REVIEW

nature medicine

Cancer stem cells revisited

Eduard Batlle[1–3] & Hans Clevers[4]

The cancer stem cell (CSC) concept was proposed four decades ago, and states that tumor growth, analogous to the renewal of healthy tissues, is fueled by small numbers of dedicated stem cells. It has gradually become clear that many tumors harbor CSCs in dedicated niches, and yet their identification and eradication has not been as obvious as was initially hoped. Recently developed lineage-tracing and cell-ablation strategies have provided insights into CSC plasticity, quiescence, renewal, and therapeutic response. Here we discuss new developments in the CSC field in relationship to changing insights into how normal stem cells maintain healthy tissues. Expectations in the field have become more realistic, and now, the first successes of therapies based on the CSC concept are emerging.

Tissues such as the intestinal epithelium and the hematopoietic system continuously self-renew through the activity of a dedicated population of tissue-specific stem cells, also known as adult stem cells[1,2]. Unlike the bulk of the cells that populate these tissues, adult stem cells are long-lived and generate cellular progeny throughout life to regenerate the multiple specialized, short-lived cells that ultimately perform tissue-specific functions.

The CSC theory states that tumor growth is similarly fueled by small numbers of tumor stem cells hidden in cancers. It explains clinical observations, such as the almost inevitable recurrence of tumors after initially successful chemotherapy and/or radiation therapy, the phenomenon of tumor dormancy, and metastasis. The first decade of this century has seen an avalanche of reports on the identification of CSCs in many common cancer types, including leukemia[3–5], breast cancer[6], colorectal cancer[7–9], and brain cancer[10]. The CSC concept has inspired the design of innovative treatment strategies for these cancers, not aimed at shrinking tumor bulk, but rather at exterminating CSCs, the cell population that sustains long-term growth.

The aim of this review is to discuss recent developments in the CSC field while bearing in mind newly emerging views on the biology of normal stem cells. Over the past few years, experiments involving lineage tracing and cell ablation in intact tumors have confirmed that many of these tumors harbor stem cells in dedicated niches[11–16]. Yet, it has gradually become clear that CSCs (similarly to normal stem cells) do not necessarily have to be rare and/or quiescent; multiple examples now show that they can be abundant and can proliferate vigorously. Furthermore, it is emerging that stem cell hierarchies may be much more plastic than previously appreciated[17], a phenomenon that complicates the identification and eradication of CSCs. This review will also focus on major advances in the development of CSC-based therapies, some of which are currently being implemented in the clinical setting.

[1]Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Barcelona, Spain. [2]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain. [3]CiberONC, Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Barcelona, Spain. [4]Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences, University Medical Center Utrecht and Princess Maxima Center, Utrecht, the Netherlands. Correspondence should be addressed to E.B. (eduard.batlle@irbbarcelona.org) and H.C. (h.clevers@hubrecht.eu).

Received 23 January; accepted 23 August; published online 6 October 2017; doi:10.1038/nm.4409

The shaping of the CSC concept

We have given an extensive history of the CSC field previously[18]. Here we briefly highlight the major influence of hematopoietic stem cell (HSC) research in shaping the adult stem cell and CSC fields.

Features of hematopoietic stem cells

HSCs were first identified 60 years ago. The prevailing view on the defining characteristics of HSCs and their downstream differentiation hierarchy can be summarized as follows[2]: the HSC is at the top of the hematopoietic hierarchy, is a rare cell type, and it divides infrequently, i.e., it is 'quiescent,' because DNA replication prior to division carries a risk of mutation. When the HSC divides, it does so in an asymmetric fashion, yielding one actively dividing daughter cell and one new, quiescent stem cell. Thus, a stem cell has the unique capacity to 'self-renew,' and its lineage is long-lived. Individual HSCs can generate all blood lineages, that is, they are multipotent. As daughter cells continue to divide, they migrate down the hierarchy and become progressively lineage restricted. This occurs through a well-orchestrated series of discrete steps, and eventually, yields the various mature blood cell types. In homeostasis, the cell hierarchy is rigid. Although these insights were obtained decades before it became feasible to study other types of adult stem cell, the defining characteristics of HSCs have served as a dominant template for interpreting experimental observations of the renewal of other mammalian tissues and cancer.

Xenotransplantation assays to assess CSC activity

In HSC research, the capacity of a given cell to reconstitute the hematopoietic lineage upon transplantation into lethally irradiated immune-deficient mice has been used as a surrogate of stem cell potential[2]. Over the past two decades, this assay has been instrumental in identifying

sets of cell-surface markers that allowed for fluorescence-activated cell sorting (FACS) of normal human HSCs and their various progenitors[2].

In pioneering studies of the properties of leukemia, it was found that most subtypes of human acute myeloid leukemia (AML) could be engrafted reliably into immune-deficient mice by transplanting a population of leukemic cells that expressed a combination of surface markers ($CD34^+CD38^-$) characteristic of normal HSCs. The frequency of these tumor-initiating cells was on the order of one per million tumor cells[3–5]. Using equivalent cell-sorting and xenografting approaches, a team showed that human breast cancer, i.e., a solid tumor, is comprised of functionally heterogeneous populations of cancer cells with varying ability to form the xenograft[6]. As few as 100 $CD44^+CD24^{-/low}$ breast cancer cells could initiate tumor growth, even in a serial xenograft setting, whereas tens of thousands of cells with alternative phenotypes could not[6]. Similar studies of other solid tumors, such as brain cancer[10], prostate cancer[19], colon cancer[7–9], pancreatic cancer[20], ovarian cancer[21], and lung cancer[22], rapidly followed. We refer the reader elsewhere for a comprehensive overview of these efforts[23].

The standard CSC model

Toward the end of the past decade, these studies supported a CSC model based on four premises that echoed the key features of the HSC hierarchy. First, a substantial fraction of cellular heterogeneity observed in tumors results from its hierarchical organization, which is often, but not always, reminiscent of the hierarchy in the tissue of origin. Second, tumor hierarchies are fueled by rare self-renewing—typically quiescent—CSCs, whereas the bulk of the tumor is composed of non-CSCs, which are capable only of transient proliferation and therefore do not contribute to long-term growth. Third, CSC identity is hardwired, as illustrated by the fact that non-CSCs seldom initiate tumors in xenograft assays. Thus, there is limited plasticity in the tumor hierarchy. Finally, CSCs are resistant to standard chemotherapy and radiation treatment, which preferentially target non-CSCs—a phenomenon that explains relapse after treatment.

Analysis of CSCs in intact tumors

The xenotransplantation approach to investigating the properties of CSCs carries inherent technical and conceptual limitations (see **Box 1** and refs. 18,24). In search of more direct evidence, several groups have studied CSCs in intact tumors through genetic-lineage tracing, the gold standard for assessing adult stem cell activity *in situ* (see **Box 1**).

Lineage-tracing studies in mouse tumors

An early study using this approach in a chemically induced tumor model for squamous skin cancer traced clones using an inducible, basal cell–specific keratin-14-Cre driver allele[11]. Although most labeled tumor

Box 1 Transplantation compared to lineage-tracing approaches to study CSCs

Prototypic CSC-transplantation assays involve sorting tumor cell populations on the basis of surface-marker heterogeneity, followed by inoculation of the isolated cell populations into immunodeficient mice in numbers sufficiently low to limit the formation of a xenograft by the bulk tumor cell population. The capacity of a given cell population to initiate a tumor under these conditions over serial passages in mice is interpreted as evidence for the presence of CSCs[3–10,19–22]. These tumor-cell transplantation assays were originally designed to study hematological malignancies, which poses several caveats regarding their utility for analyzing CSCs in solid cancers: in general, leukemias have limited genetic changes and harbor restricted intratumor genetic heterogeneity, as compared to most solid cancers. Thus, the a priori assumption of the FACS or xenografting approach (i.e., that functional differences are being scored between genetically homogeneous tumor-cell subpopulations) may be more appropriate for leukemias than for solid cancer. Although the sorting strategies in leukemias are founded on an extensive knowledge of marker combinations that define the various normal HSC and progenitor cell types, such knowledge is often absent for the tissue of origin of many types of solid cancer. The choice of testable markers for CSCs in solid cancers is often based on differential expression between different tumor-cell subpopulations and/or on knowledge of stem cell–specific expression of the marker in an unrelated tissue. Finally, transplantation assays involve dissociation of the tumor mass. Yet, cells in solid tumors rely heavily on cell-to-cell contacts, attachment to the extracellular matrix, and signals from the microenvironment. Thus, tumor-initiating potential in transplantation assays may, at best, serve as a surrogate for CSC-autonomous properties, or even simply reflect the adaptation of particular tumor-cell populations to the experimental conditions.

Genetic-lineage tracing enables the identification and study of stem cells in solid tissues *in situ* while avoiding mechanical perturbation. This technique rests crucially on the identification of a single marker gene that allows the expression of an inducible version of a recombinase (for example, Cre), which, in turn, allows for the stable activation of a reporter for the pertinent recombinase (for example, the R26R *LacZ* reporter[149]) in the cell population of interest, ultimately resulting in the labeling of the cells of interest. Importantly, stable reporter expression is maintained in all daughter cells of the marked cell. Persistence, size, and composition of cell clones generated over time are used to evaluate stemness potential. We refer the reader to excellent reviews on the advantages and technical limitations of this approach[17,150–152].

The behavior of normal adult stem cells assessed by lineage tracing differs substantially from that inferred through transplantation experiments. Hair-follicle stem cells give rise to all epidermal lineages upon transplantation[153,154], but upon lineage tracing, generate only hair-follicle lineages[155]. Similarly, although mammary basal cells are multipotent in transplantation assays[156,157], these cells are unipotent when interrogated by lineage tracing[158,159]. Of note, the use of distinct drivers and recombination strategies to study mammary stem cells in lineage-tracing experiments has generated controversy regarding the identity of this particular cell type[160,161].

Similar discrepancies were encountered when HSCs were analyzed by transplantation as compared to lineage-tracing approaches. In one study, a transplant-free tagging strategy was developed using a transposon-based approach to study hematopoiesis in mice. Steady-state blood cell production seemed to be maintained by the successive recruitment of thousands of clones, each with a minute contribution[162]. These findings contradicted the classical model of a hierarchy supplied by few HSCs inferred from transplantation assays. In a different study, the same question was addressed by genetic lineage tracing based on the HSC-specific *TIE2* gene[163]. Authors similarly found that steady-state adult hematopoiesis is maintained largely by multipotent progenitor cells, which are capable of reconstituting the blood lineages only transiently in classical transplantation assays, yet seem to be long-lived according to analysis of intact hematopoiesis by lineage tracing.

Overall, these observations imply that transplantation-based approaches may reveal the potential of stem cells, but may not necessarily unveil the fate of these cells under steady-state conditions.

cells in papillomas were lost after terminal differentiation, some survived long term and generated large clones within the growing benign tumors, indicating the existence of actual CSCs. Mathematical models built from the lineage-tracing data indicated that the papilloma CSC population divides asymmetrically to give rise to CSCs and progenitors that are committed to differentiate. Yet, at the individual level, CSCs undergo both symmetric (i.e., producing two stem cells) and asymmetric divisions in stochastic patterns[11]. This mode of division is similar to that of normal stem cells in the epidermis[25,26]. A different pattern was observed in invasive squamous cell carcinomas, consistent with the expansion of a single CSC population that has limited potential for terminal differentiation[11].

Similar findings support the presence of stem cells in primary intestinal mouse adenomas, the precursors to intestinal cancer[12]. Tumors were induced by conditional deletion of the tumor-suppressor gene *Apc*, a negative regulator of the WNT pathway that is mutated in most colon cancers. In these experiments, *Apc* deletion was targeted in intestinal stem cells (ISCs) by exploiting the specific expression of the intestinal stem cell–marker gene *Lgr5* (ref. 27) while concomitantly labeling individual *Apc*-mutated stem cells in red[12]. After the resulting single-colored tumors had grown to a substantial size, cells expressing *Lgr5* in these tumors were induced to switch their color from red to blue. Blue cells generated large clonal patches in the red tumor, providing evidence for a hierarchical organization of adenoma growth *in vivo*[12]. An unbiased lineage-tracing approach based on a mutation-induced mark during DNA replication confirmed the appearance of large dominant clones from adenoma CSCs, and it refined the model by postulating that only a small fraction of $Lgr5^+$ adenoma cells act as CSCs[13].

Another study[14] described the clonal dynamics of tumor cells over time by using intravital imaging of multicolored lineage tracing in an MMTV-PyMT mouse model of breast cancer. It was observed that some clones initially grew, yet eventually disappeared, whereas others rapidly expanded to become dominant, an observation consistent with extensive intratumor heterogeneity and the existence of CSCs in this model.

Lineage-tracing studies in human cancers

Given the complex genetic modifications required, lineage-tracing techniques had been restricted largely to genetic mouse models of cancer. A strategy for circumventing this limitation was presented in two recent studies, which implemented CRISPR–Cas9 gene-editing technology to insert cassettes into the *LGR5* locus of colorectal cancer patient–derived organoids for lineage tracing[28,29]. Xenografts generated from these organoids were subsequently used to study the behavior of human $Lgr5^+$ cells in intact tumors. These experiments revealed that $Lgr5^+$ colorectal cancer cells produce progeny, over long time periods, that progressively undergo differentiation, albeit with slower kinetics than their nontransformed counterparts[28,29]. Importantly, the number of daughter cells generated by $Lgr5^+$ tumor cells was proportional to the size of xenografts[28], whereas tumor cells expressing the terminal-differentiation-marker gene keratin 20 (*KRT20*) produced progeny that mostly persisted as single cells or vanished over time[29].

These studies confirmed previous histological and transcriptomic analyses[30,31], suggesting that human colorectal cancers are composed of heterogeneous cell populations organized into hierarchies reminiscent of the normal colonic epithelium. They also provided a strategy for analyzing CSCs in human organoids and xenografts through classical genetic approaches that, so far, had been feasible only in animal models.

Novel views of adult stem cells

The HSC-centric interpretation of stem cell biology has been widely adopted, but increasing evidence indicates that no generally applicable

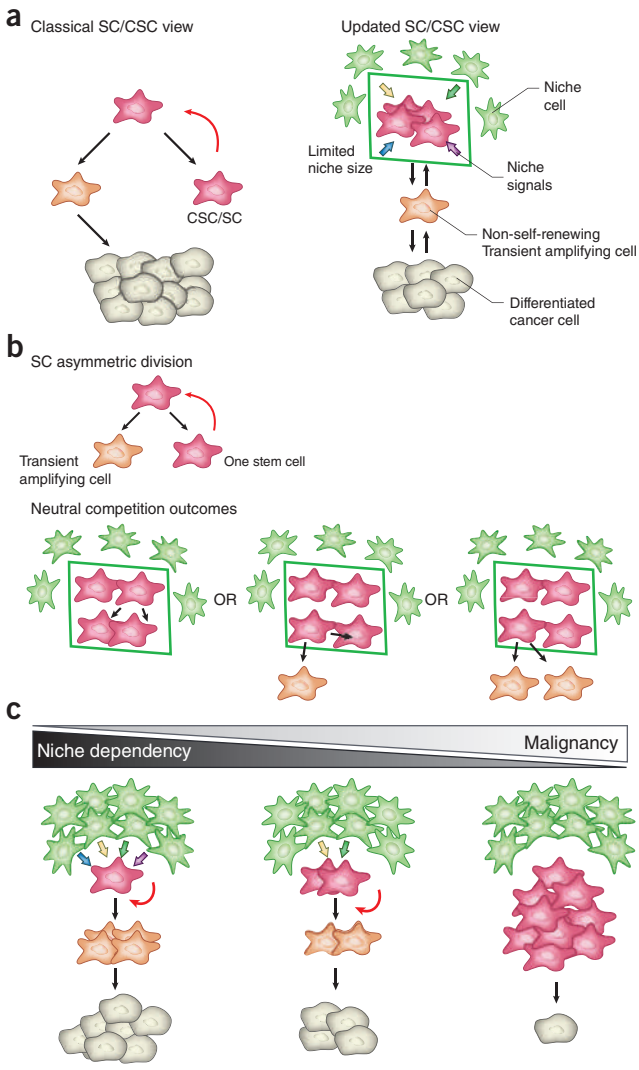

Figure 1 Emerging concepts in stem cell and CSC (stem cell/CSC) biology. (**a**) Left, a canonical hardwired stem cell/CSC hierarchy. In this type of cell hierarchy, stem cells/CSCs are rare, relatively quiescent and largely defined by intrinsic properties. Upon asymmetric division, they give rise to one stem cell and one transient amplifying (TA) cell. The latter divides rapidly, yet it is not capable of self-renewal and eventually undergoes differentiation. Nonstem cells are poorly tumorigenic and display limited functional plasticity. Right, novel features of stem cell/CSCs hierarchies; stem cells/CSCs are not necessarily rare or quiescent and are instructed by niche signals following neutral competition dynamics. TA cells and differentiated cells can be reprogrammed into stem cells by the niche through plasticity. (**b**) Modes of stem cell division. The outcome of asymmetric cell divisions is pre-established, whereas in neutral competition dynamics, the fate of (cancer) stem cell daughters is determined by niche signals, and therefore, stem cells can give rise to one, two, or no daughter stem cells, depending on the available niche space. Number of stem cells is determined by the size of the niche, i.e., in the example, the niche has available space for four stem cells, and thus stem cell progeny compete to occupy such space. Only cells that remain within the niche are specified as stem cells, whereas those that linger outside the niche undergo differentiation. (**c**) Stem cell niche factor dependency and tumor progression. In tissues such as the colon, tumor progression occurs through the acquisition of genetic alterations in the same signaling pathways that sustain self renewal of normal ISCs. This process has two consequences; first, as the tumor evolves, mutations render CSCs progressively independent of niche signals. Second, the autonomous CSC phenotype impedes differentiation, resulting in a shallow hierarchy with many CSCs and few non-CSCs.

template may exist for a given adult stem cell hierarchy, and, consequently, for a CSC-driven tumor hierarchy.

Adult stem cells can be abundant in their niches, such as in the epidermis[26] and intestinal crypts—up to 10% of crypt cells are intestinal stem cells expressing *Lgr5* (ref. 32). Adult stem cells are not obligatorily quiescent, and can actively divide throughout life, as seen in the stomach pylorus[33] and intestinal crypts[32]. In cases in which stem cell dynamics have been carefully quantified, it has been found that stem cell daughters do not display intrinsically divergent fates, but, rather, that a stem cell division can result in zero, one, or two new stem cells, depending on the available niche space (**Fig. 1a**,**b**). In other words, the stem cell progeny compete to occupy the niche, a process known as neutral competition. This mechanism contrasts sharply with the 'classical' model of the existence of a stem cell–intrinsic, asymmetric mitotic process, in which every stem cell division invariably creates one new stem cell and one daughter cell (**Fig. 1a**,**b**). The neutral competition model has been described in the epidermis[26,34], the stomach[35], and in intestinal crypts[36,37]. Stem cell hierarchies can be extensively plastic (see 'The plasticity of adult stem cells'), meaning that daughter cells—and even fully differentiated cells—can re-enter the niche and dedifferentiate to replace lost stem cells (**Fig. 1a**,**b**). Finally, fully differentiated cells (such as hepatocytes) can enter the cell cycle to replace lost tissue upon hemi-hepatectomy, without ever reverting to a distinct stem cell phenotype[38]. In sum, with some notable exceptions, such as muscle satellite cells[39] and hair-follicle stem cells[40], the search for rare, quiescent, hardwired professional stem cells in solid tissues has been less than fruitful. Apparently, evolution has resulted in many different ways of maintaining and repairing our organs.

The plasticity of adult stem cells

Lineage-tracing approaches have revealed that the potential of committed cells to move up and down the hierarchy of differentiation ('plasticity') is more widespread than previously thought. For example, the ablation of ISCs expressing *Lgr5* in the mouse intestine does not visibly affect the integrity of the epithelium[41]. In fact, committed progenitors of the secretory lineage[42,43] and of the abundant enterocyte lineage[44] readily revert into multipotent, long-lived Lgr5$^+$ stem cells upon the loss of resident ISCs. This can be understood if we consider the signals emanating from the niche: at the crypt base, ISCs receive WNT factors that promote a self-renewing undifferentiated state, obtain NOTCH ligands from neighboring cells necessary to block differentiation toward the secretory lineage, and receive potent mitogenic stimuli that trigger the activity of epidermal growth factor receptor (EGFR). The niche also protects ISCs from cytostatic and differentiation signals, such as those imposed by the bone-morphogenetic protein (BMP) and the transforming growth factor-β (TGF-β) signaling pathways. When niche signals, i.e., WNT, NOTCH, and EGFR ligands in combination with BMP/TGF-β inhibitors, are provided to progenitor cells *in vitro*, these cells rapidly regain stemness[45]. It thus seems that the proximity of committed cells to the niche at the crypt base instructs them to revert to a multipotent stem cell fate.

In other examples, lineage-tracing experiments of epithelial basal cells of the mouse adult trachea have shown that these cells self-renew and generate two differentiated cell types (club cells and ciliated cells), a finding that places basal cells at the top of the epithelial hierarchy[46]. However, differentiated club cells readily revert into functional stem cells *in vivo* upon the ablation of basal stem cells[47]. Regeneration of the proximal tubule epithelium of the kidney following ischemic reperfusion injury similarly involves the dedifferentiation of epithelial cells[48].

Therefore, plasticity in an individual tissue stem cell hierarchy may be much more common than previously appreciated.

Redefining CSC properties

Although this review discusses evidence that supports the existence of CSC hierarchies in many prevalent tumor types, there is an increasing appreciation that not every cancer adheres to the CSC model. An early skeptical report showed that, depending on the technology applied, the xenotransplantation frequency of melanoma-initiating cells could vary dramatically[49]. Under optimal conditions, up to 25% of human melanoma cells could form xenografts, which raises the possibility that either these tumors do not follow a stem cell hierarchy, or that most cells in advanced melanomas behave as CSCs[49]. As another example, the analysis of clonal dynamics during serial xenotransplantation of pancreatic cancer samples indicates that long-term growth is not driven by CSCs, but rather by the successive activation of transiently active tumor-initiating cells[50].

Plasticity of CSCs

Several studies have provided evidence that both CSCs and non-CSCs are plastic and capable of undergoing phenotypic transitions in response to appropriate stimuli. This notion is exemplified by a study in which cell populations displaying stem cell–, basal-, or luminal-like phenotypes were isolated from breast cancer cell lines[51]. *In vitro*, all three subpopulations were able to generate cells of the other two phenotypes, such that the cultures converged over time toward the proportions of cell types observed in the breast cancer line of origin. This phenotypic interconversion was stochastic and not determined by the cell phenotype of origin. Importantly, these cell phenotypes were functionally meaningful, because only the stem cell–like cells generated tumors efficiently upon xenotransplantation under standard conditions—the defining property of CSCs (see **Box 1**). By contrast, when certain environmental stimuli were modified (such as when the tumor cells were co-inoculated with irradiated cells), stem cell–, basal-, and luminal-like phenotypes were equally tumorigenic, and each tumor cell subpopulation could generate xenografts. Thus, CSC and non-CSC states are not hardwired in this model. Rather, the tumorigenic potential of cells in xenograft assays seems to reflect their adaptation to particular environmental cues.

The role of the microenvironment in CSC plasticity

Further support for the contextual functionality of CSCs has come from models of colorectal cancer. As mentioned above, WNT ligands in the crypt niche are necessary for sustaining the undifferentiated state of ISCs. Most colorectal cancers are initiated by genetic alterations that activate the WNT-signaling pathway constitutively, which imposes a crypt-progenitor phenotype onto colorectal cancer cells[52]. ISCs display increased susceptibility to transformation by activating mutations in the WNT-signaling pathway, as compared to transient amplifying cells or differentiated cells, and have been proposed to be the cells of origin of colorectal cancer[27]. However, during the onset of colorectal cancer, in which there is an inflammatory environment, NF-κB signaling can promote the tumor-initiating potential of nonstem cells by triggering their dedifferentiation[53]. Within individual colorectal cancers, tumor cells display distinct levels of WNT-pathway activity, despite sharing the same activating mutations in downstream WNT-pathway components[54]. The level of WNT signaling correlates with tumor-initiating capacity in xenotransplantation assays[55]. It is proposed that hepatocyte growth factor (HGF), secreted from fibroblasts residing in particular tumor niches, elevates WNT signaling and confers self-renewal and tumorigenic potential to non-CSCs[55].

CSC plasticity has recently been investigated through cell ablation experiments in xenografted human cancers[29,56]. A CRISPR–Cas9 approach was used to insert an inducible version of the suicide-gene caspase 9 (iCasp9) into the *LGR5* locus in human colorectal cancer

organoids[29]. In xenografts produced by these organoids, the induction of apoptosis reduced the tumor size—and yet, upon removal of the inducer, the tumors regrew. The regeneration of the tumor occurred simultaneously with the induction of proliferation in otherwise mitotically arrested, differentiated tumor cells. Lineage-tracing experiments from differentiated tumor cells that express KRT20 demonstrated that these cells regained proliferative potential and restored the LGR5$^+$ CSC pool—tell-tale signs of plasticity.

In another study, mouse colorectal cancer organoids were engineered to express the diphtheria-toxin receptor under the control of the LGR5 locus[56]. Ablation of Lgr5$^+$ CSC cells in orthotopically xenografted colorectal cancer organoids halted tumor growth, but, similarly to the above study, authors observed that tumors resumed growth upon the cessation of diphtheria-toxin treatment. This response was accompanied by a re-emergence of the cell population, indicative of plasticity[56]. Importantly, this regenerative response was not present in metastatic lesions[56], implying that the plasticity of non-CSCs is differentially regulated by the microenvironment present at primary as opposed to metastatic sites. This result suggests that genetic ablation of Lgr5$^+$ cells in metastatic lesions may result in long-lasting therapeutic effects.

In sharp contrast with the above examples, the hierarchical organization of glioblastoma is proposed to be unidirectional and largely irreversible. In a mouse model of glioblastoma, the ablation of CSCs halted tumor growth and prolonged survival without apparent regeneration of the CSC pool from other glioblastoma cells[15]. Indeed, one recent study identified a core set of transcription factors (*POU3F2*, *SOX2*, *SALL2*, and OLIG2) essential for the propagation of glioblastoma CSCs[57]. The differentiated glioblastoma cells could be reprogrammed into fully tumorigenic CSCs only upon re-expression of these four transcription factors[57].

CSCs and the epithelial-to-mesenchymal transition

Epithelial cancer cells can acquire a mesenchymal gene program that facilitates migration and invasion, a process known as epithelial-to-mesenchymal transition (EMT)[58–60]. Over the past few years, the connection between CSCs and EMT has attracted considerable attention. It is known that overexpression of EMT transcription factors not only enforces a mesenchymal-migratory phenotype, but also exacerbates the tumor-initiating potential of cell lines[61–63]. Importantly, tumor cells with elevated endogenous levels of SNAI1, the EMT master transcription factor, also displayed enhanced tumor-initiating capacity and metastatic potential in mouse and human models[64]. In breast cancer, these observations were initially interpreted to indicate that CSC properties induced by EMT echoed the mesenchymal-like phenotype of the normal stem cells that reside in the basal layer of the mammary epithelium[65]. Yet, a more detailed analysis of this process in mouse MMTV-PyMT promoter-driven breast tumors revealed different roles for EMT transcription factors in mammary stem cells from those in breast CSCs, which apparently differ in their EMT programs[64].

It is puzzling that metastases in many carcinoma types retain an epithelial organization and lack mesenchymal traits, which, in principle, implies that migratory tumor cells revert to the epithelial state upon reaching the foreign organ[66], or even that EMT is not necessary for metastasis in certain contexts, i.e., that epithelial tumor cells can migrate without adopting a mesechymal phenotype. In support of the former, cells frozen in a permanent EMT state are poorly metastatic[67,68], whereas their return to an epithelial state, mediated by the silencing of EMT inducers, is required for efficient metastatic outgrowth in experimental models[69,70]. Indeed, intravital imaging of breast cancer xenografts revealed that migratory cells, having undergone EMT, immediately reverted to an epithelial state upon reaching the metastatic site[71]. In addition, it was shown that distinct levels of the EMT master transcription factor TWIST1 regulate CSC properties and tumor progression in mouse models of skin cancer[72]. All these findings are at odds with the hypothesis that EMT is necessary to sustain the CSC phenotype, and they imply that EMT is uncoupled from stemness in many contexts.

Two observations may, however, reconcile these disparate views on the roles of the EMT in CSCs. First, EMT in cancer cells may be transient because epithelial tumor cells can adopt—depending on environmental cues—intermediate mesenchymal states that are reversible[66]. These transitions would result in a plastic CSC phenotype. For example, human basal breast cancer cells transit between non-CSC and CSC states, depending on the expression of the EMT inducer ZEB1. Non-CSCs maintain the ZEB1 promoter in a bivalent chromatin configuration, which enables cells to respond rapidly to EMT-inducing signals from the microenvironment, and, consequently, to enhance their tumor-initiating capacity[73]. Second, it has been shown that transient expression of TWIST1 primes mammary cells toward a CSC-like state that persists after TWIST1 activity is switched off and the cells have returned to the epithelial phenotype[74].

Taken together, these and other studies suggest that in many cancer types, CSC hierarchies are not rigid. Rather, interconversion of CSCs and non-CSCs might be a relatively common phenomenon that is driven by environmental stimuli, or simply by stochasticity.

CSC metabolism

Metabolism based on oxidative phosphorylation (OxPhos) is crucial for the generation of energy sufficient to support the maintenance of complex tissues. But this process also produces reactive oxygen species (ROS), which have the potential to cause stem cell dysfunction. Conventional wisdom holds that stem cells avoid OxPhos and perform glycolysis as a form of protection against ROS. Again, this concept has arisen from observations revealing HSCs that are quiescent, reside in relatively hypoxic niches, and employ glycolytic metabolism[75,76]. Proliferation and the subsequent generation of differentiated progeny by HSCs coincide with a switch to OxPhos and increased ROS production[77]. Increasing OxPhos in long-term HSCs (LT-HSCs) leads to loss of quiescence and exhaustion of the HSC pool[77,78].

These findings might not hold for the metabolic patterns of other adult stem cells. Muscle stem cells (satellite cells) are deeply quiescent, similar to LT-HSCs, yet they localize to aerobic niches close to capillary vessels. They use OxPhos, mainly through mitochondrial fatty acid oxidation (FAO)[79]. Paradoxically, the progression of satellite cells toward more committed states coincides with a switch to glycolytic metabolism, which is linked to epigenetic reprogramming[79]. In the intestinal-crypt niche, oxidative phosphorylation and glycolysis are compartmentalized. Highly proliferative Lgr5$^+$ ISCs display elevated OxPhos, whereas adjacent Paneth cells perform glycolysis while supplying lactate to ISCs for the oxidative metabolism of the latter[80]. Apparently, high ROS in ISCs do not cause damage, but seem rather to induce differentiation signals through the p38-MAPK pathway[80]. Furthermore, Paneth cells sense the organismal nutritional status through the mammalian target of rapamycin complex 1 (mTORC1) activity, and by secreting paracrine factors, they accordingly regulate the renewal of Lgr5$^+$ ISCs[81].

Altered metabolism is a hallmark of cancer and inspires novel therapeutic strategies. Most studies in this field have not considered that, similarly to normal tissues, cancers contain metabolically distinct cell populations. As a case in point, KRAS^{G12D}-mutant pancreatic tumors are mainly glycolytic. Downregulation of the *Kras*^{G12D} allele in genetic mouse models induces massive tumor regression, yet a small subset of resilient cells resists this perturbation and mediates relapse when KRAS^{G12D} is re-expressed[82]. These remaining cells display features of CSCs and rely on OxPhos (unlike the tumor bulk). Inhibitors of

oxidative metabolism blocked tumor relapse upon the re-expression of $KRAS^{G12D}$ in this experimental model[82]. A similar dependency was subsequently found in CSCs of human pancreatic cancer–derived xenografts[83].

In studies comparing CSCs to non-CSCs, no universal metabolic patterns have emerged. CSCs and non-CSCs preferentially use glycolysis or OxPhos, depending on the tumor type and model system used (as reviewed in refs. 84,85). A potential confounding effect in these studies is metabolic plasticity driven by environmental stimuli in the experimental system. For instance, glioma stem cells rely on OxPhos, but switch to glycolysis when oxidative metabolism is inhibited[86]. It was discovered that these brain CSCs adapt to nutrient-restricted conditions by upregulating the high-affinity neuronal glucose transporter GLIT3 (ref. 87). Cell plasticity through EMT has also been linked to metabolic reprogramming. The transcriptional repressor Snail silences the fructose-1,6-biphosphatase (*FBP1*) gene, which, in turn, imposes a glycolytic state onto breast cancer cells during EMT[88]. Likewise, pancreatic tumor cells lacking the EMT transcription factor ZEB1 fail to undergo EMT, and they display an impaired capacity to switch to glycolytic metabolism when OxPhos is inhibited[89]. Of note, many studies use cells cultured under conditions of high glucose and oxygen, favoring glycolysis and precluding the assessment of microenvironment effects.

Metabolic adaptation of CSCs has emerged as a particularly relevant step during metastatic colonization. One study revealed that organ-selective metastatic breast cancer cells display distinctive metabolic programs[90]. Tumor cells with tropism to the liver (but not to the lungs or bone) exhibit reduced glutamine and OxPhos metabolism while transforming glucose-derived pyruvate into lactate, a phenomenon termed the Warburg effect. This metabolic adaptation is mediated by the transcription factor HIF-1α protein[90]. Switching to glycolysis might confer an advantage to metastatic cells, enabling them to colonize gluconeogenic tissues such as the liver. Although most studies focus on glucose metabolism of cancers, two recent reports reveal that some disseminated tumor cells obtain energy through fatty acids, a process that is mediated by the expression of the fatty acid receptor CD36 in a subset of highly aggressive CSCs[91,92]. Palmitic acid, an abundant component of the western diet, boosts the metastatic potential of CSCs in experimental models of oral squamous cell carcinoma[91]. Similarly, $CD36^{+}$ leukemic SCs oxidize fatty acids from the gonadal adipose tissue, which acts as a niche for chemotherapy evasion[92]. Thus, the specific energy requirements of tumor cells, and notably, of CSCs during metastasis, may represent an opportunity to treat the late stages of disease.

CSCs and therapy resistance

Many of the current therapeutic strategies aimed at eliminating cancer cells involve treatment with standard anti-proliferative chemotherapy, which often has limited benefits. The residual population of chemotherapy-resistant tumor cells capable of regenerating the disease (relapse) is thought to be—almost by definition—enriched in CSCs. Chemotherapy and radiation resistance was initially viewed as an intrinsic property of normal stem cells and CSCs, acquired through multiple independent mechanisms, such as the upregulation of drug-efflux pumps, a superior DNA-repair capacity, or enhanced protection against ROS[93–97]. As discussed below, cell plasticity and, in particular, the ability of CSCs to adopt a quiescent state have also emerged as important drivers of drug resistance.

Work published in the 1970s on the hierarchical organization and proliferative heterogeneity of hematological tumors[98–101] predicted that slow-cycling leukemic stem cells cause tumor relapse[100,102,103]. Investigators then observed that leukemic stem cells entered into the cell cycle after chemotherapy, much like normal stem cells. The notion that recurrence after standard chemotherapy results from the persistence of quiescent CSCs has been supported recently by genetic-fate mapping in several solid tumor types. One study indicated that oxaliplatin treatment selectively favored the survival of dormant clones that became dominant after therapy[104]. Slow-cycling CSCs in mouse models of glioblastoma resist temozolomide treatment. Genetic ablation of this cell population renders glioblastomas susceptible to chemotherapy[15]. Signaling by TGF-β, a pleiotropic hormone that in epithelial cells triggers cytostatic signals[105], drives dormancy of disseminated breast cancer cells[106]. Likewise, a TGF-β-rich microenvironment slows the proliferation of CSCs localized at the leading edges of mouse squamous cell carcinomas, which, in turn, confers resistance to cisplatin[16]. Lineage-tracing experiments demonstrated that these quiescent CSCs regenerated the cancer after chemotherapy treatment[16].

In models of human glioblastoma, CSCs evade anti-proliferative therapy by adopting a slow proliferative state, which depends on Notch signaling and requires epigenetic remodeling by the H3K27 demethylases KDM6A/B[107]. In bladder cancer, chemotherapy reactivates quiescent CSCs, which, in turn, repopulate the tumor after treatment[108]. In breast and skin cancer, subpopulations of CSCs undergo EMT, which is associated not only with a migratory phenotype, but also with a slow proliferative state that confers resistance to anti-proliferative drugs[16,109].

Thus, experimental evidence increasingly supports the notion that resident quiescent CSCs indeed cause relapse after initially successful chemotherapeutic treatment. Nevertheless, non-hierarchical tumors may also contain quiescent cells that are resistant to anti-mitotic agents. For example, in melanoma, the chromatin remodeler JARID1b marks a subpopulation of slow-cycling cells required for long-term tumor growth[110] and resistance to cytotoxic therapy[111]. Consistent with the proposed lack of cell hierarchy in melanoma[49], both JARID1b-positive and JARID1b-negative single cells are tumorigenic in xenograft assays. It seems that JARID1b-negative melanoma cells re-express JARID1b and regain their tumorigenic potential, another example of plasticity[110].

Finally, analysis of the repopulation kinetics of highly proliferating tissues such as the stomach and intestine has shown that chemotherapy and radiotherapy ablate the rapidly proliferating stem cell and progenitor pool but spare cell-cycle-arrested differentiated cells[42,43,112,113]. As discussed above, differentiated cells may subsequently replace lost stem cells through plasticity. These observations immediately suggest that the differentiated CSC progeny could represent a source of chemotherapy-resistant quiescent cells that contribute to the recurrence of relapse after treatment.

Therapeutic targeting of CSCs

Despite the caveats that cell plasticity evokes toward the design of anti-CSC therapies, several pharmaceutical companies have launched programs aimed at eliminating this tumor cell population. **Table 1** summarizes the main strategies proposed to interfere with CSCs, some of which are currently being tested in patients. The idea of anti-CSC therapy arose in the 1970s and 1980s from the observation that leukemic cells were blocked in an undifferentiated state, and it is exemplified by the use of all-*trans* retinoic acid to induce terminal differentiation of CSCs[114]—currently the standard of care for the treatment of patients with acute promyelocytic leukemia. The success of all-*trans* retinoic acid therapy inspired other therapies that were based on inhibiting epigenetic regulators to induce CSC differentiation in multiple hematological malignancies (reviewed in ref. 115). As a case in point, in MLL-AF9 oncogene-driven leukemia models, the lysine-specific demethylase LSD1 is required to sustain the tumorigenic program in leukemic stem cells. Knockdown or pharmacological inhibition of LSD1 using tranylcypromine analogs induced CSC differentiation and blocked leukemia

Table 1 Therapeutic strategies against CSCs

Therapy	Potential drawbacks and limitations	Target	Progress to the clinic
Inhibition of key CSC signaling pathways	• Side effects on healthy stem cells that depend on the equivalent signals • Acquisition of resistance mechanisms • Regeneration of the CSC pool by plasticity of non-CSCs upon treatment cessation	WNT pathway	• Inhibitors of upstream WNT-signaling components (PORCN, FZD, anti-RSPO3) in clinical phases for colorectal, pancreas, and other tumor types • PRI-724, an inhibitor of β-catenin/CBP interaction in phase 1 trials for several malignancies (for reviews, see refs. 136,144)
		NOTCH pathway	• Several inhibitors of γ-secretase, NOTCH receptor or NOTCH ligands in distinct clinical phases for multiple cancer types (for reviews, see refs. 144,145)
CSC ablation using antibody–drug conjugates (ADCs)	• Toxicity associated with ADCs • Lack of CSC-specific markers • Depletion of normal stem cells that share surface markers with CSC • Regeneration of CSCs by plasticity of non-CSCs upon treatment cessation • Intratumor heterogeneity in CSC surface-marker expression	Several CSC surface markers, including CD33, LGR5, CD133, and DLL3	• ADC directed against $CD33^+$ leukemic stem cells in AML was given FDA approval but it was later withdrawn due to toxicity[146]. • Many ADCs are being tested in clinical trials[147], but it is unclear whether any target CSCs • Strategies based on bona fide CSC markers (for example, LGR5) remain at preclinical phases
Epigenetic therapy	• Toxicity owing to misregulation of gene expression in healthy stem cells • Knowledge of the epigenetic regulation of CSCs in solid tumors is sparse • Regeneration of the CSC pool by plasticity of non-CSCs upon treatment cessation	Multiple epigenetic regulators, including LSD1, HDACs, DOT1L, BET proteins, and IDH1/2	• Differentiation therapy by all-*trans* retinoic acid is standard of care in APML • HDAC inhibitors approved by FDA for several malignancies. Large number of other epigenetic regulator inhibitors in phase I-III trials for hematological and solid malignancies (for reviews, see refs. 115,148), some of which potentially target CSCs.
Targeting of quiescent CSCs	• Still limited knowledge of specific features of quiescent CSCs	Blockade of specific dependencies, such as metabolic requirements (e.g., anti-CD36 (ref. 91), inhibitors of OxPhos[82,83,111])	• Preclinical research

propagation without causing notable side effects[116]. Currently, LSD1 inhibitors are being tested in phase 1 and 2 trials in individuals with AML. The inhibition of epigenetic regulators also shows promise in the targeting of solid-tumor CSCs, as illustrated by one study demonstrating that genetic downregulation of BMI1—a subunit of the polycomb-repressor complex 1 that controls transcription by chromatin remodeling—abrogated self-renewal of CSCs in models of colorectal cancer[117]. This effect was confirmed using a small molecule, PTC-209, that acts by decreasing BMI1 protein levels in cancer[117].

Genetic ablation of CSCs in models of glioblastoma, squamous cell carcinoma, and colorectal cancer halts tumor growth[15,29,56,118]. These findings are now being translated for application in the clinic. The use of antibodies targeting LGR5 conjugated to cytotoxic drugs has demonstrated therapeutic activity in xenografts and in genetic mouse models of colorectal cancer[119,120]. Likewise, antibody–drug conjugates targeting the NOTCH ligand DLL3 eliminated tumor-initiating cells in xenograft models of pulmonary neuroendocrine cancers[121]. Although an important side effect of this approach may be damage to the normal stem cell pool, therapeutic windows could in principle be extended by targeting CSC-enriched surface genes, or through the conjugation of antibodies to drugs with some selectivity toward cancer cells.

Targeting quiescent CSCs remains a major challenge. One experimental strategy consists of 'waking up' this cell population to increase its susceptibility to chemotherapy. In experimental models of chronic myeloid leukemia (CML), quiescence results from the activity of the ubiquitin ligase FBXW7, which downregulates the levels of MYC[122,123]. Genetic ablation of *Fbxw7* promoted the re-entry of quiescent leukemic stem cells into the cell cycle and rendered the cells susceptible to imatinib[122]. The opposite strategy, i.e., preventing the activation of quiescent cells, has also been successful in experimental models. In bladder cancer, chemotherapeutic treatment raises the levels of prostaglandin E2 (PGE2), which in turn induces the proliferation of dormant CSCs, leading to tumor regeneration[108]. Blockade of PGE2 production using cyclooxygenase-2 (COX2) inhibitors abrogated this response[108]. Although this approach improves the effects of chemotherapy, keeping CSCs forever dormant may not be feasible in patients. Direct therapeutic elimination of quiescent CSCs awaits a better understanding of their vulnerabilities. For instance, several studies have shown that quiescent CSCs rely on oxidative metabolism and that inhibition of OxPhos depletes this cell population and improves responses to chemotherapeutics and targeted therapies in mouse models of cancer.[82,83,111]. Another promising example is eliminating quiescent metastatic stem cells by blocking the function of the fatty acid receptor CD36 (ref. 91).

Targeting the CSC niche

If plasticity is as extensive in tumor cells as it is in some healthy tissues, CSCs will always be re-created, as long as the tumor stem cell niche remains intact (**Fig. 2**). Thus, either CSC elimination has to be continuous to capture any newly formed CSCs, or the niche should be targeted. The latter approach may actually present an attractive alternative to directly targeting CSCs. The intestinal crypt is arguably the best-characterized stem cell niche[1]. It provides WNT and EGF signals that maintain resident stem cells, but these niche factors can also instruct progenitor cells to revert to a stem cell state when the original stem cells are lost[42–45]. Some colon CSCs still rely on stem cell–niche signals. For instance, EGFR–EGF-inhibitor therapies are effective in a subset of colon cancers—at least until tumor cells develop resistance—and, in our opinion, they should be considered as drugs that target the tumor cell niche.

A major advance toward this goal has been the recent generation of drug-like inhibitors of WNT signals, a pathway that sustains stemness in several healthy tissues, as well as in CSCs of multiple tumor types[124,125]. A subset of colon and pancreas cancers depends on paracrine WNT signaling to drive expression of their stem cell program. These tumors carry inactivating mutations in the ubiquitin ligase RNF43, which operates as a negative-feedback loop in the WNT cascade at the level of

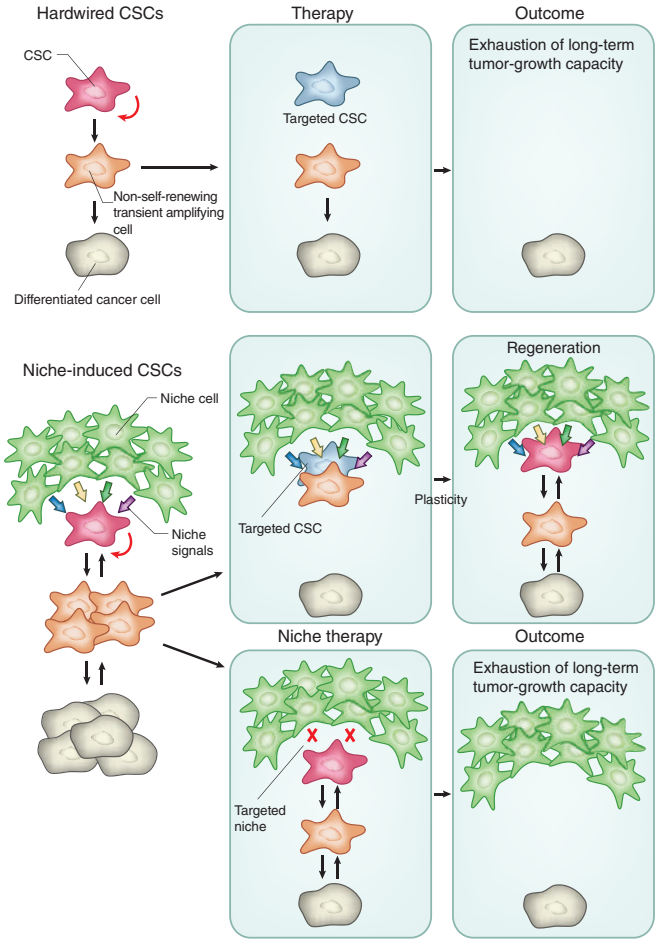

Figure 2 Consequences of anti-CSCs therapies. In tumors displaying a unidirectional hardwired CSC hierarchy, elimination of CSCs is sufficient to cure the disease. In the case of CSCs having extensive cell plasticity, niche signals will re-instruct stem cell properties to progenitor or differentiated cells after CSC loss, which will result in tumor regeneration and therapy failure. Blocking niche signals that specify or sustain CSC identity will be more effective for this class of tumors and may improve therapeutic efficacy by preventing plasticity and CSC regeneration.

WNT receptors. Blockade of Wnt secretion by small molecules that inhibit the activity of PORCN—an o-acyl transferase of WNT factors required for their secretion (reviewed in ref. 126)—effectively blocks extracellular WNT signaling and has dramatic therapeutic effects in colorectal and pancreatic organoid and xenograft models[127–129]. Recently, PORCN inhibitors have also been shown to suppress the progression of lung adenocarcinomas in genetic mouse models when the renewal of a WNT-dependent population of CSCs is blocked[130]. Several PORCN small-molecule inhibitors are currently being tested in phase 1 clinical trials in individuals with pancreatic or colorectal cancer. Likewise, antibody targeting of the WNT receptors FZD5 and FZD8 reduced the growth of pancreatic and colorectal tumors bearing *RNF43* mutations[131]. A therapeutic anti-FZD antibody (vantictumab) and a WNT decoy, FZD8- (ipafricet), are in phase 1 clinical trials for pancreatic cancer, non-small-cell lung cancer, and breast cancer.

Fusions affecting *RSPO2* and *RSPO3*, two genes that encode ligands for LGR4, LGR5, and LGR6 receptors, occur in up to 10% of colorectal cancers and sustain high levels of WNT signaling in these tumors[132]. Treatment with an anti-RSPO3-blocking antibody triggered loss of stemness, induced differentiation, and produced robust therapeutic responses in preclinical models of colorectal cancer[133]. Rosmantuzumab, an anti-RSPO3 antibody, is in phase 1 clinical trials. PRI-724, a small molecule that disrupts the β-catenin–CBP interaction[134] further downstream in the WNT-signaling cascade, is currently being tested in patients with pancreatic, colorectal, or myeloid leukemias. A plethora of other compounds and therapeutic strategies that target the WNT pathway are still in preclinical phases, but hold promise for the coming years (reviewed in refs. 135,136).

Despite these advances, it is important to consider that the mutational processes that lead to cancer progression often supplant essential stem cell–niche factors. With the advent of organoid and CRISPR technology, it has recently become feasible to grow normal human-colon epithelium *in vitro* and to sequentially introduce four of the most common mutations in colon cancer, i.e., in the *APC*, *KRAS*, *TP53*, and *SMAD4* genes[137,138]. Three of these genes control the three signaling pathways that allow for colon stem cell expansion *in vitro*: loss of APC activates WNT; activated KRAS replaces EGF; and loss of SMAD4 replaces BMP and TGF-β signaling inhibitors. Recent experiments of tumor-organoid transplantation showed that the acquisition of alterations in these four driver pathways is necessary for tumors to metastasize efficiently[139]. This notion is further supported by the analysis of niche-factor dependencies in a collection of organoids derived from adenomas, colorectal cancers, and metastases[140]. Thus, as cells progress along the normal adenoma–carcinoma sequence, they become gradually less dependent on their niches, a process that enables their growth in foreign environments. Coincident with this phenomenon, a progressively larger proportion of tumor cells exhibits CSC-like behavior (**Fig. 1c**), as observed in models of skin cancer and of colorectal cancer[11,56].

Thus, as disease progresses, targeting CSCs through the blockade of their original stem cell–niche signals may not be effective. Interestingly, CSCs develop specific vulnerabilities during metastatic dissemination, and these may represent a promising venue for new therapies. Prime examples are the extracellular-matrix proteins periostin and tenascin, two CSC niche proteins required for efficient breast cancer metastasis to lungs, but dispensable for primary tumor growth[141,142]. As discussed above, the regeneration of Lgr5$^+$ colorectal CSCs by non-CSCs occurs in primary tumors but not in liver metastases, which suggests that anti-CSC therapies might be more effective to treat the disseminated disease[56]. As another example, blockade of the fatty acid receptor CD36 has little effect on the growth of primary oral squamous carcinomas but inhibits the expansion of CSCs in foreign organs[91].

Future directions

There is now overwhelming evidence to support the existence of CSCs in many cancer types, but our understanding of the cell hierarchies present in tumors is still largely shaped by observations made of sorted, xenotransplanted tumor cells. The implementation of new technologies, such as CRISPR–Cas9, tumor organoids, and intravital imaging, opens up avenues for analyzing CSCs in their intact environment. Emerging studies using these tools have started modifying our perceptions of the features and behavior of CSCs. Paralleling the changing view on the nature of healthy stem cells[17], it is becoming evident that CSCs may not necessarily have to be rare, quiescent, and hardwired.

The simple notion that the removal of resident CSCs would cure cancer might also require rethinking. New insights into stem cell biology complicate—but do not essentially compromise—therapy designs targeting CSCs. In particular, the plasticity of tumor cells does present a major technical challenge. Increased insights into the basis of cell plasticity in normal tissues and tumors are essential for the design of smarter therapies that aim to target CSCs. How does the tumor niche specify the CSC state? To which extent are tumor-cell phenotypes reversible or

interchangeable? What type of 'regenerative' responses occur upon CSC loss in a tumor, and how are these controlled? How are the intermediate states adopted by tumor cells during EMT and the metabolic states of stem cells controlled, and what are their effects? These are burning questions in the CSC and adult stem cell fields alike.

Given the intratumor plasticity on top of the inherent mutability of cancer cells[143], it seems more attractive to modulate stem cell–niche functions rather than to pursue therapies that are based on intrinsic CSC features. Although this strategy is already rendering promising results, in their most aggressive forms, CSCs become independent of normal niche signals[56,139,140] (**Fig. 1c**). The more we learn, the more challenging it seems to outsmart cancer.

ACKNOWLEDGMENTS

Work in the laboratory of E.B. is supported by Fundación Botín and Banco Santander, through Santander Universities and the European Research Council (advanced grant no. 340176). We thank E. Sancho and J. Stanisavljevic for proofreading the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

1. Clevers, H. The intestinal crypt, a prototype stem cell compartment. *Cell* **154**, 274–284 (2013).
2. Doulatov, S., Notta, F., Laurenti, E. & Dick, J.E. Hematopoiesis: a human perspective. *Cell Stem Cell* **10**, 120–136 (2012).
3. Uckun, F.M. *et al.* Leukemic cell growth in SCID mice as a predictor of relapse in high-risk B-lineage acute lymphoblastic leukemia. *Blood* **85**, 873–878 (1995).
4. Lapidot, T. *et al.* A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* **367**, 645–648 (1994).
5. Bonnet, D. & Dick, J.E. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat. Med.* **3**, 730–737 (1997).
6. Al-Hajj, M., Wicha, M.S., Benito-Hernandez, A., Morrison, S.J. & Clarke, M.F. Prospective identification of tumorigenic breast cancer cells. *Proc. Natl. Acad. Sci. USA* **100**, 3983–3988 (2003).
7. O'Brien, C.A., Pollett, A., Gallinger, S. & Dick, J.E. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* **445**, 106–110 (2007).
8. Ricci-Vitiani, L. *et al.* Identification and expansion of human colon-cancer-initiating cells. *Nature* **445**, 111–115 (2007).
9. Dalerba, P. *et al.* Phenotypic characterization of human colorectal cancer stem cells. *Proc. Natl. Acad. Sci. USA* **104**, 10158–10163 (2007).
10. Singh, S.K. *et al.* Identification of human brain tumour initiating cells. *Nature* **432**, 396–401 (2004).
11. Driessens, G., Beck, B., Caauwe, A., Simons, B.D. & Blanpain, C. Defining the mode of tumour growth by clonal analysis. *Nature* **488**, 527–530 (2012).
12. Schepers, A.G. *et al.* Lineage tracing reveals Lgr5$^+$ stem cell activity in mouse intestinal adenomas. *Science* **337**, 730–735 (2012).
13. Kozar, S. *et al.* Continuous clonal labeling reveals small numbers of functional stem cells in intestinal crypts and adenomas. *Cell Stem Cell* **13**, 626–633 (2013).
14. Zomer, A. *et al.* Intravital imaging of cancer stem cell plasticity in mammary tumors. *Stem Cells* **31**, 602–606 (2013).
15. Chen, J. *et al.* A restricted cell population propagates glioblastoma growth after chemotherapy. *Nature* **488**, 522–526 (2012).
16. Oshimori, N., Oristian, D. & Fuchs, E. TGF-β promotes heterogeneity and drug resistance in squamous cell carcinoma. *Cell* **160**, 963–976 (2015).
17. Clevers, H. STEM CELLS. What is an adult stem cell? *Science* **350**, 1319–1320 (2015).
18. Clevers, H. The cancer stem cell: premises, promises and challenges. *Nat. Med.* **17**, 313–319 (2011).
19. Collins, A.T., Berry, P.A., Hyde, C., Stower, M.J. & Maitland, N.J. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res.* **65**, 10946–10951 (2005).
20. Li, C. *et al.* Identification of pancreatic cancer stem cells. *Cancer Res.* **67**, 1030–1037 (2007).
21. Zhang, S. *et al.* Identification and characterization of ovarian cancer-initiating cells from primary human tumors. *Cancer Res.* **68**, 4311–4320 (2008).
22. Eramo, A. *et al.* Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ.* **15**, 504–514 (2008).
23. Nassar, D. & Blanpain, C. Cancer stem cells: basic concepts and therapeutic implications. *Annu. Rev. Pathol.* **11**, 47–76 (2016).
24. Meacham, C.E. & Morrison, S.J. Tumour heterogeneity and cancer cell plasticity. *Nature* **501**, 328–337 (2013).
25. Mascré, G. *et al.* Distinct contribution of stem and progenitor cells to epidermal maintenance. *Nature* **489**, 257–262 (2012).
26. Clayton, E. *et al.* A single type of progenitor cell maintains normal epidermis. *Nature* **446**, 185–189 (2007).
27. Barker, N. *et al.* Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature* **457**, 608–611 (2009).
28. Cortina, C. *et al.* A genome editing approach to study cancer stem cells in human tumors. *EMBO Mol. Med.* **9**, 869–879 (2017).
29. Shimokawa, M. *et al.* Visualization and targeting of LGR5$^+$ human colon cancer stem cells. *Nature* **545**, 187–192 (2017).
30. Merlos-Suárez, A. *et al.* The intestinal stem cell signature identifies colorectal cancer stem cells and predicts disease relapse. *Cell Stem Cell* **8**, 511–524 (2011).
31. Dalerba, P. *et al.* Single-cell dissection of transcriptional heterogeneity in human colon tumors. *Nat. Biotechnol.* **29**, 1120–1127 (2011).
32. Barker, N., *et al.* Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* **449**, 1003–1007 (2007).
33. Barker, N. *et al.* Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. *Cell Stem Cell* **6**, 25–36 (2010).
34. Doupé, D.P., Klein, A.M., Simons, B.D. & Jones, P.H. The ordered architecture of murine ear epidermis is maintained by progenitor cells with random fate. *Dev. Cell* **18**, 317–323 (2010).
35. Leushacke, M., Ng, A., Galle, J., Loeffler, M. & Barker, N. Lgr5$^+$ gastric stem cells divide symmetrically to effect epithelial homeostasis in the pylorus. *Cell Reports* **5**, 349–356 (2013).
36. Snippert, H.J. *et al.* Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell* **143**, 134–144 (2010).
37. Lopez-Garcia, C., Klein, A.M., Simons, B.D. & Winton, D.J. Intestinal stem cell replacement follows a pattern of neutral drift. *Science* **330**, 822–825 (2010).
38. Stanger, B.Z. Cellular homeostasis and repair in the mammalian liver. *Annu. Rev. Physiol.* **77**, 179–200 (2015).
39. Brack, A.S. & Rando, T.A. Tissue-specific stem cells: lessons from the skeletal muscle satellite cell. *Cell Stem Cell* **10**, 504–514 (2012).
40. Hsu, Y.C., Li, L. & Fuchs, E. Emerging interactions between skin stem cells and their niches. *Nat. Med.* **20**, 847–856 (2014).
41. Tian, H. *et al.* A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. *Nature* **478**, 255–259 (2011).
42. van Es, J.H. *et al.* Dll1+ secretory progenitor cells revert to stem cells upon crypt damage. *Nat. Cell Biol.* **14**, 1099–1104 (2012).
43. Buczacki, S.J. *et al.* Intestinal label-retaining cells are secretory precursors expressing Lgr5. *Nature* **495**, 65–69 (2013).
44. Tetteh, P.W. *et al.* Replacement of lost Lgr5-positive stem cells through plasticity of their enterocyte-lineage daughters. *Cell Stem Cell* **18**, 203–213 (2016).
45. Sato, T. *et al.* Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature* **469**, 415–418 (2011).
46. Rock, J.R. *et al.* Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc. Natl. Acad. Sci. USA* **106**, 12771–12775 (2009).
47. Tata, P.R. *et al.* Dedifferentiation of committed epithelial cells into stem cells in vivo. *Nature* **503**, 218–223 (2013).
48. Kusaba, T., Lalli, M., Kramann, R., Kobayashi, A. & Humphreys, B.D. Differentiated kidney epithelial cells repair injured proximal tubule. *Proc. Natl. Acad. Sci. USA* **111**, 1527–1532 (2014).
49. Quintana, E. *et al.* Efficient tumour formation by single human melanoma cells. *Nature* **456**, 593–598 (2008).
50. Ball, C.R. *et al.* Succession of transiently active tumor-initiating cell clones in human pancreatic cancer xenografts. *EMBO Mol. Med.* **9**, 918–932 (2017).
51. Gupta, P.B. *et al.* Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. *Cell* **146**, 633–644 (2011).
52. van de Wetering, M. *et al.* The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. *Cell* **111**, 241–250 (2002).
53. Schwitalla, S. *et al.* Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties. *Cell* **152**, 25–38 (2013).
54. Brabletz, T. *et al.* Variable beta-catenin expression in colorectal cancers indicates tumor progression driven by the tumor environment. *Proc. Natl. Acad. Sci. USA* **98**, 10356–10361 (2001).
55. Vermeulen, L. *et al.* Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat. Cell Biol.* **12**, 468–476 (2010).
56. de Sousa e Melo, F. *et al.* A distinct role for Lgr5$^+$ stem cells in primary and metastatic colon cancer. *Nature* **543**, 676–680 (2017).
57. Suvà, M.L. *et al.* Reconstructing and reprogramming the tumor-propagating potential of glioblastoma stem-like cells. *Cell* **157**, 580–594 (2014).
58. Batlle, E. *et al.* The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. *Nat. Cell Biol.* **2**, 84–89 (2000).
59. Cano, A. *et al.* The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. *Nat. Cell Biol.* **2**, 76–83 (2000).
60. Savagner, P., Yamada, K.M. & Thiery, J.P. The zinc-finger protein slug causes desmosome dissociation, an initial and necessary step for growth factor-induced epithelial-mesenchymal transition. *J. Cell Biol.* **137**, 1403–1419 (1997).
61. Mani, S.A. *et al.* The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* **133**, 704–715 (2008).
62. Morel, A.P. *et al.* Generation of breast cancer stem cells through epithelial-mesenchymal transition. *PLoS One* **3**, e2888 (2008).
63. Puisieux, A., Brabletz, T. & Caramel, J. Oncogenic roles of EMT-inducing transcription factors. *Nat. Cell Biol.* **16**, 488–494 (2014).

64. Ye, X. *et al.* Distinct EMT programs control normal mammary stem cells and tumour-initiating cells. *Nature* **525**, 256–260 (2015).
65. Guo, W. *et al.* Slug and Sox9 cooperatively determine the mammary stem cell state. *Cell* **148**, 1015–1028 (2012).
66. Nieto, M.A., Huang, R.Y., Jackson, R.A. & Thiery, J.P. EMT: 2016. *Cell* **166**, 21–45 (2016).
67. Celià-Terrassa, T. *et al.* Epithelial-mesenchymal transition can suppress major attributes of human epithelial tumor-initiating cells. *J. Clin. Invest.* **122**, 1849–1868 (2012).
68. Tran, H.D. *et al.* Transient SNAIL1 expression is necessary for metastatic competence in breast cancer. *Cancer Res.* **74**, 6330–6340 (2014).
69. Tsai, J.H., Donaher, J.L., Murphy, D.A., Chau, S. & Yang, J. Spatiotemporal regulation of epithelial-mesenchymal transition is essential for squamous cell carcinoma metastasis. *Cancer Cell* **22**, 725–736 (2012).
70. Ocaña, O.H. *et al.* Metastatic colonization requires the repression of the epithelial-mesenchymal transition inducer Prrx1. *Cancer Cell* **22**, 709–724 (2012).
71. Beerling, E. *et al.* Plasticity between epithelial and mesenchymal states unlinks EMT from metastasis-enhancing stem cell capacity. *Cell Reports* **14**, 2281–2288 (2016).
72. Beck, B. *et al.* Different levels of Twist1 regulate skin tumor initiation, stemness, and progression. *Cell Stem Cell* **16**, 67–79 (2015).
73. Chaffer, C.L. *et al.* Poised chromatin at the ZEB1 promoter enables breast cancer cell plasticity and enhances tumorigenicity. *Cell* **154**, 61–74 (2013).
74. Schmidt, J.M. *et al.* Stem-cell-like properties and epithelial plasticity arise as stable traits after transient Twist1 activation. *Cell Reports* **10**, 131–139 (2015).
75. Suda, T., Takubo, K. & Semenza, G.L. Metabolic regulation of hematopoietic stem cells in the hypoxic niche. *Cell Stem Cell* **9**, 298–310 (2011).
76. Simsek, T. *et al.* The distinct metabolic profile of hematopoietic stem cells reflects their location in a hypoxic niche. *Cell Stem Cell* **7**, 380–390 (2010).
77. Maryanovich, M. *et al.* An MTCH2 pathway repressing mitochondria metabolism regulates haematopoietic stem cell fate. *Nat. Commun.* **6**, 7901 (2015).
78. Tothova, Z. *et al.* FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell* **128**, 325–339 (2007).
79. Ryall, J.G. *et al.* The NAD^+-dependent SIRT1 deacetylase translates a metabolic switch into regulatory epigenetics in skeletal muscle stem cells. *Cell Stem Cell* **16**, 171–183 (2015).
80. Rodríguez-Colman, M.J. *et al.* Interplay between metabolic identities in the intestinal crypt supports stem cell function. *Nature* **543**, 424–427 (2017).
81. Yilmaz, O.H. *et al.* mTORC1 in the Paneth cell niche couples intestinal stem-cell function to calorie intake. *Nature* **486**, 490–495 (2012).
82. Viale, A. *et al.* Oncogene ablation-resistant pancreatic cancer cells depend on mitochondrial function. *Nature* **514**, 628–632 (2014).
83. Sancho, P. *et al.* MYC/PGC-1α balance determines the metabolic phenotype and plasticity of pancreatic cancer stem cells. *Cell Metab.* **22**, 590–605 (2015).
84. Peiris-Pagès, M., Martinez-Outschoorn, U.E., Pestell, R.G., Sotgia, F. & Lisanti, M.P. Cancer stem cell metabolism. *Breast Cancer Res.* **18**, 55 (2016).
85. Sancho, P., Barneda, D. & Heeschen, C. Hallmarks of cancer stem cell metabolism. *Br. J. Cancer* **114**, 1305–1312 (2016).
86. Vlashi, E. *et al.* Metabolic state of glioma stem cells and nontumorigenic cells. *Proc. Natl. Acad. Sci. USA* **108**, 16062–16067 (2011).
87. Flavahan, W.A. *et al.* Brain tumor initiating cells adapt to restricted nutrition through preferential glucose uptake. *Nat. Neurosci.* **16**, 1373–1382 (2013).
88. Dong, C. *et al.* Loss of FBP1 by Snail-mediated repression provides metabolic advantages in basal-like breast cancer. *Cancer Cell* **23**, 316–331 (2013).
89. Krebs, A.M. *et al.* The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer. *Nat. Cell Biol.* **19**, 518–529 (2017).
90. Dupuy, F. *et al.* PDK1-dependent metabolic reprogramming dictates metastatic potential in breast cancer. *Cell Metab.* **22**, 577–589 (2015).
91. Pascual, G. *et al.* Targeting metastasis-initiating cells through the fatty acid receptor CD36. *Nature* **541**, 41–45 (2017).
92. Ye, H. *et al.* Leukemic stem cells evade chemotherapy by metabolic adaptation to an adipose tissue niche. *Cell Stem Cell* **19**, 23–37 (2016).
93. Borst, P. Cancer drug pan-resistance: pumps, cancer stem cells, quiescence, epithelial to mesenchymal transition, blocked cell death pathways, persisters or what? *Open Biol.* **2**, 120066 (2012).
94. Holohan, C., Van Schaeybroeck, S., Longley, D.B. & Johnston, P.G. Cancer drug resistance: an evolving paradigm. *Nat. Rev. Cancer* **13**, 714–726 (2013).
95. Diehn, M. *et al.* Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature* **458**, 780–783 (2009).
96. Li, X. *et al.* Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. *J. Natl. Cancer Inst.* **100**, 672–679 (2008).
97. Bao, S. *et al.* Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* **444**, 756–760 (2006).
98. Clarkson, B., *et al.* Studies of cellular proliferation in human leukemia .3. Behavior of leukemic cells in three adults with acute leukemia given continuous infusions of 3H-thymidine for 8 or 10 days. *Cancer* **25**, 1237–1260 (1970).
99. Killmann, S.A., Cronkite, E.P., Robertson, J.S., Fliedner, T.M. & Bond, V.P. Estimation of phases of the life cycle of leukemic cells from labeling in human beings *in vivo* with tritiated thymidine. *Lab. Invest.* **12**, 671–684 (1963).
100. Clarkson, B.D. Review of recent studies of cellular proliferation in acute leukemia. *Natl. Cancer Inst. Monogr.* **30**, 81–120 (1969).
101. Clarkson, B. The survival value of the dormant state in neoplastic and normal populations. in *Control of Proliferation in Animal Cells.* (eds. Clarkson, B., Baserga, R.) 945–972 (Cold Spring Harbor Laboratory, New York, NY, 1974).
102. Clarkson, B.D. & Fried, J. Changing concepts of treatment in acute leukemia. *Med. Clin. North Am.* **55**, 561–600 (1971).
103. Cronkite, E.P. Acute leukemia: is there a relationship between cell growth kinetics and response to chemotherapy? *Proc. Natl. Cancer Conf.* **6**, 113–117 (1970).
104. Kreso, A. *et al.* Variable clonal repopulation dynamics influence chemotherapy response in colorectal cancer. *Science* **339**, 543–548 (2013).
105. Massagué, J. TGFβ signalling in context. *Nat. Rev. Mol. Cell Biol.* **13**, 616–630 (2012).
106. Bragado, P. *et al.* TGF-b2 dictates disseminated tumour cell fate in target organs through TGF-β-RIII and p38α/β signalling. *Nat. Cell Biol.* **15**, 1351–1361 (2013).
107. Liau, B.B. *et al.* Adaptive chromatin remodeling drives glioblastoma stem cell plasticity and drug tolerance. *Cell Stem Cell* **20**, 233–246 (2017).
108. Kurtova, A.V. *et al.* Blocking PGE2-induced tumour repopulation abrogates bladder cancer chemoresistance. *Nature* **517**, 209–213 (2015).
109. Creighton, C.J. *et al.* Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. *Proc. Natl. Acad. Sci. USA* **106**, 13820–13825 (2009).
110. Roesch, A. *et al.* A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. *Cell* **141**, 583–594 (2010).
111. Roesch, A. *et al.* Overcoming intrinsic multidrug resistance in melanoma by blocking the mitochondrial respiratory chain of slow-cycling JARID1B(high) cells. *Cancer Cell* **23**, 811–825 (2013).
112. Stange, D.E. *et al.* Differentiated Troy+ chief cells act as reserve stem cells to generate all lineages of the stomach epithelium. *Cell* **155**, 357–368 (2013).
113. Wei, L. *et al.* Inhibition of CDK4/6 protects against radiation-induced intestinal injury in mice. *J. Clin. Invest.* **126**, 4076–4087 (2016).
114. Nowak, D., Stewart, D. & Koeffler, H.P. Differentiation therapy of leukemia: 3 decades of development. *Blood* **113**, 3655–3665 (2009).
115. Stahl, M. *et al.* Epigenetics in cancer: a hematological perspective. *PLoS Genet.* **12**, e1006193 (2016).
116. Harris, W.J. *et al.* The histone demethylase KDM1A sustains the oncogenic potential of MLL-AF9 leukemia stem cells. *Cancer Cell* **21**, 473–487 (2012).
117. Kreso, A. *et al.* Self-renewal as a therapeutic target in human colorectal cancer. *Nat. Med.* **20**, 29–36 (2014).
118. Boumahdi, S. *et al.* SOX2 controls tumour initiation and cancer stem-cell functions in squamous-cell carcinoma. *Nature* **511**, 246–250 (2014).
119. Junttila, M.R. *et al.* Targeting LGR5+ cells with an antibody-drug conjugate for the treatment of colon cancer. *Sci. Transl. Med.* **7**, 314ra186 (2015).
120. Gong, X. *et al.* LGR5-targeted antibody-drug conjugate eradicates gastrointestinal tumors and prevents recurrence. *Mol. Cancer Ther.* **15**, 1580–1590 (2016).
121. Saunders, L.R. *et al.* A DLL3-targeted antibody-drug conjugate eradicates high-grade pulmonary neuroendocrine tumor-initiating cells *in vivo*. *Sci. Transl. Med.* **7**, 302ra136 (2015).
122. Takeishi, S. *et al.* Ablation of Fbxw7 eliminates leukemia-initiating cells by preventing quiescence. *Cancer Cell* **23**, 347–361 (2013).
123. Reavie, L. *et al.* Regulation of c-Myc ubiquitination controls chronic myelogenous leukemia initiation and progression. *Cancer Cell* **23**, 362–375 (2013).
124. Reya, T. & Clevers, H. Wnt signalling in stem cells and cancer. *Nature* **434**, 843–850 (2005).
125. Anastas, J.N. & Moon, R.T. WNT signalling pathways as therapeutic targets in cancer. *Nat. Rev. Cancer* **13**, 11–26 (2013).
126. Nile, A.H. & Hannoush, R.N. Fatty acylation of Wnt proteins. *Nat. Chem. Biol.* **12**, 60–69 (2016).
127. Jiang, X. *et al.* Inactivating mutations of RNF43 confer Wnt dependency in pancreatic ductal adenocarcinoma. *Proc. Natl. Acad. Sci. USA* **110**, 12649–12654 (2013).
128. Koo, B.K., van Es, J.H., van den Born, M. & Clevers, H. Porcupine inhibitor suppresses paracrine Wnt-driven growth of Rnf43;Znrf3-mutant neoplasia. *Proc. Natl. Acad. Sci. USA* **112**, 7548–7550 (2015).
129. van de Wetering, M. *et al.* Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell* **161**, 933–945 (2015).
130. Tammela, T. *et al.* A Wnt-producing niche drives proliferative potential and progression in lung adenocarcinoma. *Nature* **545**, 355–359 (2017).
131. Steinhart, Z. *et al.* Genome-wide CRISPR screens reveal a Wnt-FZD5 signaling circuit as a druggable vulnerability of RNF43-mutant pancreatic tumors. *Nat. Med.* **23**, 60–68 (2017).
132. Seshagiri, S. *et al.* Recurrent R-spondin fusions in colon cancer. *Nature* **488**, 660–664 (2012).
133. Storm, E.E. *et al.* Targeting PTPRK-RSPO3 colon tumours promotes differentiation and loss of stem-cell function. *Nature* **529**, 97–100 (2016).
134. Emami, K.H. *et al.* A small molecule inhibitor of beta-catenin/CREB-binding protein transcription [corrected]. *Proc. Natl. Acad. Sci. USA* **101**, 12682–12687 (2004).
135. Novellasdemunt, L., Antas, P. & Li, V.S. Targeting Wnt signaling in colorectal cancer. A review in the theme: cell signaling: proteins, pathways and mechanisms. *Am. J. Physiol. Cell Physiol.* **309**, C511–C521 (2015).
136. Kahn, M. Can we safely target the WNT pathway? *Nat. Rev. Drug Discov.* **13**, 513–532 (2014).
137. Matano, M. *et al.* Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. *Nat. Med.* **21**, 256–262 (2015).
138. Drost, J. *et al.* Sequential cancer mutations in cultured human intestinal stem cells. *Nature* **521**, 43–47 (2015).
139. Fumagalli, A. *et al.* Genetic dissection of colorectal cancer progression by orthotopic transplantation of engineered cancer organoids. *Proc. Natl. Acad. Sci. USA* **114**, E2357–E2364 (2017).
140. Fujii, M. *et al.* A colorectal tumor organoid library demonstrates progressive loss of niche factor requirements during tumorigenesis. *Cell Stem Cell* **18**, 827–838 (2016).
141. Oskarsson, T. *et al.* Breast cancer cells produce tenascin C as a metastatic niche

component to colonize the lungs. *Nat. Med.* **17**, 867–874 (2011).
142. Malanchi, I. *et al.* Interactions between cancer stem cells and their niche govern metastatic colonization. *Nature* **481**, 85–89 (2011).
143. McGranahan, N. & Swanton, C. Clonal heterogeneity and tumor evolution: past, present, and the future. *Cell* **168**, 613–628 (2017).
144. Takebe, N. *et al.* Targeting Notch, Hedgehog, and Wnt pathways in cancer stem cells: clinical update. *Nat. Rev. Clin. Oncol.* **12**, 445–464 (2015).
145. Andersson, E.R. & Lendahl, U. Therapeutic modulation of Notch signalling--are we there yet? *Nat. Rev. Drug Discov.* **13**, 357–378 (2014).
146. Laszlo, G.S., Estey, E.H. & Walter, R.B. The past and future of CD33 as therapeutic target in acute myeloid leukemia. *Blood Rev.* **28**, 143–153 (2014).
147. de Goeij, B.E. & Lambert, J.M. New developments for antibody-drug conjugate-based therapeutic approaches. *Curr. Opin. Immunol.* **40**, 14–23 (2016).
148. Nervi, C., De Marinis, E. & Codacci-Pisanelli, G. Epigenetic treatment of solid tumours: a review of clinical trials. *Clin. Epigenetics* **7**, 127 (2015).
149. Soriano, P. Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat. Genet.* **21**, 70–71 (1999).
150. Blanpain, C. & Simons, B.D. Unravelling stem cell dynamics by lineage tracing. *Nat. Rev. Mol. Cell Biol.* **14**, 489–502 (2013).
151. Tata, P.R. & Rajagopal, J. Cellular plasticity: 1712 to the present day. *Curr. Opin. Cell Biol.* **43**, 46–54 (2016).
152. Llorens-Bobadilla, E. & Martin-Villalba, A. Adult NSC diversity and plasticity: the role of the niche. *Curr. Opin. Neurobiol.* **42**, 68–74 (2017).
153. Blanpain, C., Lowry, W.E., Geoghegan, A., Polak, L. & Fuchs, E. Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. *Cell* **118**, 635–648 (2004).
154. Morris, R.J. *et al.* Capturing and profiling adult hair follicle stem cells. *Nat. Biotechnol.* **22**, 411–417 (2004).
155. Ito, M. *et al.* Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. *Nat. Med.* **11**, 1351–1354 (2005).
156. Stingl, J. *et al.* Purification and unique properties of mammary epithelial stem cells. *Nature* **439**, 993–997 (2006).
157. Shackleton, M. *et al.* Generation of a functional mammary gland from a single stem cell. *Nature* **439**, 84–88 (2006).
158. Van Keymeulen, A. *et al.* Distinct stem cells contribute to mammary gland development and maintenance. *Nature* **479**, 189–193 (2011).
159. Prater, M.D. *et al.* Mammary stem cells have myoepithelial cell properties. *Nat. Cell Biol.* **16**, 942–950 (2014).
160. Rios, A.C., Fu, N.Y., Lindeman, G.J. & Visvader, J.E. *In situ* identification of bipotent stem cells in the mammary gland. *Nature* **506**, 322–327 (2014).
161. Wuidart, A. *et al.* Quantitative lineage-tracing strategies to resolve multipotency in tissue-specific stem cells. *Genes Dev.* **30**, 1261–1277 (2016).
162. Sun, J. *et al.* Clonal dynamics of native haematopoiesis. *Nature* **514**, 322–327 (2014).
163. Busch, K. *et al.* Fundamental properties of unperturbed haematopoiesis from stem cells in vivo. *Nature* **518**, 542–546 (2015).