141], has important implications. First, by disseminating at early stages, DTCs that survive and eventually divide may evolve divergently from the primary tumour. This may generate metastases with different characteristics from those of the primary lesion and may explain the lack of success of treating metastasis with therapies designed exclusively on the basis of primary tumour characteristics. Second, the vast majority of early DTCs in mouse models seem to be dormant, and clinical evidence supports this hypothesis[65,66]. This suggests that persistence in a dormant state even with interspersed division such as that observed in adult haematopoietic stem cells[119] may allow these DTCs to remain unscathed after treatment, contributing to late recurrence of disease. Furthermore, pre-metastatic niches may in fact be conditioned or created by early DTCs. Thus, early DTCs might influence metastasis development even if they themselves remain dormant or senescent. This supports a cooperative model between early and later progressed DTCs for metastatic niche development and escape from dormancy to fuel metastasis.

The heterogeneity of primary and secondary tumours is also expected to exist in residual dormant cancer (FIG. 1). Although clinical dormancy is well documented[1,5], this clinical definition is of little use without a mechanistic understanding. Tumour dormancy was originally defined by Willis in the late 1940s and then redefined by Hadfield in the early 1950s as a temporary mitotic arrest[6] and a growth arrest[1] (BOX 2). Dormancy was later divided into three categories[4]: cellular dormancy, where intrinsic and/or extrinsic mechanisms drive solitary or small groups of DTCs to enter quiescence (BOX 2); angiogenic dormancy, where the tumour mass is kept constant by a balance between dividing cells and cells that die due to poor vascularization; and immune-mediated dormancy, where the immune system keeps a proliferating tumour mass constant via a persistent cytotoxic activity. These categories are not static, as processes that affect single cells may share underlying mechanisms with processes that affect the tumour mass. Clinical evidence supports the idea that DTCs are non-proliferative, as determined by the lack of

proliferation markers when DTCs are profiled at the single-cell level[7–9], arguing that they enter cellular dormancy. In particular, quiescence may be more fitting than senescence as a functional definition for cellular dormancy (BOX 2). Similar to senescence, quiescence is a stable, non-proliferative cellular state, but in contrast to senescence, quiescence is reversible.

Box 2

Dormancy: cell cycle arrest due to reduced mitogenesis?

Is dormancy simply growth arrest due to a lack of mitogenic signalling? Growth arrest due to senescence, a replicative arrest that is triggered by signals such as oncogene-induced stress[142], does not emerge as a central mechanism in cellular dormancy studies. As senescence is an irreversible barrier to transformation, this suggests tumour cells would not re-activate the senescence programme to become dormant [143–145]. The fact that disseminated tumour cells (DTCs) are found in a non-proliferative or possibly a slow-cycling phase suggests that G0/G1 arrest will be an inescapable part of this biology. However, a dormancy signature that predicts late relapse in breast cancer, for example[44,111], seems to contain genes other than those differentially regulated in cells deprived of growth factors[146]. These differentially regulated genes include those involved in regulating the quiescence of normal stem cells[44,131]. For example, ~62% of the genes induced in quiescent normal muscle, hair follicle and haematopoietic stem cells[131] are upregulated in dormant head and neck squamous cell carcinoma cells[44]. Signals in normal stem cell niches seem to regulate dormancy[71,147], and these same cues repress oncogene signalling via quiescence induction[120]. Perhaps dormancy of DTCs may be an active programme that recapitulates quiescence of normal stem cells. However, in dormant DTCs cell cycle arrest is coupled to a persistent but latent form of tumour-initiating or pluripotent capacity that provides an adaptive and survival advantage that eventually fuels tumour growth.

Current therapies target proliferating tumour cells with varying success. Thus, the idea of ‘awakening’ dormant cells in order to kill them[10] in the absence of effective and specific cytotoxic therapies could worsen patient outcome (BOX 3). However, and perhaps counter-intuitively, if DTCs could be kept dormant or could be eradicated while dormant, this would constitute a novel strategy to prevent metastasis[11]. Achieving such a clinical goal requires the answers to several important questions, which are explored in this Review. Here, we analyse data focusing on stress signalling and autophagy pathways linked to solitary tumour cell dormancy; microenvironments supportive of or restrictive for dormancy; and the role of stem cell, immune and vascular niches in the regulation of dormancy phenotypes. For information on the link between cancer stem cells, epithelial–mesenchymal transition (EMT) and cancer dormancy, please see recent reviews on these topics[12,13]. Here, we integrate the literature published since we last reviewed the field[4] to provide avenues for answering important questions on the biology and relevance of tumour cell dormancy.

Box 3

Therapy-induced dormancy and the disadvantages of awakening dormant cells to kill them

A key question for which there are limited data, is whether cancer therapy forces surviving residual tumour cells into dormancy by activating stress signalling[31,44,71,79,148]. An example of this may be tumour cells that are known as drug-tolerant persisters that survive targeted therapies by altering epigenetic mechanisms[149]. These drug-tolerant residual tumour cells could be compared with disseminated tumour cells (DTCs) from patients in remission to determine mechanisms that are common to both.

Another frequent question is whether 'awakening' dormant tumour cells may facilitate their eradication with conventional anti-proliferative therapies. However, the clinical evidence suggests that chemotherapy treatment of highly proliferative local and distant lesions does not always fully stop their progression. In addition, residual DTCs are genetically heterogeneous[9,65,66,133,150] and awakening dormant DTCs would expand the genetic, and probably epigenetic, repertoire of mechanisms that would allow systemic disease to resist therapy. In support of this, awakened dormant tumour cells seem to rapidly fuel tumour recurrence in experimental systems[18,46,151]. Thus, we argue against awakening dormant DTCs and instead support keeping them dormant or eradicating them while dormant as an alternative strategy.

Coordination of quiescence and autophagy

Reduced PI3K–AKT signalling has been linked to dormancy-like phenotypes[14–17]. In the presence of nutritional stress, cancer cells secrete factors that inhibit the PI3K pathway, resulting in quiescence and autophagy induction[14]. In tumour cell spheroids, loss of adhesion and nutrient deprivation promoted short-term growth arrest. This arrest was linked to epidermal growth factor receptor (EGFR)-Y1086 autophosphorylation, which inhibited AKT activation and cyclin D1 induction[15]. Although *in vivo* validation is still lacking, these findings might be clinically relevant. Balz *et al.*[16], were able to obtain cell lines from human bone marrow-derived breast cancer DTCs, despite their lack of proliferation in that compartment. Although difficult, cell lines from DTCs dormant *in vivo* can be expanded in culture and they appear to still carry characteristics of the *in vivo* phenotype[18]. Similarly, cancer cell lines that grow easily *in vitro* can show dormant behaviour *in vivo*[19–21]. For example, Balz *et al*., found that these DTC-derived cell lines contained weak or undetectable AKT-S473 phosphorylation[16]. Although this does not affect *in vitro* proliferation where growth factors are in excess, constitutive reduction in the ATK signalling pathway may predispose these cells to entering quiescence *in vivo*. Although correlative, the non-proliferative status of bone marrow DTCs in the bone marrow of patients, as determined by the absence of proliferation markers such as Ki67 and PCNA[8], together with the reduction of phosphorylated AKT in these cells, suggests that PI3K–AKT inhibition might be linked to DTC quiescence[14,16]. Furthermore, in dormant head and neck squamous cell carcinoma (HNSCC) tumour cells the levels of AKT phosphorylation were reduced[17]. However, these

quiescent tumour cells maintained mTOR activation independently of AKT by upregulating the small GTPase RHEB via the transcription factor ATF6α. Both RHEB and ATF6α promoted the survival of the dormant HNSCC cells by protecting them from apoptosis[17]. Thus, specific rewiring of pathways that coordinate metabolic homeostasis and survival during growth arrest might be a feature of dormant DTCs.

Tumour cells detached from the extracellular matrix (ECM) can survive by inhibiting AKT signalling and by inducing autophagy and an antioxidant response[22]. Floating tumour spheroids of ovarian cancer cell lines or spheroids isolated directly from patients' peritoneal fluid (ascites) were shown by one study to enter a G0/G1 arrest[23]. This arrest was due to AKT inhibition and increased p130 (also known as RBL2) and p27 (also known as KIP1) protein levels, which sequester RB and inhibit cyclin-dependent kinase 4 (CDK4) and/or CDK6 activity to induce G0/G1 arrest. If tumour cells re-adhered, AKT was activated and proliferation ensued[23], with the cells escaping the G0/G1 growth arrest. Because ovarian cancer metastases are usually in the omentum and mesothelium[24] it is important to establish whether dormant DTCs persist in these anatomical sites or whether cellular dormancy is only a property of suspended cells in the peritoneum.

Autophagy might allow dormant DTCs to maintain metabolic fitness while inhibiting PI3K–AKT signalling, although reducing this signal may not always inhibit mTOR activation[17]. For instance, ARHI (also known as DIRAS3 and RHOI), a RAS homologue that is downregulated in 60–70% of ovarian cancers, was found to be upregulated along with p21 (a CDK inhibitor) in dormant ovarian tumours in mice[25]. ARHI inhibited PI3K–mTOR signalling and induced the expression of autophagy-related gene 4 (ATG4) and LC3 (also known as ATG8), suggesting that it induces autophagy[25]. RNA interference (RNAi)-mediated downregulation of ARHI interrupted dormancy and tumours resumed growth in mice. Interestingly, inhibition of autophagy using chloroquine in tumours that had been rendered dormant by AHRI expression *in vivo* resulted in the avoidance of tumour re-growth. Thus, autophagy may be a survival mechanism during dormancy[25] and enable tumour cells to survive targeted therapies. For example, the treatment of mouse transgenic gastrointestinal stromal tumours with imatinib (a KIT and BCR-ABL inhibitor) promoted cell death[26]. However, residual tumour cells *in vivo* displayed a reversible quiescent phenotype and their survival was dependent on ATG7 and ATG12. Thus, the autophagy signalling machinery might integrate quiescence and survival signals to promote damage repair, for example, via ATG7 regulation of p53 (REFS 27,28) and metabolic fitness by generating an alternative route for amino acid turnover into ATP[29,30]. Further, AHRI or ATG expression might mark persistent and dormant minimal residual disease.

Blocking survival signals during drug-induced dormancy is an attractive possibility[31]. Bortezomib (a proteasome inhibitor) induces quiescence in surviving multiple myeloma cells[31], which have dephosphorylated eIF2α (eukaryotic translation initiation factor 2α) and reduced endoplasmic reticulum (ER) stress-related apoptotic gene induction (BOX 3). Importantly, salubrinal, a GADD34-PP1c (eIF2α phosphatase) inhibitor, not only potentiated bortezomib cell killing, but also induced apoptosis in the quiescent tumour cells that survived bortezomib[31]. Similarly, in non-small-cell lung cancer (NSCLC) xenografts treated with an EGFR inhibitor (erlotinib), tumour cells entered a quiescence-like state, and

cells that survived this treatment eventually grew after a prolonged arrest (BOX 3) but were eliminated when erlotinib was combined with the BH3-mimetic ABT-737 (a BCL-2 and BCL-X_L inhibitor)[32]. The erlotinib-surviving fractions displayed increased levels of phosphorylated signal transducer and activator of transcription 3 (STAT3), a cancer cell survival regulator[32]. It is possible that microenvironmental or drug-induced stress conditions induce survival in quiescent tumour cells via autophagy or other stress-related proteins (for example, increased BCL-2 expression or decreased proteinase-activated receptor 4 (PAR4)[33] expression).

Quiescence and stress tolerance signalling

Although reduced mitogenic signalling can trigger quiescence, specific kinases such as dual specificity tyrosine-phosphorylation-regulated kinase 1B (DYRK1B) can actively induce this state[34,35]. In pancreatic and ovarian cancer cells DYRK1B blocks the G0/G1/S transition machinery proteins, including cyclin D1, CDK4 and p27 (REFS 34,36–38). A related kinase, DYRK1A, can also induce quiescence or RAS-induced senescence via the activation of the DREAM (DP, RB, E2F and MuvB) complex that induces G0 arrest during quiescence by repressing cell cycle genes[34,39]. Interestingly, DYRK1B also coordinates survival via an antioxidant response, and inhibition of this kinase, through unknown mechanisms, seems to specifically kill quiescent pancreatic cancer cells but not normal quiescent cells[34]. Thus, DYRK1A and DYRK1B may be markers of dormant cells and testing the expression and function of these proteins in DTCs may identify a role for quiescence in residual disease.

Pausing proliferation to repair damage is a stress response conserved across evolution[40]. Farnesyl transferase inhibitors (FTIs) can induce a growth arrest in breast cancer cells for about 15 days *in vitro*. Cells that entered this arrest established a $RHOC^{hi}/RHOA^{low}$ signalling ratio that activated the stress-activated kinase JUN N-terminal kinase (JNK) that was required for the growth arrest[41]. Similarly, upstream kinases such as MKK4 (also known as MAPKK4) that can also activate JNKs can induce dormancy in other systems[42,43]. This supports the hypothesis that the activation of stress signalling pathways[11,18,44–46] could induce a sustained state of quiescence linked to dormancy.

The integration of differentiation and stress pathways has been shown to regulate dormancy, but these pathways may have cell type-specific effects. This is particularly relevant in leukaemias as opposed to carcinomas. For example, in normal T cells, in contrast to epithelial cells, enhanced p38 activation can block T cell differentiation and induce growth arrest of immature thymocytes[47], whereas ERK1 and ERK2 activation favours T cell differentiation[47]. In T cell acute lymphoblastic leukaemia (T-ALL), ERK1, ERK2 and p38 are inactivated by the ERK and p38 phosphatase MKP1 (also known as DUSP1)[48]. In dormant T-ALL cells, MKP1 is downregulated[48]. MKP1 in turn can be upregulated by NOTCH3, and this upregulation has been shown to induce dormancy escape in T-ALL cells *in vivo*. These data argue that during T-ALL dormancy maintaining high activation of both ERK and p38 signals simultaneously keeps these cells quiescent but uncommitted to full differentiation.

Activation of p38 and/or the unfolded protein response (UPR), an adaptive ER stress tolerance mechanism[49], might promote the survival of dormant tumour cells[50]. A proteomic analysis revealed that p38-induced dormancy resulted in a UPR. Induction of protein disulphide isomerase (PDI) and GRP78 (also known as BiP) in dormant cells due to p38 activation resulted in the survival of these cells when exposed to chemotherapy and/or nutrient deprivation via BAX inhibition[50], possibly through BIK upregulation[51]. This survival response was concomitant with ATF6α activation, an inducer of GRP78 (REF. 17). Proteomic analysis of bone marrow DTC cell lines obtained from patients with breast, prostate or lung cancer, revealed the upregulation of PDI, GRP78 and GRP94 (REFS 52,53). This may be due to hypoxia[54] found in the bone marrow microenvironment[55], which is known to induce a UPR owing to low nutrient and ATP availability[56,57]. Interestingly, expression of GRP78 and GRP94 was increased in DTCs isolated from bone marrow aspirates from patients with breast cancer[52] and, clinically, GRP78 upregulation in primary breast cancer correlates with a poor prognosis[58]. Cytokeratin 19 (CK19) upregulation also coordinated a UPR to promote the survival and the growth arrest of breast carcinoma cells by activating p38 and XBP1 (a UPR-regulated transcription factor), which in turn induce the PDI-like protein ERP29 (REF. 59). The above studies suggest that the activation of stress signalling might explain how DTCs survive therapy-induced or microenvironment-induced stress in different cancer types.

Microenvironmental regulation of dormancy

DTCs that survived dissemination and a new tissue microenvironment seem to undergo a default dormancy response before reactivation[60–62]. The ability of different organs to support DTC growth has led to the classification of microenvironments as dormancy-permissive or dormancy-restrictive[18,63,64]. This distinction is clinically relevant because in many cancer types DTC incidence in various organs is much higher than metastasis incidence in those organs, suggesting that the microenvironment influences DTC fate[4]. For example, the incidence of bone marrow DTCs is high in patients with gastric cancer, but clinically the incidence of bone metastasis is rare and bone marrow DTCs are commonly negative for proliferative markers, suggesting that they survive in a non-proliferative state[1,4,7,65,66]. Mouse models of other tumour types concur with these data, as DTCs are found in animals with pre-malignant mouse mammary tumour virus (MMTV)-ERBB2 (also known as HER2)-positive lesions that are considered non-invasive[66] (FIG. 1). The number of $ERBB2^+$ DTCs was never found to be higher than 10^4 per bone marrow sample and bone metastasis did not develop in any cases. However, when DTCs were transplanted from MMTV-ERBB2 mice into lethally irradiated wild-type siblings, $ERBB2^+$ bone marrow transplant recipients developed bone marrow carcinosis; this was not observed in non-irradiated recipient mice[66]. These data suggest that signals encoded in specific microenvironments dictate DTC fate.

Dormancy-permissive microenvironments

The signals that regulate haematopoietic stem cell (HSC) dormancy in the bone marrow, including growth arrest-specific protein 6 (GAS6), bone morphogenetic protein 4 (BMP4), BMP7 and transforming growth factor-β2 (TGFβ2), could induce residual disease dormancy

in different types of cancer[18,67–72] (FIGS 2,3). GAS6, which is a stromal-derived ligand of the MER, TYRO3 and AXL (also known as UFO) tyrosine kinase receptors, regulates HSC survival[73]. Leukaemia cells with the E2A–PBX1 fusion protein express high levels of MER[67], and when these cells are in contact with human osteoblasts, which produce GAS6 (FIG. 2), they enter a G0/G1 arrest and are protected from chemotherapy-induced apoptosis[67]. Similar studies with prostate cancer cell lines showed that GAS6 induces prostate cancer DTC dormancy but only when AXL receptor signalling predominates. This evidence supports the idea that HSC niche signals could induce dormancy[68–70,74].

Along the same lines, BMP7, a TGFβ family member secreted by normal bone marrow stromal cells, induced ERK inhibition and p38 activation (a low ERK/p38 signalling ratio)[75,76] and dormancy of prostate DTCs (PC3 and other human prostate cancer cell lines) injected into the bone[71]. This was dependent on the induction of the metastasis suppressor protein NDRG1 and the CDK inhibitor p21 (REF. 71). Knockdown of the BMP7 receptor BMPR2 inhibited BMP7-induced DTC dormancy [71] and an inverse correlation between BMPR2 expression and bone metastasis was found in patients with prostate cancer[71]. Thus, loss of BMPR2 may allow DTCs to escape dormancy. NDRG1 also suppresses metastasis in prostate cancer and breast cancer models by binding to LRP6 and blocking WNT-induced metastasis[77]. Similarly to BMP7, BMP4 is another BMPR2 ligand, and inhibition of BMP4 by the secreted antagonist COCO (also known as DAND5) induces the proliferation of dormant mouse mammary 4T07 DTCs in the lungs, a tissue with abundant BMP4 (REF. 72). However, in this model NDRG1 had an opposing function and was linked to COCO-induced metastasis. It is possible that NDRG1 functions differently in mouse versus human epithelial cancer cells or in different organs.

A third signal that regulates HSCs and induces dormancy of HNSCC DTCs in the bone marrow is TGFβ2 (REF. 18). TGFβ2 induced a low ERK/p38 signalling ratio, which induced the metastasis suppressor DEC2 (also known as SHARP1 and BHLHE41)[18]. In turn, DEC2 repressed CDK4 and induced p27 to induce bone marrow DTC quiescence. Importantly, inhibition of p38 or TGFβR1 (a TGFβ2 receptor) induced HNSCC multi-organ (liver, spleen, bone marrow and lung) proliferation of DTCs in mice[18]. In mouse lung, host-derived TGFβ2 was less abundant than in the bone marrow, providing clues for the organ-specific ‘seed and soil’ mechanisms[78] (FIG. 2). Other studies showed that inhibiting the lysophosphatidic acid receptor (EDG2; also known as LPAR1) in breast cancer DTCs also caused dormancy owing to the induction of a low ERK/p38 signalling ratio[79]. These studies provide a new molecular description of how signals that control normal tissue function or the inhibition of signals that stimulate reactivation may enforce a dormancy phenotype in solitary DTCs lodged in those niches (FIGS 2,3).

Dormancy-restrictive microenvironments

The switch that regulates escape from dormancy has also been studied. For example, upregulation and activation of the adhesion protein vascular cell adhesion protein 1 (VCAM1) induced escape from dormancy in meta-static breast cancer cell lines that were dormant in mouse bone marrow[19] (FIGS 2,3). In this model, these bone marrow VCAM1^{hi} DTCs bound to α4β1 integrin expressed by osteoclasts[19] and recruited osteoclast

progenitors, which together resulted in bone metastases[19]. However, the mechanisms underlying VCAM1 silencing and re-expression in these bone marrow DTCs are unknown. Another study has shown that dormancy escape[80] is promoted by periostin (POSTN), which is produced by fibroblasts[81] or endothelial tip cells that surround single dormant DTCs (FIGS 2,3).

DTCs can also remodel their microenvironment to support proliferation after dormancy exit. Green *et al.* showed that the inhibition of myosin light chain kinase (MLCK) induced the dormancy of solitary DTCs in the lung, thus reducing the number of breast cancer lung macro-metastases[82] (FIGS 2,3). Thus, MLCK regulates a dormancy-to-proliferation switch. Furthermore, using *in vitro* three-dimensional (3D) modelling they showed that production of fibronectin and β1 integrin activated MLCK and stress fibre formation to maintain proliferation[82]. Others[83] reproduced the first studies[84,85] showing that FAK, SRC and β1 integrins are required to prevent dormancy onset[46,86]. Importantly, pharmacological inhibition of SRC alone induced the dormancy or slow cycling of breast cancer cells, while inhibition of both MEK1 and SRC caused apoptosis of DTCs[46], suggesting that this pathway can be specifically targeted in DTCs in different niches (FIG. 2).

That the cytoskeletal architecture can signal to reactivate dormant tumour cells suggests that ECM stiffness might promote dormancy escape (FIGS 2,3). In hepatocellular carcinoma cells (HCCs), increased matrix stiffness was linked to increased TGFβ1 signalling, induction of cyclin D1 and cyclin D3, and proliferation *in vitro*[87]. Thus, microenvironments that are less rigid might support DTC quiescence. Although this hypothesis awaits *in vivo* validation, it was shown in a breast cancer model that TGFβ1-induced fibrotic lungs (that are associated with type I collagen deposition) could support dormancy escape[88]. Thus, like in primary tumours where ECM stiffness promotes proliferation[88], a dense type I collagen ECM might antagonize solitary DTC dormancy[83].

The multiplicity of signals that DTCs must activate to escape dormancy, including VCAM activation, TGFβ1 expression[80] and POSTN production, might explain why dormancy is such a stable phenotype (FIG. 2). The reviewed data support the general principle that solitary cell dormancy can be traced back to the reciprocal crosstalk between cells and their niches and the regulation of mitogenic and stress signalling pathways (FIGS 2,3).

Immune modulation of tumour dormancy

The above data focused on ECM or soluble ligands as cues within the microenvironment. However, the immune cells are a key component of the microenvironment[89,90] (FIG. 2). The immune system is a default constituent of the tumour microenvironment having both pro-tumour and antitumour functions[90]. Recipients of organs from donors who were disease-free but who had previously had melanoma develop melanoma metastasis in the transplanted organ after immunosuppression, suggesting that dormant cells were kept in check by the immune system[91]. However, it is unclear whether DTC reactivation is immune system-dependent or whether it is due to other microenvironmental changes from transplantation. There are few studies with clinically relevant models that have examined this. One example is of dissemination in uveal melanoma in RET. AAD mice that constitutively express the

RET oncogene in melanocytes and thus develop spontaneous uveal melanoma[92]. DTCs in these mice were found in almost all organs, including the lungs, reproductive tract and skin, as early as 2 weeks after primary tumours appeared[92]. Maintenance of dormancy in the DTCs in the lung and reproductive tract required $CD8^+$ T cells, as mice depleted of these cells with antibodies developed metastasis with a much shorter latency than those with $CD8^+$ cells (FIG. 2). Interestingly, cutaneous metastases grew despite $CD8^+$ T cell depletion, suggesting an organ-specific function for $CD8^+$ T cells and DTC fate. However, whether $CD8^+$ T cells kill or induce the growth arrest of single DTCs remains unclear (FIG. 2). This is important because there is no direct clinical proof that the immune system is involved in the growth arrest of non-proliferative solitary DTCs or in controlling their numbers via immune editing.

Tumour mass dormancy might also depend on adaptive immunity. In immunogenic 3′-methylcholantrene-induced sarcoma models, around 80% of small stable tumour masses remained static for >150 days before spontaneous regression to undetectable levels. The static tumours had a high apoptotic fraction and a low Ki67 index (a marker of G0 exit), which was consistent with $CD8^+$ T cell-mediated cytotoxicity and cytostasis. However, the small tumours resumed growth after $CD8^+$ and $CD4^+$ T cell depletion[93] (FIG. 2).

T cell-induced tumour mass dormancy may also involve crosstalk with endothelial cells and angiogenesis inhibition. In the pancreatic RIP-Tag2 cancer mouse model, pre-malignant adenomas develop by week 6–8 and adenocarcinomas by week 10 (REF. 94), and injection of $CD4^+$ T cells at week 7–8 arrested tumour progression independently of $CD8^+$ T cells via tumour necrosis factor receptor 1 (TNFR1) and interferon-γ (IFNγ) signalling[94] (FIG. 2). $CD4^+$ T cell-mediated antitumour effects were due to the release of the potent angiogenesis inhibitors CXCL9 and CXCL10 and to reduced αvβ3 integrin expression[94]. A similar function for $CD4^+$ T cells but not $CD8^+$ T cells was observed with a vaccination protocol that targeted tumour blood vessels[95]. Finally, IFNγ derived from T helper 1 cells can induce tumour cell senescence in a T antigen-induced pancreatic cancer model that is irreversible, unlike quiescence[96]. These studies show that these immune-mediated microenvironmental responses can induce marked growth arrest phenotypes[96] (FIG. 2). Interestingly, they seem to converge on IFNγ signalling as a powerful inducer of a growth arrest and potentially a dormancy-inducing signal (FIG. 2). Although these studies did not address residual disease dormancy, they may provide mechanistic information to model the regulation of solitary DTC fate, the stage at which the immune system might have a better chance to curtail metastasis development.

The above data suggest that further work is necessary to conclusively determine whether the effects of $CD4^+$ and $CD8^+$ T cells on DTC behaviour require active cytotoxicity or whether they are the result of the immune system creating a microenvironment non-permissive for tumour expansion.

Cellular dormancy and angiogenic dormancy

Like normal tissues, tumours require a functional vasculature. When a DTC grows into a micrometastasis it recruits a new vasculature. If angiogenesis fails, cell death proceeds and

equilibrium between proliferation and apoptosis can keep a small tumour mass constant and clinically dormant[97]. This could persist until genetic, epigenetic or microenvironmental signals trigger the angiogenic switch. However, there is no evidence to support the persistence of angiogenic dormancy for years or decades in patients. A study of angiogenic-poor dormant tumour masses[98] in *in vivo* models of breast cancer, gliobastoma, osteosarcoma and liposarcoma that remain dormant in immunodeficient mice for >90 days[99] showed that thrombospondin (TSP) and angiomotin were upregulated in these tumours, although their functional relevance was unclear. Recently, Ghajar *et al.*[80] found that breast cancer DTCs reside in a dormant state on the microvasculature in organs to which they have metastasized. Using *in vitro* 3D tissue modelling they found that endothelial cells in the stable microvasculature produce TSP, which keeps solitary DTCs in a dormant state. This effect of the endothelium on the tumour cells may at least partly explain the previously reported effect of TSP on the dormancy of small tumour masses that show low vascular densitiy[100,101]. By contrast, in sprouting neovasculature, the tip cells produce TGFβ1 and POSTN, which may allow the solitary tumour cells to initiate proliferation[80] (FIG. 2). This suggests that angiogenesis regulators such as TSP that control a vascular switch triggered by poor oxygenation might also regulate solitary DTC behaviour (cellular dormancy (FIG. 2)). This suggests that molecules such as TSP might influence DTC activity and that tumour angiogenesis is the consequence of DTCs switching from dormancy to proliferation. Additional work could determine how TSP and POSTN regulate solitary DTC quiescence versus tumour mass dormancy and whether tumour mass dormancy is indeed occurring in patients.

Overexpression of the Notch signalling ligand delta-like 4 (DLL4) in endothelial cells can promote T-ALL cells to exit dormancy by binding to the NOTCH3 receptor on T-ALL cells[102] (FIG. 2). Furthermore, this overexpression of DLL4 can be induced by vascular endothelial growth factor (VEGF)[102]. Thus, modulating the endothelial cell and dormant DTC interactions by using, for example, VEGF inhibitors, may be a new avenue to prevent dormancy exit.

To further study how endothelial cells might drive tumour cell entry and exit from dormancy, one study showed that expression of the angiogenesis inducers epoxyeicosatrienoic acids (EETs) in the endothelium[103] stimulated exit from tumour mass dormancy in mice[99]. EETs stimulated metastasis of various xenograft tumours, including Lewis lung carcinomas and B16-F10 melanomas[103].

These studies suggest that endothelial cell–DTC interactions might substantially predate any micro-metastasis that arises and remains dormant because of a reduced vasculature[104] (FIG. 2). In addition to endothelial cells, other cell types may also foster a switch from dormancy, for example, recruitment of vascular ($CD105^+$) and myeloid ($CD11b^+$ and $F4/80^+$) cells by tissue factor signalling can interrupt the dormancy of glioma cells *in vivo*[98,105] (FIG. 2).

We propose that before angiogenic dormancy, which may be short-lived on the basis of mathematical modelling of human cancer kinetics[106,107], endothelial cells might establish niches that support cellular dormancy. Accordingly, live *in situ* imaging of melanoma and breast cancer DTCs in the mouse brain showed that these cells survive by establishing strict

perivascular localization and direct contact with endothelial cells[108]. These DTCs remained dormant even though they are directly attached to endothelial cells in blood vessels (also observed in REF. 80) (FIG. 2). Findings such as these challenge the angiogenic dormancy theory that proposes that decreased oxygenation is the dominant driver of tumour cell dormancy. Thus, targeting the survival niche of solitary DTCs might be a more efficient strategy to stop metastatic growth.

Targeting dormancy

Recent studies have identified cues that induce dormancy[18,44,71,72,109] and specific organs that are restrictive sites for metastasis due to dormancy induction[18,67]. This knowledge could help to develop therapies (BOX 3) that mimic the dormancy programme to sustain, rather than awaken, dormant DTCs and thereby prevent relapse.

Drugs already in the clinic may be useful to induce dormancy in DTCs (BOX 4). One study showed that, in both primary cells and breast cancer and leukaemia cell lines, the DNA methylation inhibitor 5-azacytidine alone caused decreased expression of G0 to G1 exit genes — such as *DNMT1* and *FOXM1* — that are also downregulated in haematological and epithelial tumour dormancy models[44,110]. Remarkably, expression of other key genes upregulated in the p38-induced dormancy signature[44,111], such as *RARB* and *CDKN1A*, were induced by 5-azacytidine[110]. Thus, demethylating agents coupled to specific RARα and RARβ agonists could be used to reprogramme tumour cells into dormancy (BOX 4). In fact, our unpublished work indicates that a combination of all-*trans* retinoic acid and 5-azacytidine recapitulates the hallmarks of dormancy *in vivo* identified in bone marrow and p38-induced dormancy[18,44,111] and induces a prolonged and stable quiescence.

Box 4

DTC and niche markers that may dictate DTC targeting

Patients with disseminated tumour cell (DTC)-negative bone marrow commonly have a favourable outcome and could be monitored for DTC appearance[63]. However, bone marrow aspirates positive for DTCs (at the time of surgery or after treatment) are considered to be indicative of a poor prognosis. In this group, DTCs from different types of cancer (see the table) could be profiled for markers that indicate whether DTCs are proliferating (such as increased expression of COCO, fibronectin, periostin (POSTN), transforming growth factor-β1 (TGFβ1) or phosphorylated ERK (P-ERK) and low expression of phosphorylated p38 (P-p38)) or dormant (such as low expression of P-ERK, high expression of P-p38, TGFBR3, bone morphogenetic protein receptor type 2 (BMPR2), TGFβ2 or BMPs)[63]. If proliferative markers are found then DTCs could be targeted with conventional anti-proliferative treatments in combination with a dormancy-inducing therapy. If DTCs displayed dormancy markers then drugs to induce dormancy could be used to prolong dormancy. Markers or expression profiles from DTCs might help to identify specific targets in the unfolded protein response (UPR) and chaperone pathways that, when blocked, could eradicate DTCs. This could be followed by dormancy-inducing or dormancy-maintaining treatments. DTCs positive for interferon-γ (IFNγ) receptors may be sensitive to immune therapy that affect DTCs via cytostatic and

cytotoxic mechanisms or via the regulation of vascular niches. Specific examples are provided in the table for ovarian cancer where dormant tumour cells may undergo autophagy to survive. Cloroquine may eliminate autophagic dormant DTCs, and SRC and MEK inhibitors may cause the eradication of DTCs. These strategies might work if markers for DTC phenotypes and therapeutic options to target DTCs were available.

Type of cancer	DTC and niche markers	Potential therapy	Potential patient outcome
Dormant DTC phenotype			
Prostate, breast and HNSCC	• DTC: TGFβR3 ↑[18], BMPR2 ↑[71], P-ERK ↓, P-p38 ↑[18] and GRP78 ↑[50] • Niche: TGFβ2 ↑, BMP4 and BMP7 ↑[18,71,72]	• TGFβ2, BMP4 and BMP7 inducers or active biologicals • Chaperone and MEK–ERK inhibitors	Chronic asymptomatic MRD
Breast, glioblastoma, osteosarcoma and liposarcoma	DTC: POSTN ↓[80] and TSP receptors ↑[98]	POSTN blockers, TSP and angiomotin inducers	Chronic asymptomatic MRD
Ovarian	DTC: ARHI ↑[25] and ATG genes ↑[26]	Chloroquine[26]	MRD eradication
Pancreatic	• DTC: IFNR ↑ and TNFR ↑[94] • Niche: active $CD4^+$ $CD8^+$ T cells	• IFNγ • TBVA-derived peptide vaccines[95]; OX40 agonist[152]	Chronic asymptomatic MRD
Active DTC phenotype			
Breast	DTC: EDG2 ↑[79], P-Src ↑[46], P-ERK ↑ and P-p38 ↓[18]	AZD0530/6244 (REF. 46)	MRD eradication
HNSCC and breast	• DTC: P-ERK ↑ and P-p38 ↓ • Niche: fibronectin ↑, COCO ↑[72] and TGFβ1 ↑	• 5-Aza-C[110], HDAC inhibitors, anti-RARα and anti-RARβ retinoids • Anti-β1, anti-α5β1 or anti-TGFβ1 mAbs	Chronic asymptomatic MRD

5-Aza-C, 5-azacytidine; ATG, autophagy-related gene; BMP, bone morphogenetic progenitor; HDAC, histone deacetylase; HNSCC, head and neck squamous cell carcinoma; mAbs, monoclonal antibodies; MRD, minimal

residual disease; TBVA, tumour-associated blood vessel antigen; TGFβ, transforming growth factor-β; TNFR, tumour necrosis factor receptor; TSP, thrombospondin.

Uveal melanoma is another type of cancer in which epigenetic mechanisms might be manipulated for dormancy induction with currently available drugs. For example, inactivating mutations in BAP1 (REF. 112) promote epigenetic deregulation, leading to poorly differentiated metastatic tumours that resemble primitive neuroectoderm. Histone deacetylase (HDAC) inhibitors, such as LBH-589, converted uveal melanoma lines from class 2 (high metastatic risk) to class 1 (low metastatic risk)[113] and induced a G0/G1 arrest and epigenetic reprogramming consistent with melanocytic differentiation. Thus, HDAC inhibitors or DNA demethylating agents might represent alternative adjuvant therapies to induce prolonged dormancy of uveal melanoma or other types of DTCs (BOX 4).

Future directions

The work reviewed here reveals an apparent ‘fine-tuning’ of cancer dormancy research into specific areas. An emerging theme includes how the interaction of endothelial cells with DTCs affects their dormancy (FIG. 2). Analysis of dormancy-inducing cues identified BMP4, BMP7 and TGFβ2, among others[18,71,72,109]. These are ligands involved in regulating normal stem cell biology[114–117]. This identifies another emerging theme in which dormant tumour cells might recapitulate evolutionary conserved programmes of normal stem cell quiescence, coordinating growth arrest and pluripotency for prolonged periods[118,119] (BOX 2; FIG. 2). A characteristic of dormant DTCs, like normal adult stem cells, is their remarkable persistence over time. Moreover, quiescence insulates normal adult stem cells from transformation by powerful oncogenes[120]. Interestingly, morphogenetic cues, such as BMPs and TGFβ family members, which induce dormancy in tumour cells despite genetic alterations, regulate the ‘oncogene-insulating’ function of quiescence[120].

Dormancy may require robust epigenetic programming. Although much is unknown about the epigenetics of DTCs there is sufficient expertise in other areas of biology that could be applied to DTCs[121–123], such as the mechanisms underlying stem cell plasticity and EMT[124–127]. For example, p38 limits self-renewal[128], which is a property of stem cells, and a p38-regulated dormancy gene signature was associated with longer metastasis-free periods in patients with oestrogen receptor-positive breast cancer[44,111]. In addition, several genes in this p38-regulated dormancy signature are epigenetic regulators such as *NR2F1*, *TGFB2* and *DNMT1* (REFS 121,129,130), Specifically, TGFβ2 and NR2F1 can limit induced pluripotent stem cell (iPSC) reprogramming via the regulation of chromatin remodelling[121], and NR2F1 is associated with a dormant phenotype that is characterized by repressive chromatin[127] (M.M.S., P.B. and J.A.A.-G., unpublished observations). These similarities between dormant tumour cells and adult stem cells, might assist in determining which epigenetic mechanisms regulate the long-term commitment of DTCs to quiescence while retaining growth potential[115,131] (BOX 2; FIG. 2).

A question linked to early dissemination (BOX 1; FIG. 1), is whether the genetic alterations that are present in early DTCs might predispose these cells to dormancy. Perhaps a subpopulation of DTCs that evade therapy and evolve in parallel to the primary tumour

could result in delayed relapse, as evolution in distant organs may follow different kinetics. DTCs in patients with breast cancer without distant metastasis carry specific chromosomal gains such as 5cen-5q23.3 or 18q[132,133]. Could some of these gains induce DTC dormancy? *NR2F1*, a gene that is induced in dormancy models, and that is known to induce quiescence and lineage commitment, is located in the 5q region and was independently found to be linked to a locus that limits progression in breast cancer[134]. Perhaps DTCs overexpressing NR2F1 may remain dormant until additional genetic and/or epigenetic changes override this signal.

The current research provides insight into the complexities of cancer dormancy but also into the opportunities that might come from understanding this process. To apply this knowledge, clinicians will need markers informative of the dormancy status of DTCs[63] (BOX 4). Concomitantly, drug development must focus on targeting these cells, because markers without a therapeutic option will be of limited benefit[63]. Finally, proper clinical trials to test dormancy therapies are essential. As recently explained[135,136], clinical trials that use the time to first metastasis and the time to next metastasis as clinical parameters may help to determine whether new drugs are affecting dormant DTCs or whether they are inducing dormancy of proliferative DTCs. Importantly, not all cancers may be clinically dormant after diagnosis and treatment as some, like triple-negative breast cancer or pancreatic cancer, can recur as early as 3 years after ending treatment. However, the timing of recurrence is tied to the time of diagnosis. Early recurrence does not negate the fact that the metastases may have initiated from DTCs, such as early DTCs that persisted in a dormant state for a long time and gave rise to metastasis only a few years after diagnosis.

Minimal residual disease represents a new and challenging area of cancer biology that awaits exploration. The detection of DTCs in patients has been strongly suggested to be a risk factor for metastasis[137–139]. However, the enumeration and risk analysis will only be able to be addressed when this information is linked to detailed mechanistic analysis of DTC biology. The data summarized in this Review show that the field is moving promisingly in that direction. Although not yet considered a ‘hallmark’ of cancer[140], in many cancer types tumour cell dormancy may be a rite of passage for cancer relapse. Understanding cancer dormancy will answer long-standing questions regarding how to treat the most life-threatening hallmark of cancer[140], namely, untreatable metastatic disease.

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Glossary

Disseminated tumour cells (DTCs)	Cells that have physically separated from the primary tumour mass and have spread to other anatomical locations through the circulation. In the context of this Review these tumour cells are not

	yet considered to be micrometastases as they must expand to form a small population of cells
Dormant	A state of suspended animation or low activity of a cell or organism
Niches	A term borrowed from ecology, it refers to a unique optimal tissue microenvironment with defined nurturing and positional cues in a given anatomical location that allows a cell or a group of cells to survive and function
Tumour cell spheroids	Clusters of cells in a ball-like structure that can exist in suspension, for example, in the peritoneal fluid of patients with ovarian cancer. These spheroids commonly shed cells into the peritoneal cavity
Omentum	A layer of tissue from the peritoneum that connects abdominal viscera and the stomach that is a protective and supporting cover
Mesothelium	A mesoderm-derived epithelium that lines the body cavities
Minimal residual disease	Remnant tumour cells that remain after treatment and that cannot be detected by conventional methods in routine clinical testing. These cells can persist in the primary site or as disseminated tumour cells and might have proliferative and/or dormant phases
Unfolded protein response	(UPR). A cellular response to stress unique to the endoplasmic reticulum (ER) that senses the misfolding of proteins in the ER caused, for example, by overloading of the ER, oxidative damage or protein unfolding during synthesis. It activates a series of pathways that will help cells to survive by correcting the proteotoxicity caused by unfolded proteins or by activating mechanisms of cell death
$CD8^+$ T cells	Cytotoxic immune cells from the T cell lineage that are specialized in killing target cells (that is, virus-infected cells)
Angiogenesis	The formation of new blood vessels that create new pathways for blood flow during normal tissue development or remodelling or during pathological conditions such as tumour growth or retinopathy
Micrometastasis	A small group of tumour cells that have grown in secondary organs but that are too small to be seen or detected by currently available detection methods. These lesions derive from a disseminated tumour cell and might not produce clinical symptoms
Angiogenic switch	Occurs when a small tumour mass senses the lack of appropriate blood supply and activates a series of transcriptional programmes that allows the production of signals that will recruit new blood vessels

Transformation	A process in which cells that have been immortalized acquire additional genetic and epigenetic alterations that allow them to form primary tumours

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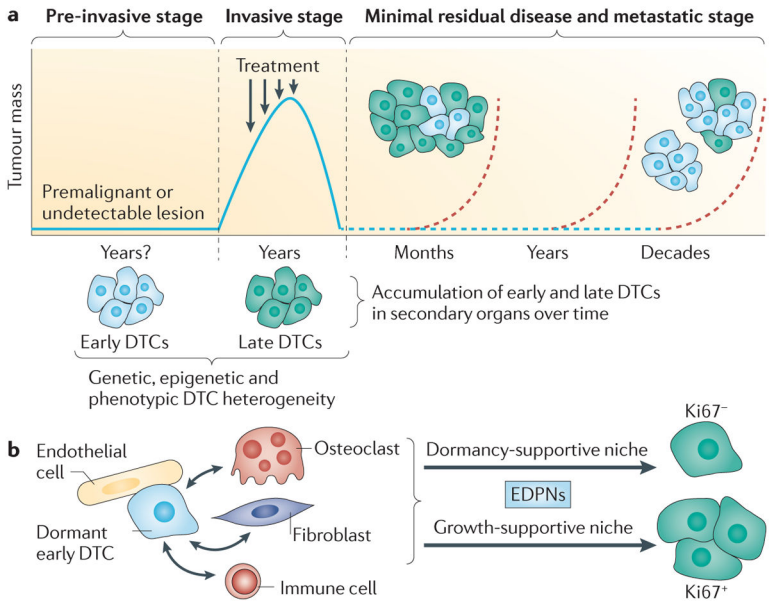

Figure 1. Dormancy of heterogeneous DTC subpopulations

a | Metastases may be initiated by and may evolve from dormant disseminated tumour cells (so-called ‘early DTCs’) from pre-invasive lesions, rather than established primary tumours[9,65]. The time required for premalignant or undectable lesions is unknown and is indicated in the figure as ‘Years?’. In fact, at the time of diagnosis, tumour cell dissemination has occurred in >50% of patients[1,9]. After primary tumour surgery and/or treatment (indicated by arrows), the tumour mass decreases and residual disease characterized by solitary dormant DTCs can be detectable for long periods (dashed blue line). After months, years or decades the metastatic tumour mass then increases (dashed red lines and tumour cell clusters). DTCs that originate from different stages of tumour evolution could form these heterogeneous masses[1,9]. For example, late DTCs, which arise from the established primary tumour may have higher metastatic potential and give rise to metastatic lesions earlier (within months of ending treatment of the primary tumour). By contrast, early DTCs (light blue) from pre-invasive lesions that remain dormant may generate metastatic tumours decades after first diagnosis. These DTCs may be the first colonizers of distant organs but may have remained in a dormant state while the primary tumour progressed. As the primary lesions progress, additional DTCs reach the target organs and contribute to dormant and proliferative DTC heterogeneity[65,66]. **b** | A hypothetical possibility discussed in BOX 1 is that early DTCs may, by colonizing target organs, become the founders of early DTC pre-metastatic niches (EDPNs) by crosstalk (arrows) with different host cell types such as endothelial cells, immune cells, fibroblasts and/or other stromal cells. EDPNs could support the dormancy and/or outgrowth of later arriving DTCs from later stages of evolution. Note that in part **b**, the same process can proceed for early DTCs that go on to form metastases independently of late DTCs (blue tumour mass in part **a**).

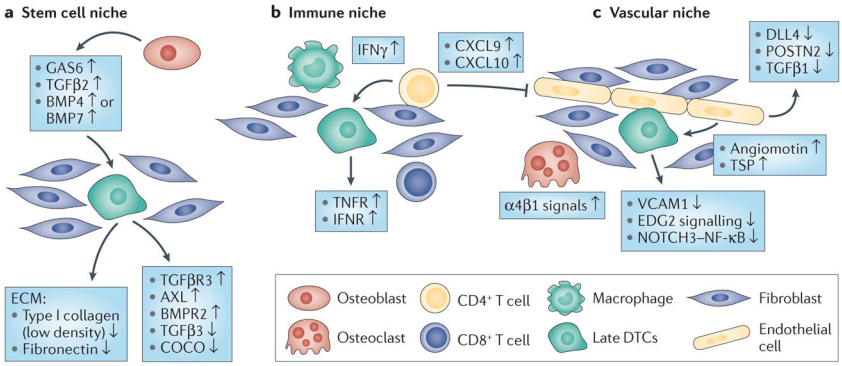

Figure 2. Permissive microenvironments and cues for DTC dormancy

Three non-mutually exclusive niches or microenvironments may actively support dormancy that contain active inducers of cellular dormancy and may lack signals that induce reactivation. **a** | Stem cell niches contain signals that also control haematopoietic stem cell (HSC) self-renewal and quiescence such as bone morphogenetic proteins (BMPs) or transforming growth factor-β2 (TGFβ2)[18,72] produced by stromal cells (for example, osteoblasts). Other factors which may induce dormancy include the expression of the AXL[109], TGFβR3 or bone morphogenetic protein receptor type 2 (BMPR2) receptors[18,71]. In addition, this environment may also lack proliferation reactivation signals such as type I collagen-dense microenvironments[88], fibronectin[153], COCO or TGFβ3 ligand[147]. **b** | The immune niche contains macrophages, $CD4^+$ cells or $CD8^+$ cells that may produce interferon-γ (IFNγ), which may induce dormancy in tumour cells[93,94]. In particular, tumour necrosis factor receptor 1-positive ($TNFR1^+$) tumour cells may be susceptible to dormancy entry in this niche. **c** | In the vascular niche, extracellular matrix (ECM) components (for example, thrombospondin (TSP)) produced by endothelial cells not engaged in active sprouting may induce quiescence of DTCs[80]. In addition, dormant DTCs in this niche have downregulated expression of vascular cell adhesion protein 1 (VCAM1) and lysophosphatidic acid receptors (EDG2)[19,79]. The immune and vascular niches may crosstalk, as dormancy has been linked to $CD4^+$ T cell-dependent production of CXCL9 and CXCL10, which inhibits angiogenesis[152] (inhibitory arrow).

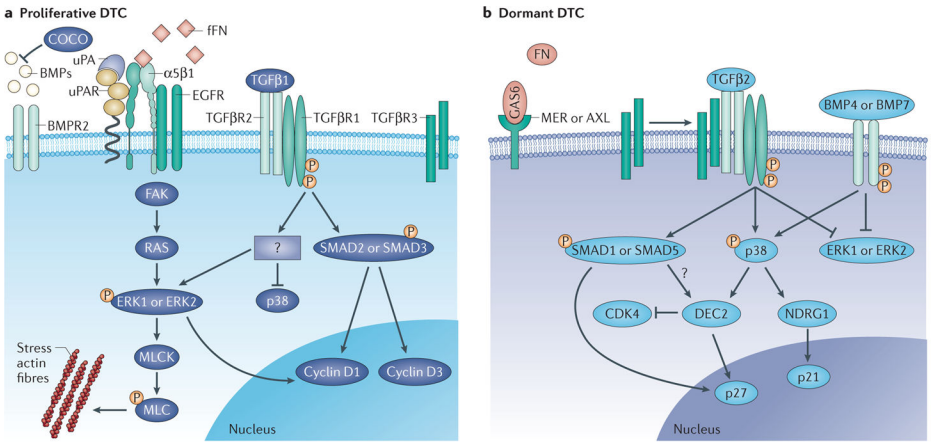

Figure 3. Intracellular pathways present in dormant and proliferative DTCs

a | In order to escape dormancy and become proliferative dormant disseminated tumour cells (DTCs) can inhibit bone morphogenetic protein (BMP) signalling by expressing inhibitors such as COCO[147]. Proliferative DTCs also require activation of growth factor signalling via ERBB family receptors such as epidermal growth factor receptor (EGFR), which can be coupled to uPAR and β1 integrin signalling that can be activated by extracellular matrix (ECM) molecules such as fibrillar fibronectin (fFN) via α5β1 integrins or by heterotypic interaction with vascular cell adhesion protein 1 (VCAM1)[153]. Receptor tyrosine kinases like the ERBB family funnel proliferative signals through FAK, SRC and the MEK–ERK modules to cyclin D1 and cyclin D3 or through myosin light chain kinase (MLCK). Other growth-promoting pathways activated in proliferative DTCs include the canonical transforming growth factor-β1 (TGFβ1) signalling pathway. Signals between the EGFR and TGFβ signalling pathways are integrated through as yet unknown mechanisms. **b** | In dormant DTCs TGFβ2 and bone morphogenetic protein 4 (BMP4) or BMP7 signals predominate, activating p38, inhibiting ERK1 or ERK2 (for example, by TGFβ2, BMP7 or EDG2 inhibition), and inducing p21 and p27 cyclin-dependent kinase (CDK) inhibitors (in all cases)[18]. Specific TGFβ2 canonical (SMAD1 or SMAD5) and non-canonical signalling result in the upregulation of DEC2 and BMP7 signalling[71] and induce NDRG1 that subsequently leads to the induction of cell cycle inhibitors and prostate cancer cell dormancy. Paradoxically, in mouse mammary cancer models NDRG1 was also shown to be induced by COCO[147] and to support metastasis. Importantly, dormant DTCs may also be sensitive to the growth arrest-specific protein 6 (GAS6)[109] or become unable to derive proliferative signals from fibronectin in the ECM through α5β1 integrins (previously reviewed in REF. 4). Question marks indicate unknown mechanisms or factors.