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Stem cell fate in cancer growth, progression and therapy resistance

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Abstract

Although we have come a long way in our understanding of the signals that drive cancer growth, and how these signals can be targeted, effective control of this disease remains a key scientific and medical challenge. The therapy resistance and relapse that are commonly seen are driven in large part by the inherent heterogeneity within cancers that allows drugs to effectively eliminate some, but not all, malignant cells. Here, we focus on the fundamental drivers of this heterogeneity by examining emerging evidence that shows that these traits are often controlled by the disruption of normal cell fate and aberrant adoption of stem cell signals. We discuss how undifferentiated cells are preferentially primed for transformation and often serve as the cell of origin for cancers. We also consider evidence showing that activation of stem cell programmes in cancers can lead to progression, therapy resistance and metastatic growth and that targeting these attributes may enable better control over a difficult disease.

Introduction

Over the past several decades, cancer has largely been treated as a disease of aberrant proliferation and survival, and the therapies most commonly used today — radiation and chemotherapy — mainly target these properties. Despite the successes of cytotoxic therapies, which include cures achieved in childhood acute lymphoblastic leukaemia (ALL)[1] and lymphoma[2], it is also clear that we are reaching the limits of how effective these approaches can be, at least in their current form. Thus, it has become important to examine aspects of oncogenesis beyond aberrant survival and proliferation.

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Author contributions

N.K.L., A.B. and T.R. researched data for the article, wrote the article and reviewed or edited the manuscript before submission. N.K.L. and T.R. also made substantial contributions to the discussion of content.

Competing interests

The authors declare no competing interests.

One critical aspect of the changes that occur as benign lesions transition to malignant ones is the progressive acquisition of the undifferentiated state. Benign lesions are often more differentiated, while malignancies are more undifferentiated, suggesting a reversal of the differentiation signals put in place during development. As many of the signals that drive the undifferentiated state are also key to conferring a stem cell fate, it is perhaps not surprising that many cancers show an acute dependence on these signals to maintain their more aggressive state.

In addition, stem cell signals are also integrally linked to cancer initiation, propagation and therapy resistance. While driver mutations are key to initiating oncogenesis, the cells in which these mutations occur are of equal importance; thus, mutations that cannot transform differentiated cells can transform undifferentiated ones[3–6], suggesting that the stem or progenitor cell state provides a more permissive context for transformation. Even after cancer establishment, perpetuation of a stem cell state in some cells creates cancer stem cells (CSCs), ‘driver cells’ that are preferentially aggressive and pose a high risk of therapy resistance and disease relapse[7]. Thus, understanding and targeting the signals critical to sustaining these cells are essential to improving outcomes. Here, we focus on how regulation of stem cell fate can not only influence cancer initiation but also serve as a driver event for disease progression, therapy resistance and metastatic growth.

Stem cell states in cancer initiation

Transcriptional context and the cell of origin.

Key studies have shown that subsets of cells with stem and progenitor characteristics in normal tissues are particularly susceptible to oncogenic transformation. Beginning with work in haematologic malignancies, where chronic myeloid leukaemia (CML) arose only when the *BCR–ABL* mutation occurred in stem cells[8,9], this paradigm has now been extended to other haematological[10,11] and solid cancers[3,12]. Defining the cell of origin can be critical for understanding both the environments that are permissive for transformation and the signals required for transformation. *BCR–ABL* provides an interesting example of an oncogene that produces different outcomes depending on the cell in which it is expressed. While *BCR–ABL* rapidly triggered CML when introduced into stem cells, it triggered B cell ALL (B-ALL) when expressed in progenitor cells[13]. Interestingly, this difference in cell of origin is closely coupled to differential signal dependencies: while loss of β-catenin blocked CML propagation, it did not impact B-ALL to the same extent[13]. Thus, the cell of origin can define both the nature of the cancer and its dependencies.

The link between the cell of origin and tumour types holds true across some solid cancers. For example, expression of KRAS in the context of p53 loss triggers squamous cell carcinoma when targeted to either interfollicular epidermis cells or hair follicle cells[14]. Despite both cell types giving rise to squamous cell carcinoma, interfollicular epidermis-derived tumours were largely epithelial in nature and have limited metastatic potential, whereas hair follicle-derived tumours contained a mixture of both epithelial and mesenchymal tumour cells and were associated with a higher incidence of metastasis. Similarly, the cell of origin in glioblastoma can dictate sensitivity to transformation and the type of tumour formed. Concurrent inactivation of *Trp53, Nf1* and *Pten* in neural stem

cells, neural progenitor cells or oligodendrocyte progenitors triggered the development of unique subtypes of glioblastoma with distinct gene expression profiles that were predictive of differential molecular dependencies[15,16]. These studies suggest that the transcriptional context of the cell of origin can be selectively permissive for specific tumour types and can be at least as strong a determinant of tumorigenesis as the driver mutations themselves (FIG. 1a).

By contrast, activation of Hedgehog signalling via genetic deletion of its receptor, protein patched homologue 1 (PTC1, which is encoded by *Ptch1*), in either neural stem cells or granule neural precursors leads to development of aggressive medulloblastomas with similar molecular profiles[17]. This suggests that in some cases certain driver mutations, rather than the cell of origin, define the tumour profile by leading to a convergence of cell states[17,18] (FIG. 1b). However, it remains unclear which tumours are predominantly determined by the cell of origin versus by the relevant mutations. It is possible that certain mutations are powerful enough in terms of defining cell fate that they can override the transcriptional context of the cell of origin; for example, in the cases above, mutations in the Hedgehog pathway could have a dominant impact on fate because they can control stem cell programmes (FIG. 1b). Given the impact of these early tumorigenic events in determining tumour evolution, it may be important to better understand the factors that control tumour cell fate.

Epigenetic mechanisms and the cell of origin.

In addition to the transcriptional context dictating susceptibility to transformation, epigenetic states may also direct tumour-initiating capacity and mutational state. Recent studies have shown that changes in DNA[19] or histone[20] methylation patterns can override oncogene-induced senescence programmes. Moreover, transformed cells that escape senescence have increased DNA methylation, leading to inactivation, at promoters of differentiation-associated genes[19]. This suggests that the epigenetic landscape is a critical determinant of both transformation susceptibility and the acquisition of a stem or progenitor phenotype.

Work in zebrafish models has shown that there is an early permissive epigenetic signature within tumour-initiating cells in melanoma[21]. In a field of melanocytes expressing driver mutations, only cells harbouring an epigenetic profile that enabled SRY-box 10 (Sox10)-driven expression of the fetal oncogene crestin were sensitive to transformation[21]. Furthermore, chromatin accessibility in melanocytes substantially overlaps with mutational density in human melanoma samples, suggesting that the combination of mutations that drive tumorigenesis mirrors the permissive epigenetic landscape of the cells within the tissue of origin[22]. Consistent with this, the epigenetic landscape of normal cells and the mutational status of tumours from the same tissue were also congruent in liver cancer, multiple myeloma, colorectal cancer, oesophageal cancer, glioblastoma, lung adenocarcinoma and lung squamous cell carcinoma[22]. Highlighting the importance of the tumour-initiating cell in defining the molecular profile of the tumour, a survey of over 10,000 tumour samples across cancers revealed that the methylome, transcriptome and proteome all cluster by the tissue of origin[23].

Importantly, the epigenetic alterations that precede transformation may act as the functional equivalent of a driver mutation. Bronchial epithelial cells chronically exposed to cigarette smoke display altered methylation patterns that lead to aberrant KRAS, WNT and epidermal growth factor receptor (EGFR) signalling[24]. The altered epigenetic state sensitized the cells to transformation with just one mutation instead of the three normally required[24]. Thus a deeper understanding of how epigenetic mechanisms contribute to the acquisition or maintenance of a stem cell phenotype is critical for developing strategies to disable the early permissive states for effective early intervention or prevention strategies.

Stem cell states in tumour propagation

Genetic and epigenetic control of cell fate in cancer.

Beyond their role in establishing the cell of origin and initiating oncogenesis, programmes that control cell fate are critical for cancer propagation via both genetic and epigenetic mechanisms. Multiple stem cell signals such as WNT or NOTCH or those of the Hedgehog pathway are activated in various cancers through mutations.

For example, loss of adenomatous polyposis coli (APC) in colon cancer activates the WNT pathway[25], activating mutations in smoothened homologue (SMO) or glioma-associated oncogene (GLI1), or loss of PTC1 trigger aberrant Hedgehog signalling in medulloblastoma[26] and basal cell carcinoma[27], and NOTCH mutations are prevalent in T cell ALL (T-ALL)[28]; in each of these contexts, the signals serve as driver mutations, highlighting the powerful influence of stem cell signals in promoting oncogenic growth.

While in some cancers genes encoding members of stem cell signalling pathways are recurrently mutated, in other cancers, these same genes are often activated epigenetically (FIG. 2a). For example, *NOTCH1* is epigenetically activated in breast cancers and pancreatic cancer[29,30], as is WNT signalling in leukaemias[31], and targeting these factors therapeutically using monoclonal antibodies against the NOTCH ligand delta-like protein 4 (DLL4) or antagonists of CREB-binding protein (CBP) and β-catenin, respectively, is currently being tested in clinical trials[32,33]. More recently, defined stem cell signals, such as the RNA-binding protein Musashi homologue (MSI), have also been shown to be both genetically and epigenetically modified in cancers; for example, blast crisis CML can harbour translocations in *MSI2* (REF.[34]), but *MSI2* can also be epigenetically activated in the absence of mutations[34–36]. The discussion below focuses on how epigenetic mechanisms can influence expression and activation of stem cell signalling pathways to support cancer propagation.

DNA methylation can also influence the acquisition of the stem cell state in cancer. Alterations in DNA methylation may occur early in tumour development: inactivating mutations in *DNMT3A* (which encodes DNA (cytosine-5)-methyltransferase 3A) lead to altered methylation and leukaemia onset[37,38], and *Dnmt3A* deletions can trigger a spectrum of haematologic malignancies in mouse models[39–41] (FIG. 2b). At a molecular level, mutant DNMT3A leads to decreased DNA methylation[42], which may confer stem cell fate by activating stem cell genes such as *HOXB*[43] and leaving pro-differentiation genes hypermethylated[37]. Promoter hypomethylation may also be a mechanism by which other key stem cell genes are reactivated in high-grade cancer: for example, hypomethylation

of the *MSI1* locus is linked to high expression in triple-negative breast cancer[44], as is hypomethylation of the *CD133* (also known as *PROM1*) locus in glioblastoma stem cells[45]. Although many studies suggest that DNMT3A promotes differentiation and acts as a tumour suppressor, DNMT3A and methylation status may have different consequences depending on the cellular context, as DNMT3A can be found overexpressed in multiple cancers, including breast, colon and liver cancers[46]. Functionally, DNMT3A can also lead to a differentiation blockade such as that seen in hepatocellular carcinoma[47], and its deletion blocked tumour progression in a model of colon cancer[48]. Consistent with these findings, hypomethylating agents have been shown to promote differentiation and increase sensitivity to chemotherapies in some cancer cells[49]. Given the context-specific impact of de novo DNA methylation, further work is clearly needed to define the programmes that differentially inhibit or promote tumorigenesis and to identify the cellular contexts most responsive to disruption of methyltransferase activity.

As a result of efforts to pharmacologically target epigenetic states, inhibitors of several broad-acting modulators such as enhancer of zeste homologue 2 (EZH2)[50], bromodomain-containing protein 4 (BRD4)[51] and histone deacetylases (HDACs)[52] have been shown to have a profound impact on tumour burden by promoting differentiation or by eroding stem cell programmes[51,53–57]. Interestingly, perturbation of epigenetic programmes via either gain or loss of histone acetylation using HDAC inhibitors or bromodomain inhibitors, respectively, can deplete CSCs[55,58]. Similarly, loss or gain of DNA methylation via deletion or activation, respectively, of DNMT3A can trigger a collapse of oncogenic programmes and can impact CSCs preferentially relative to bulk tumour cells[41,59]. The bidirectional nature of these dependencies suggests that cancer cells harbouring stem cell traits depend on tightly regulated networks, and either gain or loss of epigenetic modifications can be deleterious. Further, because epigenetic regulators control large-scale programmes, targeting them may be particularly effective for perturbing the stem cell state in cancers.

Asymmetric division and stem cell fate.

In addition to epigenetic programmes, a key way in which stem cell fate can be controlled is through asymmetric division, a post-translational mechanism critical for diversification through differential segregation and inheritance of proteins during cell division (BOX 1). Misappropriation of asymmetric division by oncogenic events can be a potential force driving cancer. When asymmetric divisions are balanced, tumours are heterogeneous, containing both CSCs and bulk cancer cells. However, when the balance is shifted towards symmetric division, this results in the expansion of CSCs that subsequently drive a more aggressive, undifferentiated state.

The connection between aberrant asymmetric division and cancer was originally identified in *Drosophila melanogaster*[60–63], and has since been linked to mammalian cancers as well. The possibility that the differentiation arrest in aggressive cancers may be driven by disrupted asymmetric division was initially suggested by observations in haematologic malignancies (FIG. 3; TABLE 1). While division patterns were not altered in chronic-phase CML, introduction of a second mutation leading to blast crisis CML triggered an imbalance favouring symmetric renewal[64]. Mechanistically, this shift was driven by MSI[35], which

repressed the pro-differentiation signal protein numb homologue (NUMB) to promote an aggressive undifferentiated state. Though dysregulation of asymmetric division may result in a more aggressive cancer, the balance can be corrected: thus, both increased expression of NUMB or loss of MSI as well as inhibition of the dynein-binding protein lissencephaly 1 protein (LIS1; also known as PAFAH1B1), which leads to increased asymmetric division, served to halt the progression of aggressive myeloid disease in vivo[35,65].

As in leukaemia, a common theme in solid cancers involves disruption of NUMB leading to increased self-renewal. Receptor tyrosine-protein kinase ERBB2-mutant breast cancer cells display increased symmetric renewal divisions[66] triggered by symmetric NUMB inheritance. MSI signalling has also been implicated in other aggressive cancers such as pancreatic cancer, where it is an indicator of poor prognosis[55,67]. p53 may also act in part by influencing symmetric renewal, with p53 loss reducing the frequency of asymmetric divisions and thus reducing differentiation in cells in the brain[68,69]. These data suggest that hijacking asymmetric division can be a point of control for classic tumour suppressors and oncogenes and raise the possibility that enforced asymmetric division could be a strategy for controlling certain aggressive cancers.

Stem cell states in metastasis

Stem cell programmes and epithelial—mesenchymal transition.

The conventional paradigm for metastasis was based originally on observations in breast cancer, and it postulated that cancer cells within primary tumours undergo epithelial–mesenchymal transition (EMT) and that this was necessary to enter circulation and transit to secondary sites[70]. Although recent studies have raised doubts about the necessity of EMT during metastasis[71,72], there is substantial evidence for a gradient of tumour cells[73,74] expressing both epithelial and mesenchymal markers within the primary tumour, in circulation and at the secondary site[75–77] However, in order for these mesenchymal cells to establish an epithelial tumour at the secondary site, genes responsible for maintaining a mesenchymal cell state must be switched off[78,79]. These findings led to the idea that EMT occurs at the primary site and is followed by mesenchymal–epithelial transition at the secondary site for successful metastatic growth. This model bears striking parallels with the stem cell model, which postulates that a subpopulation of cells within the tumour has preferential capacity for driving tumour growth and regrowth at a new site and can effectively recreate tumour heterogeneity (FIG. 4).

The congruence between the stem cell and the EMT models of metastasis is supported by multiple observations showing that most disseminated tumour cells express stem cell markers[55,80,81] and that functionally cells expressing stem cell markers like aldehyde dehydrogenase (ALDH) are highly enriched in their ability to form metastases[82,83]. Consistent with the idea that the stem cell state is a critical part of EMT and metastatic potential, genome-wide analysis of cells undergoing EMT[84] and circulating tumour cells[85] revealed a remarkably congruent transcriptomic profile between these cells and primary CSCs[86]. Circulating tumour cells isolated from patients with breast cancer[84] or from xenografts derived from patients with breast cancer[85] overexpress both EMT markers (such as twist-related protein 1 (TWIST1), AKT2 and PI3K) and stem cell markers

(such as ALDH, epithelial cell adhesion molecule (EPCAM), CD44, CD47 and MET) or exhibit stem cell properties such as chemoresistance. Genes associated with EMT are also highly expressed in CSCs[87]. Vimentin[88], transforming growth factor-β (TGFβ)[89] and the transcription factors TWIST1 (REF.[90]), zinc-finger protein SNAI1 and SNAI2 (REFS[91,92]), and zinc-finger E-box-binding homeobox 1 (ZEB1) and ZEB2 (REF.[93]) are enriched in and support the maintenance of CSCs from multiple cancers. Conventional EMT factors such as TWIST1, SNAI1, SNAI2 and ZEB1 can lead to acquisition of stem cell traits such as tumoursphere formation[94,95] and activate expression of stem cell programmes driven by transcription factor SOX2 and krueppel-like factor 4 (KLF4)[96].

Metastatic stem cells.

Although the discussion above strongly suggests that the stem cell state and EMT are in fact overlapping concepts developed by different fields, it is possible that these cells represent populations with substantial but not complete overlap. A ‘metastatic stem cell’ (REF.[97]) has been proposed as a population with increased metastatic capabilities that may not overlap with other CSC properties such as therapy resistance or immediate capacity to propagate tumours. The metastatic stem cell could in fact be a subpopulation of stem cells or one that evolves with new mutations needed to trigger metastasis. For example, $CD133^+$ pancreatic CSCs isolated from primary patient samples preferentially propagate tumours and are highly resistant to chemotherapy[98]. At the invasive front of tumour growth, $CD133^+$ cells are enriched for CXC-chemokine receptor 4 (CXCR4) expression, and this double-positive population is more migratory than $CD133^+CXCR4^-$ cells. Patients with more $CD133^+CXCR4^+$ cells had more metastatic disease, indicating the relevance of these cells for human disease[98]. A similar subpopulation of colorectal CSCs expressing CD26 was identified as the population responsible for liver metastasis and was predictive of distant metastasis in patients[99].

Exposure to spatially distinct microenvironmental cues throughout the tumour could be one trigger for heterogeneity within CSCs. In this regard, $CD133^+CXCR4^+$ or $CD133^+CXCR4^-$ cells may not be two distinct populations but might rather represent a gradient of stem cell programmes that are expressed at higher or lower levels in response to intra-cellular and inter-cellular signals. Emerging technologies using unbiased single-cell sequencing have independently supported the existence of intratumoural heterogeneity among cells with stem-like properties[100–103]. New insights into the state of tumour cells driving metastasis and which programmes and cues may promote functionally distinct capacities will likely develop by applying these same unbiased technologies to metastatic tumour cells.

Stem cell states in therapy resistance

A major challenge in cancer therapy is the fact that not all cells within a tumour are equivalently sensitive to or effectively targeted by most therapies (FIG. 5a). In large part, the cells that are not eliminated contribute to residual disease and are the key drivers of cancer relapse. Thus, understanding the basis of differential sensitivity to drugs is critical to more efficient therapies and control of tumour growth. While some cytotoxic therapies have been thought to directly induce mutations that can lead to acquired resistance[104–106],

other studies have revealed pre-existing resistant clones within the tumour that drive tumour regrowth following therapy[105–109]. Beyond genomic heterogeneity, it is becoming clear that epigenetic heterogeneity[110,111] is a key driver of differential sensitivity of cancer cells to multiple therapies. Such epigenetically driven resistance often depends on hijacked properties of normal stem cells such as the expression of drug transporters[112] (FIG. 5b), heightened DNA damage repair capacity[113] (FIG. 5c) and recruitment of a protective niche[114].

Resistance to chemotherapy and radiotherapy.

Cytotoxic drug efflux is frequently controlled by ATP-binding cassette (ABC) transporters, including the efflux pumps P glycoprotein 1 (also known as ABCB1) and ABC subfamily member 2 (ABCG2), which are highly expressed on normal and malignant haematopoietic and neural stem cells[115–117]. Because ABC transporters are generally promiscuous, they have the capacity to nonspecifically clear a range of toxic agents. Thus, cytotoxic chemotherapies are moderately successful at eliminating bulk tumour cells but leave behind aggressive CSCs that continue to express high levels of ABC transporters (FIG. 5b). In primary cell lines derived from patients with neuroblastoma, an $ABCG2^{hi}ABCA3^{hi}$ side population of tumour cells is able to sustain long-term expansion ex vivo and rapidly clear the cytotoxic drug mitoxantrone[118]. Interestingly, this population divides through asymmetric division to give rise to $ABCG2^{hi}ABCA3^{hi}$ stem cells and more differentiated $ABCG2^{low}ABCA3^{low}$ daughter cells, suggesting that drug pump expression is specifically inherited asymmetrically by the self-renewing daughter cell.

Resistance to radiation has been well studied, and its links to stem cell traits are perhaps best explored in glioblastoma, where radiation is a standard of care. While radiotherapy improves overall survival and quality of life, most patients relapse even following full remission[119]. $CD133^{+}$ cancer cells, a key population driving tumour growth in human disease[120], are highly enriched following radiation in vitro and in patient xenografts[121]. This enrichment appears to be driven by the preferential ability of the stem cell population to repair DNA damage (FIG. 5c) by activating checkpoint kinase 1 (CHK1) and CHK2. While preclinical studies indicated that these stem cells could be radio-sensitized with CHK1 and CHK2 inhibitors[121], this therapeutic approach failed in trials owing to high toxicity[122]. Recent studies suggest that glioma stem cells also rely on PCNA-associated factor (PAF)-driven translesion DNA synthesis for preferential survival following radiation[123]: pharmacologic inhibition of translesion DNA synthesis leads to radio-sensitization and depletion of glioma stem cells and thus represents a novel therapeutic approach for patients with glioblastoma. Efforts to identify new strategies to erode programmes that enable enhanced DNA repair in stem cells remain critical to improving the durability of non-targeted as well as some targeted therapies.

Targeted and immunotherapies.

In the past few decades, the greatest strides in molecularly targeted therapies have been led by the discovery of imatinib, the first tyrosine kinase inhibitor. Imatinib effectively blocks BCR–ABL activity in CML and leads to remarkably effective prevention of CML progression[124]. However, among patients with CML and minimal evidence of disease,

approximately half relapsed within the first year of imatinib withdrawal[125]. This relapse was found to be driven by residual disease comprising leukaemia stem cells[126–128]. Although imatinib is effective in blocking BCR–ABL in the stem cell fraction[129], CML stem cells are insensitive to imatinib because they are not addicted to BCR–ABL. Instead, resistant leukaemia stem cells activate several alternative signals to enable survival and renewal including β-catenin, SMO and arachidonate 5-lipoxygenase (ALOX5)[130–133]. These broad patterns have also been observed in lung cancer, in which therapies targeting EGFR mutations lead to enrichment of stem-like cells that are dependent on NOTCH3 (REFS[134,135]), and this resistance can be overcome by inhibiting Notch signalling[136]. This provided an early and important example of drug resistance without the evolution of any new mutations and is one of the best examples of a disease in which the stem cell fraction is the key contributor to residual disease.

With the advent of new cancer therapies exploiting the innate ability of the immune system to track and kill cancer cells[137–140], understanding resistance to such therapies has become an increasing focus, and stem cell signals appear to be relevant in this context. A machine-learning algorithm used to identify epigenetic and transcriptomic signatures revealed that a stem-high, undifferentiated tumour landscape is associated with lower immune infiltration and downregulated programmed cell death 1 ligand 1 (PD-L1) signalling[141], are characteristics that predict a poor response to immunotherapy[142,143]. This link is supported by earlier data in melanoma, in which tumours with high T cell infiltration responded to immune checkpoint inhibitors[143], and T cell infiltration was found only in tumours with low WNT–β-catenin signalling[144]. These data suggest that CSC signals can alter the tumour microenvironment by directly modulating tumour infiltrating lymphocytes. Bladder CSCs also modulated tumour infiltrating lymphocytes by producing inflammatory mediators like interleukin-6 (IL-6) and IL-8, which led to infiltration of pro-tumorigenic myeloid cells[145]. In many ways, these studies exemplify the interplay between stem cells and the stem cell niche and highlight the importance of mapping the complex interactions CSCs make in vivo that influence the rise of resistance.

The microenvironment in resistance.

While intrinsic mechanisms of therapy resistance have been more frequently linked to increased survival of CSCs, emerging studies suggest that the microenvironment may be equally critical. In brain tumours, endothelial cells have been shown to interact closely with stem-like cells and secrete factors that support maintenance of stem cell traits[114,146–150] (FIG. 5d). For example, endothelial cells can induce expression of stem cell programmes in glioma cells by secreting nitric oxide to promote Notch signalling[149] or by secreting the CD44 ligand osteopontin[150]. By contrast, endothelial cell inhibition through the use of the vascular endothelial growth factor (VEGF) inhibitor bevacizumab[151] may also promote stem-like characteristics in non-stem cells through anti-VEGF-triggered hypoxia, which can block CSC differentiation[152,153] (FIG. 5e). As an example, hypoxia triggers β-interferon gene positive regulatory domain I-binding factor (BLIMP1; also known as PRDM1) expression in pancreatic cancer cells[154], which subsequently activates EMT genes associated with therapy resistance. These examples highlight the challenges of interpreting

studies involving signals from the tumour microenvironment, as they can be pleiotropic and involve multiple cell types.

In addition to endothelial cells, recent studies have highlighted important roles for other niche components in therapy resistance. Non-stem cells help maintain a pool of CSCs by secreting supportive signals such as WNT in lung adenocarcinoma[155] and brain-derived neurotrophic factor (BDNF) in glioblastoma[156]. Analysis of cancer-associated fibroblasts from breast cancer samples before and after chemotherapy revealed an enrichment of fibroblasts in therapy-resistant tumours[157]. This population was not only resistant to chemotherapy but also created a therapy-resistant niche by closely interacting with CSCs and secreting factors such as IL-6 and IL-8 that promoted CSC survival[157]. Fibroblasts have also been shown to promote CSC survival and expansion in non-small-cell lung cancer[158], basal cell carcinoma[159] and colorectal cancer[160].

Although the microenvironment is generally thought to be particularly important for therapy resistance in solid cancers, emerging evidence shows that leukaemia cells, which are generally considered to be highly motile, may in fact share this dependency. For example, genetic loss of CD98, a hub for integrin signalling, triggers defects in interactions of acute myeloid leukaemia (AML) stem cells with endothelial cells and leads to their depletion[161]. Similarly, tetraspanin 3 (TSPAN3) loss blocked AML localization to CXC-chemokine ligand 12 (CXCL12)-rich bone marrow regions and led to impaired leukaemia and AML stem cell growth[162]. In addition to myeloid leukaemia, T-ALL-initiating cells are dependent on CXCR4-mediated cell motility for survival, and microenvironment-derived CXCL12 is essential for CXCR4 activation[163]. In human B cell precursor-ALL (BCP-ALL) and T-ALL, long-term dormant cells are preferentially therapy resistant when associated with microenvironmental cells, suggesting that the microenvironment can drive therapy resistance[164]. These studies highlight the importance of niche signals for leukaemia stem cell homing, proliferation and survival.

New technologies

The recent development of culture conditions that support long-term expansion of normal and neoplastic organoids[165,166] has provided a new platform for identifying drivers of therapy resistance and improving prediction of good responders. Importantly, patient-derived organoid cultures from colorectal cancer[167], pancreatic cancer[166,169], breast cancer[170], liver cancer[171] and bladder cancer[172] have been shown to retain genetic mutations present in the parental tumour sample. As expected, colorectal cancer organoids with wild-type p53 responded well to nutlin-3a, and those with activating mutations in the WNT pathway were sensitive to WNT inhibitors[167]. Additionally, in vitro drug screens using patient-derived organoids recapitulated in vivo xenograft drug response[170,172], which supports the robust nature of this system for accurately predicting therapy response. Interestingly, much of the variability in therapy response in tumour organoids can only marginally be explained by mutation burden[167,172], suggesting diverse mechanisms of therapy resistance that reflect patient diversity. This was supported by unbiased longitudinal tracking of patients and a matched pancreatic cancer organoid response to common chemotherapies[169]: organoids that were markedly responsive or resistant to specific chemotherapies coincided with patient

outcome accordingly. Moreover, parallel transcriptome analysis led to the identification of transcriptional signatures that correlated with patient response[169]. Because organoids are specifically derived from CSCs in the colon[167] and the pancreas (N.K.L., T.R. and Rajbhandari, unpublished observations), the studies discussed above provide a unique platform for measuring the drug responsiveness of a heterogenous population that is sustained by stem cell programmes. Thus, drug-sensitive organoid signatures provide a unique perspective on inter-tumoural stem cell heterogeneity and may allow us to better predict vulnerabilities.

Perspectives

The discussion above provides a view into how stem cell programmes can enable cancer initiation, therapy resistance and metastasis. The compelling biology in this rapidly moving field has already led to the development of agents targeting stem cell signals that have emerged as an important new class of differentiation therapies. Among these, the SMO antagonists, which inhibit the Hedgehog pathway, are furthest along, are approved for use in the treatment of advanced basal cell carcinoma. These antagonists have been in trials for several other cancers as well, including medulloblastoma and lung cancer[173–180] on the basis of findings from studies identifying the importance of the Hedgehog pathway in these cancers[133,181–183]. The Notch pathway has been inhibited using γ-secretase inhibitors, which prevent cleavage of NOTCH though are not specific to Notch signalling[32]. More recently, anti-DLL4 monoclonal antibodies, which more specifically target the Notch pathway, have also been developed and are in trials for multiple advanced malignancies including metastatic colorectal cancer and ovarian cancer[184]. The development of WNT inhibitors, while critical given its extensive mutation in colon cancer and activation in multiple other cancers, has been a more challenging undertaking[185]. However, the development of a CBP–β-catenin antagonist (PRI-724), which interferes with the binding of β-catenin with CBP and not p300 (REF.[186]), has allowed clinical testing of WNT pathway inhibition in advanced myeloid malignancies. Additional trials have tested the impact of inhibiting the WNT pathway at the level of WNT secretion or receptor binding using an anti-frizzled 7 (FZD7) receptor monoclonal antibody (vantictumab)[187], a WNT ligand antagonist (ipafricept)[188] or a protein-serine O-palmitoleoyltransferase porcupine inhibitor (LGK974)[189] in pancreatic and breast cancers.

At a broader level, it is worth considering the fact that despite the intense focus on identifying key signalling events and targeting these as potential strategies for therapeutic intervention, the rate of failure in trials remains high. It is likely that many drugs could be very effective, but inefficient delivery and trials in advanced stage disease likely reduce their impact on tumour growth. Improving methods of delivery through nanoparticle or lipid-mediated delivery, antibody–drug conjugate strategies and local delivery efforts represents a crucial area to explore to improve outcomes. The issue of early intervention has important ramifications for treatment outcomes in general. Among targeted therapies, imatinib is extraordinary in leading to remarkable long-term remissions that have allowed a majority to patients to live normal lives. Though usually considered a poster child of targeted therapies, the success of imatinib may have more to do with it being a true early intervention, as CML can be detected in the indolent and benign chronic phase, and imatinib is far less successful

in controlling the disease as CML progresses into blast crisis[190]. This highlights the need for a greater focus on early detection methods and raises the possibility that strategies to detect stem cell signatures could be useful as indicators of disease progression. Combining the development of innovative early detection tools with an understanding of the signals that drive benign disease to a more malignant phase would enable effective early intervention and provide a more balanced approach to controlling cancer.

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Glossary terms

Stem cell	A cell that has the ability to perpetuate itself through self-renewal and to generate differentiated cells. Stem cells are relatively rare among other cell types and can be more quiescent and resistant to toxins and chemicals as well as display enhanced DNA repair.
Stem cell signals	Also called stem cell programmes, these are signals or gene expression programmes that are often associated with the undifferentiated state in embryonic and adult stem cells. Many stem cell programmes or signalling pathways are reactivated in oncogenesis.
Cancer stem cells	(CSCs) Cells with enriched functional capacity to drive tumour growth and recreate its heterogeneity. CSCs generally share many of the defining characteristics of normal stem cells including increased drug resistance and DNA repair.
Asymmetric division	A method of cellular diversification via differential segregation and inheritance of fate determinants leading to differently fated daughter cells. Controlled asymmetric division can be critically important during development but can become dysregulated during tumour initiation and progression.
Symmetric division	A method of cell division in which fate determinants are equivalently segregated. The resulting pair of daughter cells can either be undifferentiated (symmetric renewal) or differentiated daughter cells (symmetric commitment).
Tumour heterogeneity	Here, refers to the presence of functionally distinct malignant cells within a tumour. Heterogeneity can be

driven by different genomic, transcriptomic or epigenetic landscapes.

Side population — A small population of cells detected via flow cytometry that has increased dye efflux, a property that is associated with an increased expression of drug transporters. Functionally, the side population is enriched for cells with the ability to self-renew and differentiate. As these are key features of stem cells, the side population has traditionally been found to be enriched in stem cells and cancer stem cells.

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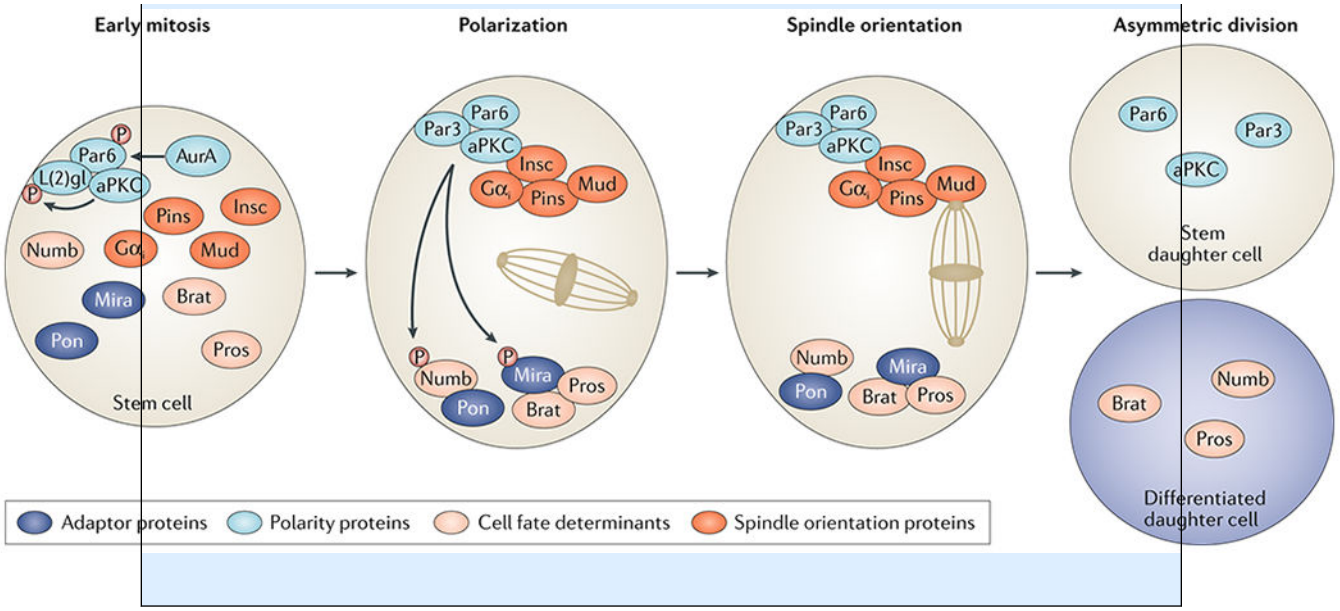
Box 1 |

Asymmetric division

In *Drosophila melanogaster*, the clearest elucidation of the sequence of events leading to asymmetric division has come from studies of the neuroblast[191–193]. These cells undergo many rounds of asymmetric division in embryogenesis, generating one neuroblast and another ganglion mother cell that in turn gives rise to neurons and glia[194]. Though these asymmetric divisions occur at distinct stages during development and adult life, similar mechanisms drive the balance of divisions, and homologues for most of the key regulators of these pathways exist in humans, suggesting conserved mechanisms of asymmetric division[193].

In the *Drosophila melanogaster* neuroblast, within a cell that will divide, atypical protein kinase C (aPKC) and Par6 are positioned at the apical cell cortex, a position inherited from a previous cell division. Here, they form a complex with Lethal (2) Giant Larvae Protein (L(2)gl), which prevents phosphorylation of Numb by aPKC. Upon entry into mitosis, the kinase Aurora A (AurA) phosphorylates Par6, which in turn triggers aPKC to phosphorylate L(2)gl (see the figure). Phosphorylated L(2)gl is then released from the complex and replaced with Par3. Polarization results when aPKC phosphorylates Numb and the adaptor protein Miranda (Mira), restricting their localization to the basal region along with the adaptor protein Partner of Numb (Pon). Miranda recruits Prospero (Pros) and Brain Tumour (Brat) to the basal membrane, allowing for the accumulation of these cell fate determinants by late prometaphase. The adaptor protein Inscuteable (Insc) then links the Par3—Par6—aPKC complex to the Gαi—Partner of Inscuteable (Pins) protein complex, which then interacts with Mushroom Body Defect (Mud), thereby linking the entire complex to the mitotic spindle and establishing its apical-basal orientation. Following cell division, the asymmetric inheritance of Numb acts to inhibit Notch signalling, and this in combination with the transcriptional activity of Pros promotes differentiation of the daughter cell.

The connection between aberrant asymmetric division and cancer was originally identified via a screen for genes that promote brain tumour development in *Drosophila melanogaster*[60–63]. Deletion of *l(2)gl, brat, prospero* and *numb* resulted in a loss of differentiation, uncontrolled cell proliferation and eventual development of brain tumours. Despite these early studies, progress in defining the link between division pattern and cancers in mammalian systems has been slow. However, over the past few years, emerging data have shown that this is an important regulator of cancer progression, and mutations in key regulators of this process are associated with oncogenesis[35,60–65].



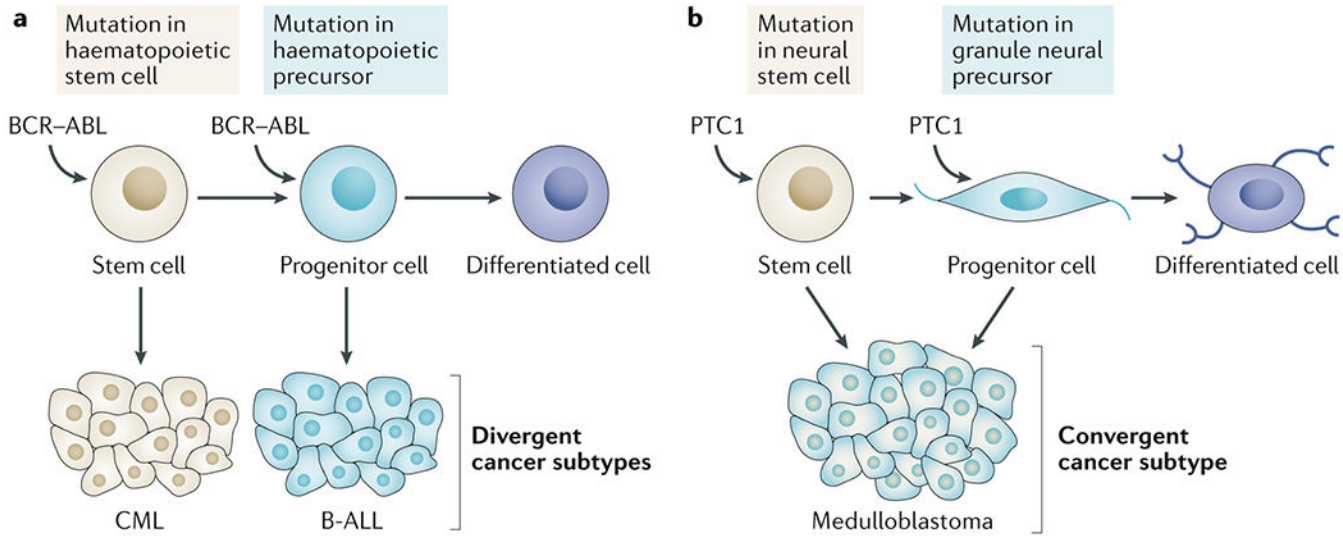


Fig. 1 |. Impact of the cell of origin on cancer development.

a | Oncogenic mutation can drive distinct cancer subtypes depending on the epigenetic and transcriptomic profile of the cell of origin. For example, in haematologic malignancies, when BCR–ABL is introduced into stem cells, it results in chronic myeloid leukaemia (CML); however, when this same mutation is introduced into progenitor cells, it results in B cell acute lymphoblastic leukaemia (B-ALL). **b** | Alternatively, oncogenic mutation in distinct cells of origin can lead to a convergence of cell states that results in the same cancer subtype. For example, in medulloblastoma, deletion of protein patched homologue 1 (PTC1) in either neural stem cells or granule neural precursors leads to the development of aggressive medulloblastoma. P, phosphorylation.

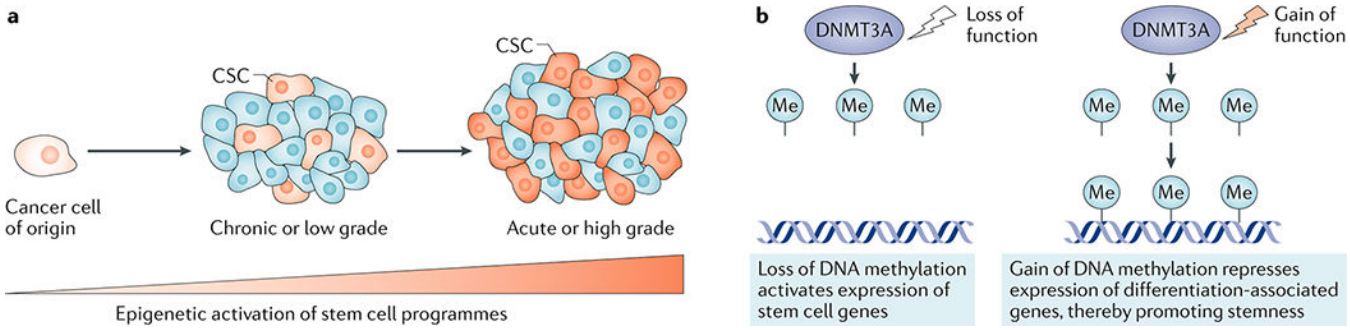


Fig. 2 |. Epigenetic regulation of the stem cell state in cancer.

a | During normal development, stem cell programmes are extinguished during differentiation; in cancers, such as myeloid leukaemia, epigenetic reactivation of stem cell programmes can promote propagation and progression to an aggressive state. The activation of these programmes in a subpopulation (cancer stem cells (CSCs), shown in orange) is associated with chronic myeloid leukaemia (CML), a low-grade disease, while widespread activation of these programmes — illustrated by the expanded pool of CSCs in the figure — is associated with blast crisis CML, an aggressive, high-grade disease. **b** | Epigenetic regulation of stem cell programmes may also be mediated through modification of DNA. For example, mutation of the DNA methyltransferase DNA (cytosine-5)-methyltransferase 3A (DNMT3A) can promote the stem cell state through either loss of function mutations (which can lead to hypomethylation and activation of genes that promote the stem cell state; shown on the left) or gain of function mutations (which can lead to hypermethylation and silencing of genes associated with differentiation; shown on the right). Me, methylation.

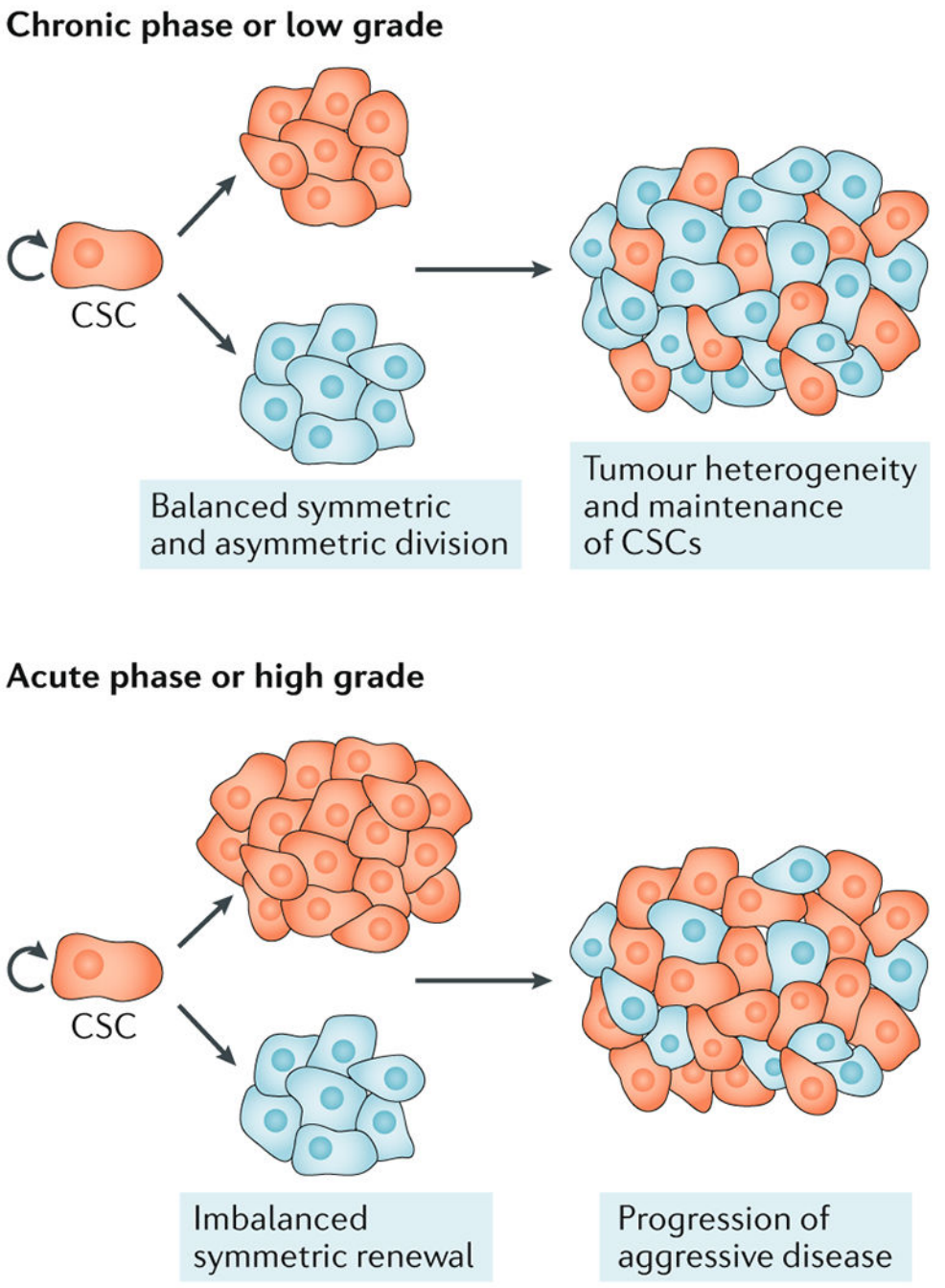


Fig. 3 |. Asymmetric division in cancer.

The disruption of asymmetric division is one way in which cancer may progress to an aggressive state. In low-grade cancers, symmetric renewal and asymmetric divisions are fairly balanced, resulting in both tumour heterogeneity and the maintenance of cancer stem cells (CSCs). However, in high-grade cancers, this balance may be shifted towards increased symmetric renewal, resulting in the expansion of CSCs, which may result in a more aggressive disease state. While imbalances in asymmetric division leading to the progression of cancer have been clearly demonstrated in haematologic malignancies, there is

evidence to suggest that disruption of asymmetric division can promote an aggressive state in some solid tumours as well.

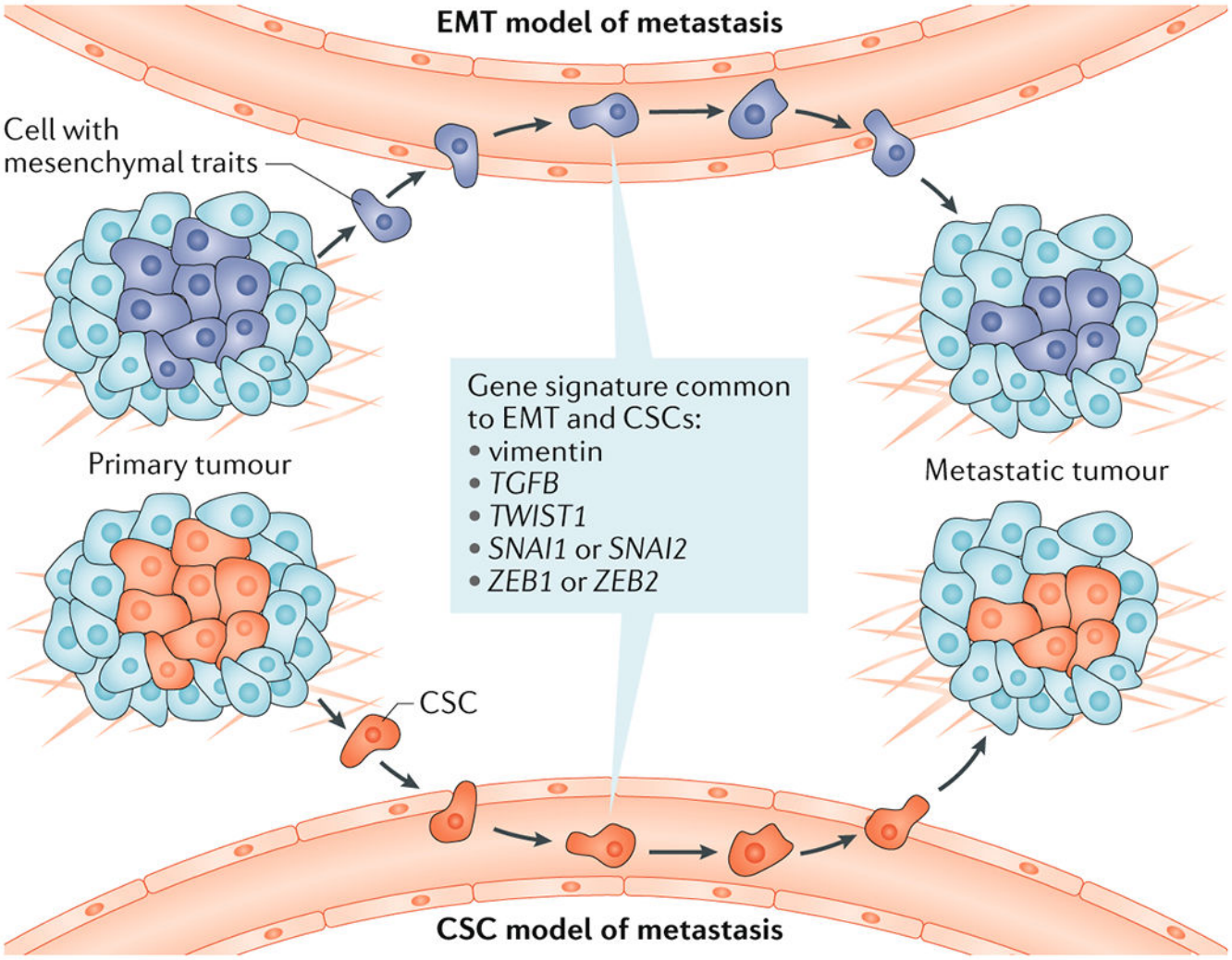


Fig. 4 |. Metastasis and cancer stem cells.

The classic epithelial–mesenchymal (EMT) model of metastasis (top) posits that the dissemination of cancer cells requires loss of epithelial cell traits commensurate with gain of mesenchymal cell traits (dark blue), which enables the cells to detach from the primary tumour and invade surrounding tissue, intravasate and survive in circulation, and, finally, extravasate and localize to a distant metastatic site. Several genes (shown in the centre box) have been shown to drive EMT, and their expression serves as a marker of the process. Interestingly, cancer stem cells (CSCs) (bottom) are also enriched in disseminated tumour cells and express the EMT gene signature. Further, the capacity for tumour propagation, which is required for establishment of a tumour at a distant site, is a salient feature of CSCs. The parallels between EMT cells and CSCs raise the possibility that they represent overlapping concepts.

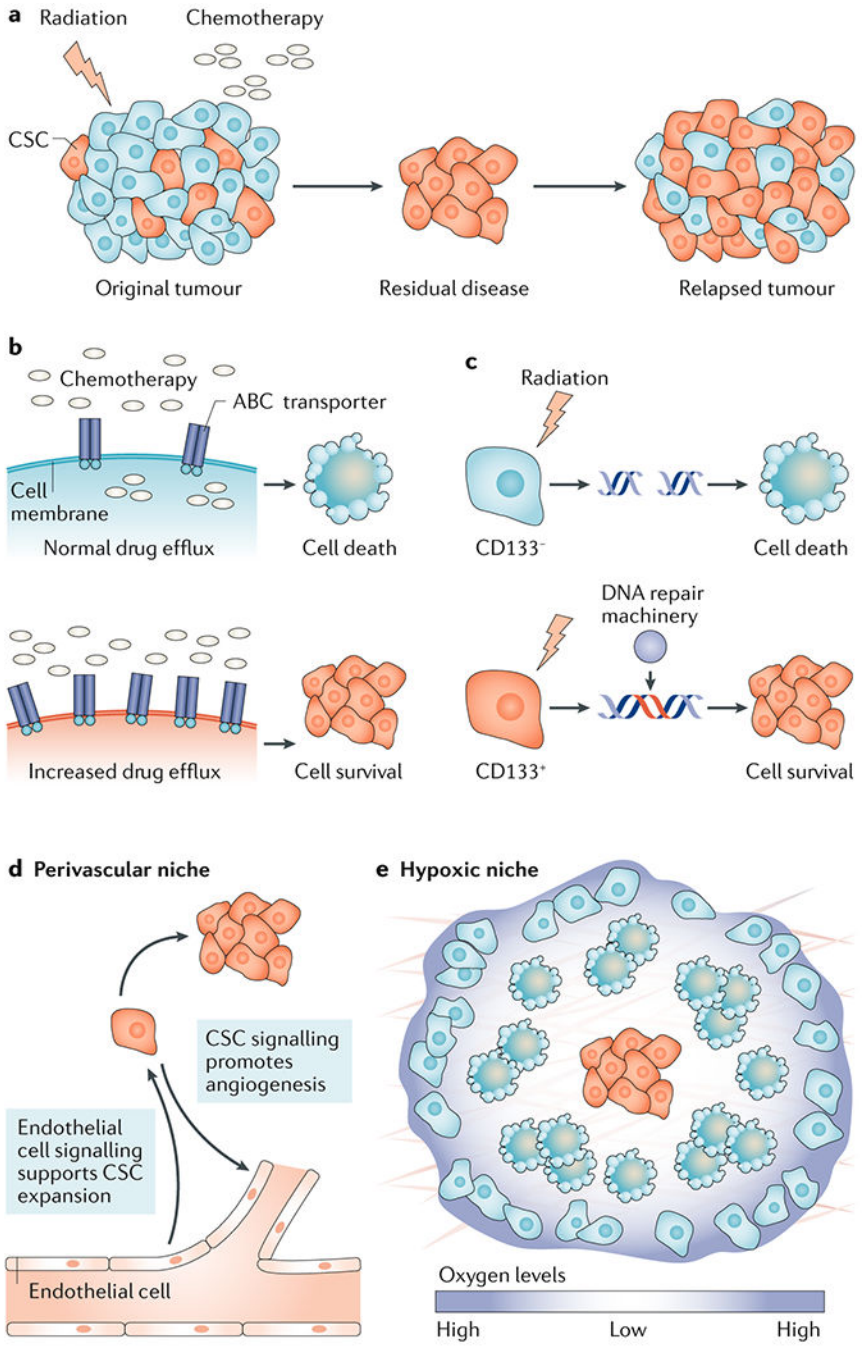


Fig. 5 |. Therapy resistance in cancer stem cells.

a | Cytotoxic agents such as radiation and chemotherapy are commonly used to treat cancer, efficiently targeting bulk cancer cells (blue cells) but not cancer stem cells (CSCs) (orange cells). The residual disease can be enriched in CSC populations that can drive a more aggressive disease, triggering recurrence. **b** | Stem cell properties are commonly hijacked in cancer. One such property is increased drug efflux. Chemotherapeutic agents target bulk cancer cells with normal levels of drug efflux, resulting in cell death (top). In CSCs, higher expression of ATP-binding cassette (ABC) transporters can increase drug efflux capacity,

increasing cell survival (bottom). **c** | Enhanced DNA repair can also be hijacked in cancer. In glioblastoma, radiation generates unrepaired double strand breaks in $CD133^-$ bulk cancer cells, leading to cell death (top). In $CD133^+$ CSCs (bottom), the DNA damage checkpoint is activated, allowing for repair that leads to increased cell survival. **d** | CSCs utilize the tumour microenvironment for increased survival. In brain tumours, the endothelial cells of the perivascular niche promote the survival of CSCs. Endothelial cell signalling supports the stem cell properties of the cancer, which allows CSC expansion. CSCs can promote angiogenesis by secreting factors such as vascular endothelial growth factor (VEGF) and stromal cell-derived factor 1 (SDF1). **e** | Hypoxic environments can support CSCs. Although hypoxia (represented by the descending oxygen gradient shown in blue) induces some cell death within the tumour, it also promotes CSC expansion and triggers expression of genes that promote therapy resistance.

Table 1 |

Asymmetric division genes in cancer

Protein	Function in asymmetric division	Cancer type	Effect on asymmetric division	Dysregulation in cancer	Refs
LLGL1	Cell polarity	Leukaemia	Promotes asymmetric division	Decreased expression	195
NUMB	Cell fate	Leukaemia, colon cancer and breast cancer	Promotes differentiation	Decreased expression	35,66,199
MSI	Cell fate	Leukaemia	Promotes stemness	Increased expression	35
LIS1	Dynein binding and spindle orientation	Leukaemia	Promotes symmetric renewal	Critical for propagation of CSCs	65
TRIM 3	Cell fate	Brain cancer	Promotes asymmetric division	Decreased expression	200
p53	Cell fate	Brain cancer, colon cancer and breast cancer	Promotes asymmetric division	Decreased expression	66,69,196
miR-34a	Cell fate	Colon cancer and brain cancer	Promotes differentiation (targets NOTCH)	Decreased expression	197–199
miR-146 a	Cell fate	Colon cancer	Promotes symmetric renewal (targets NUMB)	Increased expression	199
lnc34a	Cell fate	Colon cancer	Promotes symmetric renewal (targets miR-34a)	Increased expression	201

CSCs, cancer stem cells; LLGL1, lethal(2) giant larvae protein homologue; LIS1, lissencephaly 1 protein; lnc, long non-coding RNA; miR, microRNA; MSI, RNA-binding protein Musashi homologue; NUMB, protein numb homologue; TRIM3, tripartite motif-containing protein 3.