Cancer Stem Cells revisited

Eduard Batlle[1,2,3] and Hans Clevers[4]

[1]Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, 08028 Barcelona, Spain

[2]Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain.

[3] CiberONC

[4]Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences, University Medical Center Utrecht and Princess Maxima Center, Utrecht, The Netherlands.

Corresponding authors: eduard.batlle@irbbarcelona.org and h.clevers@hubrecht.eu

Abstract

The Cancer Stem Cell (CSC) concept was proposed five decades ago and states that tumor growth, analogous to 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, yet their identification and eradication is not as obvious as had initially been hoped. Recent 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, yet the first successes of therapies based on the CSC concept are emerging.

Introduction

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 cells that populate these tissues, adult stem cells are long-lived and generate cellular progeny throughout life in order 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 within cancers. It explains clinical observations, such as the almost inevitable recurrence of tumors after initially successful chemo 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[3,4,5], 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 sustain long-term growth.

The aim of this review is to discuss recent developments in the CSC field 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 harbor stem cells in dedicated niches[11-16]. Yet, it has gradually become clear that CSCs (like normal stem cells) do not necessarily have to be rare and/or quiescent, as 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 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**.**

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 (HSCs) research in shaping the adult stem cell- and CSC fields.

Features of hematopoietic stem cells

HSCs were first identified sixty 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, it is rare cell type and it divides infrequently, i.e. it is ‘quiescent’, since DNA replication previous to division carries the risk of mutation. When the HSC divides, it does so in

an asymmetric fashion, yielding one actively dividing daughter cell and a 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 cells, the defining characteristics of HSCs have served as a dominant template to interpret experimental observations of renewal of other mammalian tissues and of 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 Fluorescent Activated Cell Sorting (FACS) of normal human HSCs and their various progenitors[2]. In pioneering studies on 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 in the order of one per million tumor cells[3,4,5]. Using equivalent cell sorting and xenografting approaches, it was shown 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 one hundred $CD44^+CD24^{-/low}$ breast cancer cells could transfer the tumor, even in a serial xenograft setting, whereas tens of thousands of cells with alternative phenotypes could not[6]. Similar studies on other solid tumors such as brain cancer[10], prostate cancer[19], colon cancer[7-9], pancreas cancer[20], ovary cancer[21] and lung cancer[22] rapidly followed. We refer the reader elsewhere for a comprehensive recent overview of these efforts[23].

The standard CSC model

Towards 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, while the bulk of the tumor is composed of non-CSCs, which are only capable of transient proliferation and therefore don't contribute to long-term growth. Third, CSC identity is hard-wired as illustrated by the fact that non-CSCs seldom initiate tumors in xenograft assays. Thus, there is limited plasticity within 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 xeno-transplantation approach to investigating the properties of CSCs carries inherent technical and conceptual limitations (**see Box 1** and references[18,24]). In search of more direct evidence, several groups have studied CSCs in intact tumors through genetic lineage tracing, the gold standard to assess adult stem cell activity *in situ* (**see Box 1**).

Linage 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]. While most labeled tumor 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 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 precursor 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 to intestinal stem cells (ISCs) by exploiting the specific expression of the intestinal stem cell marker gene *Lgr5*[27] at the same time that individual *Apc*-mutant stem cells were labelled red[12]. After the resulting single-colored tumors had grown to a significant size, cells expressing Lgr5 in these tumors were induced to switch their color from red to blue. Blue cells generated large clonal patches within the red tumor, providing evidence for a hierarchical organization of adenoma growth *in vivo*[12]. Unbiased lineage tracing approach based on a mutation-induced mark during DNA replication confirmed the appearance of large dominant clones from adenoma CSCs and 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 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, while others rapidly expanded to become dominant, consistent with extensive intra-tumor 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 largely been restricted to genetic mouse models for solid cancers. A strategy to circumvent this limitation was presented in two recent studies, which implemented CRISPR/Cas9 technology to insert cassettes in 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 (Figure 1). These experiments revealed that LGR5+ colorectal cancer cells produce progeny over long time periods that progressively undergo differentiation, albeit with slower kinetics than their normal 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 remained 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 (Figure 1). They also provide a strategy to analyze CSCs in human organoids and xenografts through classical genetic approaches that so far had only been feasible in animal models.

Novel views on adult stem cells

The HSC-centric interpretation of stem cell biology has been widely adopted, but increasing evidence indicates that no generally applicable template may exist for a given adult stem cell hierarchy, and consequently for a CSC-driven tumor hierarchy.

Adult stem cells can be abundant within their niches such as in the epidermis[26] and intestinal crypts: up to 10% of crypt cells are intestinal stem cells expressing Lgr5[32]. Adult stem cells are not obligatorily quiescent, and can be actively dividing throughout life, as seen in the stomach pylorus[33], and in intestinal crypts[32]. In cases where stem cell dynamics have been carefully quantified, it was found that stem cell daughters do not display intrinsically divergent fates but rather a stem cell division can result in zero, one or two new stem cells, depending on available niche space. 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. The neutral competition model has been described in epidermis[26,34], stomach[35] and intestinal crypts[36 37]. Stem cell hierarchies can be extensively plastic (see below), meaning that daughter cells -and even fully differentiated cells- can re-enter the niche and dedifferentiate to replace lost stem cells. 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, hard-wired professional stem cells in solid tissues has been less than

fruitful. Apparently, evolution has resulted in many different ways to maintain and repair 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 believed. For example, 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 a multipotent, long-lived Lgr5+ stem cells upon loss of resident ISCs. This can be understood if the signals emanating from the niche are considered: 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 towards the secretory lineage and receive potent mitogenic stimuli that trigger the activity of the 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-beta (TGF-beta) signaling pathways. When niche signals, i.e. Wnt, Notch and EGFR ligands combined with BMP/TGF-beta inhibitors, are provided to progenitor cells *in vitro*, these cells rapidly regain stemness[45]. It thus appears that proximity to the niche at the crypt base instructs committed cells to revert to a multipotent stem cell fate.

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

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

Redefining CSC properties

While this review discusses evidences that support 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 applied technology- the xenotransplantation frequency of melanoma initiating cells could differ dramatically[49]. Under optimal conditions, up to 25% of human melanoma cells could form xenografts raising the possibility that either these tumors do not follow a stem cell hierarchy or that most cells in advanced melanomas behave as a CSCs[49]. In a another example, analysis of clonal dynamics during serial xenotransplantation of pancreatic cancer samples indicate 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, 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 towards 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 as only the stem cell-like cells generated tumors efficiently upon xeno-transplantation under standard conditions, the defining property of CSCs (**see box1**). In contrast, when certain environmental stimuli were modified (such as when the tumor cells were co-inoculated with irradiated cells), stem-, basal- and luminal-like phenotypes were equally tumorigenic and xenografts could be generated by each tumor cell subpopulation. Thus, CSC and non-CSC states are not hard-wired in this model. Rather, the tumorigenic potential of cells in xenograft assays appears to reflect 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 to sustain the undifferentiated state of ISCs. Most colorectal cancers are initiated by

genetic alterations that activate the Wnt signaling pathway constitutively, which impose a crypt progenitor phenotype onto colorectal cancer cells[52]. ISCs display increased susceptibility to transformation by activating mutations in the WNT signaling pathway 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-KB signaling can promote tumor initiating potential of non-stem 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 signals 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, induction of apoptosis reduced the tumor size yet - upon removal of the inducer- 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 KRT20+ tumor cells demonstrated that these differentiated cells regained proliferative potential and restored the LGR5+ CSCs 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 cessation of diphtheria toxin treatment. This response was accompanied by reemergence of the LGR5+ cell population, indicative of plasticity[56]. Importantly, this regenerative response was not present in metastasic lesions[56], implying that plasticity of non-CSCs is differentially regulated by the microenvironment present at

primary versus metastastic 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, 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 only be reprogrammed into fully tumorigenic CSCs upon re-expression of these four transcription factors[57].

CSC 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 know that overexpression of EMT transcription factors enforces not only a mesenchymal migratory phenotype, but also exacerbates the tumor-initiating potential of cell lines[61,62,63]. 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 and 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. 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 the return to an epithelial state through 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 TWIST1 levels 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 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 intermediate mesenchymal states that are reversible, depending upon environmental cues[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, enabling 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 towards a CSC-like state that persists after TWIST1 activity is switched off and the cells return to the epithelial phenotype[74].

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 driven by environmental stimuli, or simply by stochasticity.

CSC metabolism

Metabolism based on oxidative phosphorylation (OxPhos) is critical for the generation of sufficient energy to support the maintenance of complex tissues. Yet, it produces reactive oxygen species (ROS), with the potential to cause stem cell dysfunction. Conventional wisdom holds that stem cells avoid OxPhos and perform glycolysis as a way of protection against ROS. Again, this concept has arisen from observations of 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 LT-HSCs leads to loss of quiescence and exhaustion of the HSC pool [77,78].

These findings may not hold for metabolic patterns of other adult stem cells. Muscle stem cells (satellite cells) are deeply quiescent like LT-HSCs, yet localize within aerobic niches close to capillary vessels. They utilize OxPhos, mainly through mitochondrial Fatty Acid Oxidation (FAO)[79]. Paradoxically, progression of satellite cells towards 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 rather appear to induce differentiation signals through the P38 MAPK pathway[80]. Furthermore, Paneth Cells sense the organismal nutritional status through mammalian target of rapamycin complex 1 (mTORC1) activity and regulate accordingly the renewal of Lgr5+ ISCs by secreting paracrine factors[81].

Altered metabolism is a hallmark of cancer and inspires novel therapeutic strategies. Most studies in this field have not considered that, like 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 Kras-G12D re-expression in this experimental model[82]. A similar dependency was subsequently found in CSCs of human pancreatic cancer-derived xenografts [83].

In studies where CSCs have been compared to non-CSCs, no universal metabolic patterns have emerged. CSCs and non-CSCs preferentially use of glycolysis or OxPhos, depending on tumor type and model system used (reviewed in [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 Glut3 [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 display 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 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 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 HIF1a [90]. Switching to glycolysis may represent an advantage for metastatic cells to colonize a gluconeogenic tissue such as the liver. While most studies focus on glucose metabolism of cancers, two recent reports reveal that some disseminated tumor cells in oral squamous cell carcinoma 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]. Likewise 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 CSCs during metastasis may represent an opportunity to treat the late stages of the disease.

CSCs and therapy resistance

Much 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 an enhanced protection against ROS [93,94] [95-97]. As discussed below, cell plasticity and in particular the ability of CSCs to adopt a quiescent state have also emerged as an important driver of drug resistance.

Work published in the 1970s on the hierarchical organization and proliferative heterogeneity of hematological tumors[98,99,100,101] 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 recently been supported 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-beta, a pleiotropic hormone that in epithelial cells triggers cytostatic signals[105], drives dormancy of disseminated breast cancer cells[106]. Likewise, a TGF-beta-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, that –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 that is 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-possitive and -negative single cells are tumorigenic in xenograft assays. It appears that Jarid1b-negative melanoma cells re-express Jarid-1b and regain tumorigenic potential, another example of plasticity[110].

Finally, analysis of the kinetics of repopulation 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 recurrence of relapse after treatment.

Therapeutic targeting of CSCs

Despite the caveats that cell plasticity evokes towards 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 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 acute promyelocytic leukemia patients. The success of All-Trans Retinoic Acid acid therapy inspired therapies 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 propagation without significant side effects[116]. Currently, LSD1 inhibitors are being tested in phase I and II trials in patients with AML. Inhibition of epigenetic regulators also shows promise in the targeting of solid tumor CSCs, as illustrated by one study that demonstrated 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 colorectal cancer models[117]. This effect was confirmed using a small molecule – PTC-209 – that acts by decreasing BMI1 protein levels[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]. Whereas an important side effect of this approach may be damage to the normal stem cell pool, therapeutic windows could be in principle extended by targeting CSC-enriched surface genes or through conjugation of antibodies to drugs with some selectivity towards cancer cells.

Targeting quiescent CSCs remains a major challenge. One experimental strategy consists in 'waking up' this cell population to increase 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 reentry 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 levels of PGE2, which in turn induces proliferation of dormant CSCs leading to tumor regeneration[108]. Blockade of PGE2 production with cyclooxygenase-2 (COX2) inhibitors abrogated this response[108]. Whereas 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[82, 83, 111]. Another promising example is the elimination of quiescent metastatic stem cells by blocking the function of the fatty acid receptor CD36[91].

Targeting the CSC niche

If plasticity amongst tumor cells is as extensive as it is in some healthy tissues, CSCs will always be re-created as long as the tumor stem cell niche remains intact **(Figure 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 a very attractive alternative to direct targeting of 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[42-44,45]. 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 targeting the tumor cell niche.

A major advance towards 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 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 by blocking the renewal of a WNT-dependent population of CSCs[130]. Several PORCN small molecule inhibitors are currently being tested in Phase I clinical trials in pancreatic and colorectal cancer patients. 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-Fc (Ipafricet) are in Phase I clinical trials for pancreatic cancer, non-small cell lung cancer, and breast cancer.

Fusions affecting the RSPO2 and RSPO3 genes, that encode ligands for LGR4/5/6 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 stem-ness, induced differentiation and produced robust therapeutic responses in preclinical models of colorectal cancer [133]. Rosmantuzumab, an anti-RSPO3 antibody is in phase-I clinical trials. PRI-724, a small molecule that disrupts the beta-catenin/CBP interaction[134], further downstream in the Wnt signaling cascade, is currently being tested in patients with pancreatic, colorectal and myeloid leukemias. A plethora of other compounds and therapeutic strategies that target the WNT pathway are still in preclinical phases yet 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 very 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, P53 and SMAD4 genes[137,138]. Three of these genes control the three signaling pathways that allow colon stem cell expansion *in vitro*: Loss of APC activates Wnt; activated KRAS replaces EGF and loss of SMAD4 replaces BMP and TGF-beta signaling inhibitors. Recent experiments of tumor organoid transplantation showed that acquisition of alterations in these four driver pathways are necessary 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, while cells progress along the normal-adenoma-carcinoma sequence, they become gradually less dependent on their niches, a process that allows growth in foreign environments. Coincident with this phenomenon, a progressively larger proportion of tumor cells exhibit CSC-like behavior **(Figure 1c)**, as observed in models of skin cancer and of colorectal cancer [11,56].

Thus, as the disease progresses, targeting CSCs through blockade of their original stem cell niche signals may not be effective. Interestingly, CSCs develop specific vulnerabilities during metastatic dissemination that 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, regeneration of LGR5+ colorectal CSCs by non-CSCs occurs in primary tumors but not in liver metastases, which suggest 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 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 on sorted, xeno-transplanted tumor cells. The implementation of new technologies such as CRISPR/Cas9, tumor organoids and intravital imaging opens up avenues to analyze CSCs in their intact environment. Emerging studies using these tools have started modifying our perception 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 hard-wired.

The simple notion that removal of resident CSCs will cure cancer may also require rethinking. New insights into stem cell biology complicate –but do not essentially compromise- therapy designs targeting CSCs. In particular, 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 smarter designs of 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 within 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 fields and in the adult stem cell fields alike.

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

ACKNOWLEDGMENTS

Work in the laboratory of Eduard Batlle is supported by Fundación Botín and Banco Santander, through Santander Universities and the European Research Council (advanced grant 340176). We apologize to authors whose papers could not be cited due to space restrictions.

BOX 1 - Transplantation versus 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 sufficient low to limit the formation of a xenografts by the bulk tumor cell population. The capacity to initiate a tumor of a given cell population in this 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 to analyze CSCs in solid cancers: In general, leukemias have limited genetic changes and harbor restricted intra-tumor genetic heterogeneity, as compared to most solid cancers. Thus, the a priori assumption of the FACS sorting/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 cancers. While the sorting strategies in leukemias are founded on an extensive knowledge of marker combinations that define the various normal HSC/progenitor cell types, such knowledge is often absent for the tissue of origin of many types of solid cancers. 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 within 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 of CSC autonomous properties, or even simply reflect 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 (e.g. Cre), which in turn allows stable activation of a reporter for the pertinent recombinase (e.g. 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 the 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 only generate 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 in lineage tracing experiments to study mammary stem cells has generated controversy on the identity of this particular cell type[160,161.]

Similar discrepancies were encountered when HSCs were analyzed by transplantation versus 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 appeared 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 haematopoiesis is largely maintained by multipotent progenitors cells, which are only capable of transiently reconstituting the blood lineages in classical transplantation assays yet appear to be long-lived in analysis of intact hematopoiesis by lineage tracing.

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

FIGURE LEGENDS

Figure 1. Emerging concepts in stem cell/CSC biology. a. Left panel illustrates a canonical hardwired stem cell/CSC hierarchy. In this type of cell hierarchy, stem cells/CSCs are rare, relative 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. Non-stem cells are poorly tumorigenic and display limited functional plasticity. Right panel represents novel features of stem cell/CSCs hierarchies; stem cells/CSCs are not necessarily rare or quiescent and are instructed by niche signals following a 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 none 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, niche has available space for four stem cells, and therefore stem cell progeny competes 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 involves genetic alterations in the same signaling pathways that sustain self-renewal of normal stem cells. 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.

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 extensive cell plasticity, niche signals will re-instruct stem cell properties on 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.

TABLE I. 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 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 beta-catenin/CBP interaction in phase-I trials for several malignancies (for reviews see refs [136,144])
		NOTCH pathway	• Several inhibitors of gamma-secretase and Notch receptor/ligands in distinct clinical phases for multiple cancer types (for reviews see refs [144,145])
CSC ablation using antibody-drug conjugates (ADC)	• Toxicity associated to 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	Several CSC surface markers including CD33, LGR5, CD133, 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 if any target CSCs • Strategies based on bona-fide CSC markers (e.g. Lgr5) remain at preclinical phases.

	• Intratumor heterogeneity in CSC surface marker expression		
Epigenetic therapy	• Toxicity due to misregulation of gene expression in healthy stem cells • Knowledge on the epigenetic regulation of CSCs in solid tumors is sparse • Regeneration of 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 on specific features of quiescent CSCs	Blockade of specific dependencies such as metabolic requirements (ex. anti-CD36[91], inhibitors of OxPhos [82, 83, 111])	• Preclinical research

REFERENCES

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. *Nature medicine* **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 U S A* **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.U.S.A* **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-beta 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. *Nature medicine* **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. Mascre, 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* (2017).
29. Shimokawa, M.*, et al.* Visualization and targeting of LGR5+ human colon cancer stem cells. *Nature* **545**, 187-192 (2017).
30. Merlos-Suarez, 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. *Nature biotechnology* **29**, 1120-1127 (2011).
32. Barker, N.*, et al.* Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* **449**, 1003-1010U1001 (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. Doupe, 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. *Annual review of physiology* **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. *Nature medicine* **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* (2010).
46. Rock, J.R.*, et al.* Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc Natl Acad Sci U S A* **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 U S A* **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* (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 U S A* **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. Melo, F.S.*, et al.* A distinct role for Lgr5+ stem cells in primary and metastatic colon cancer. *Nature* **543**, 676-680 (2017).
57. Suva, 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).

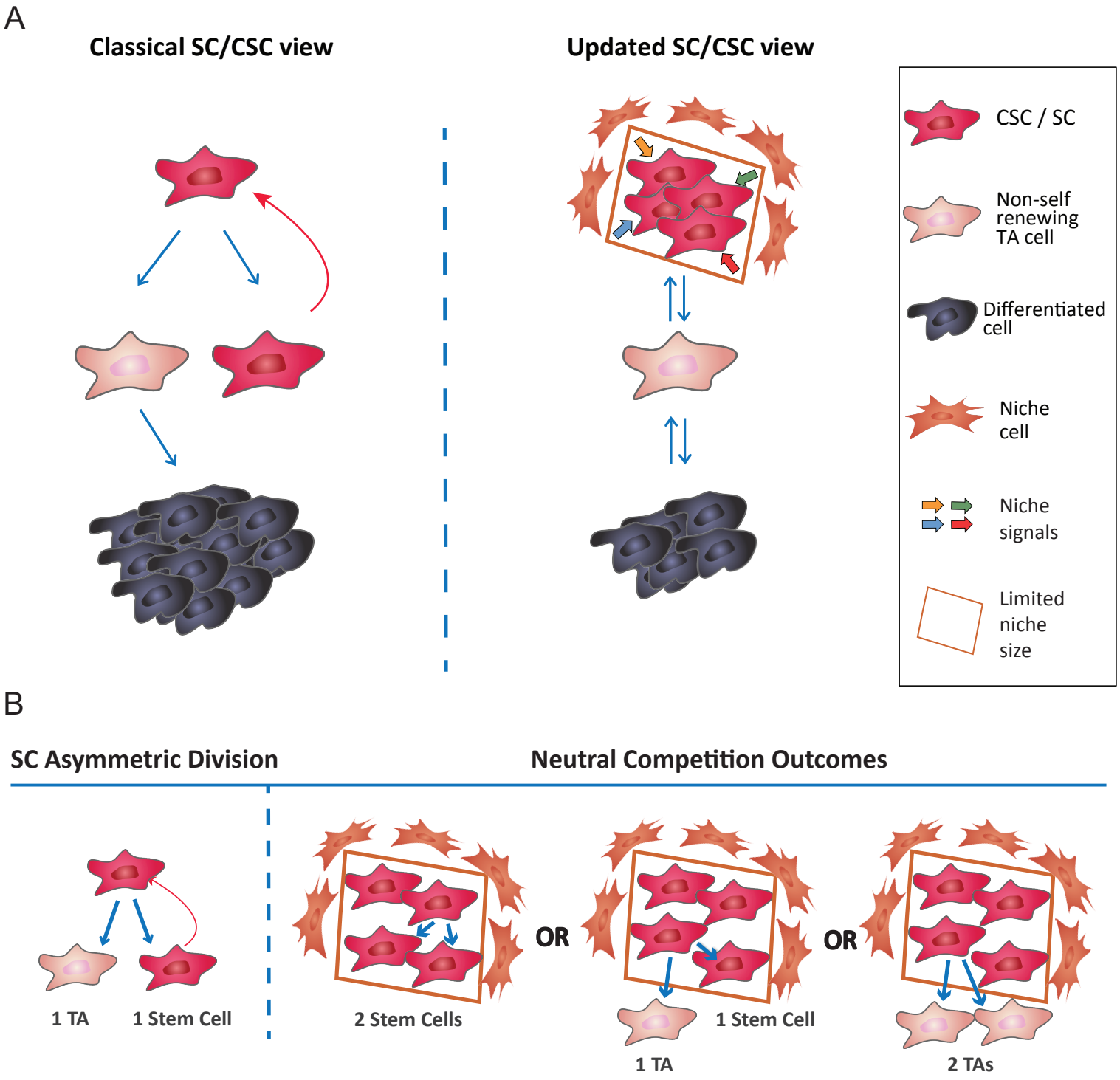
67. Celia-Terrassa, T.*, et al.* Epithelial-mesenchymal transition can suppress major attributes of human epithelial tumor-initiating cells. *J Clin Invest* **122**, 1849-1868 (2012).
68. Tran, H.D.*, et al.* Transient SNAIL1 expression is necessary for metastatic competence in breast cancer. *Cancer Res* **74**, 6330-6340 (2014).
69. Tsai, Jeff H., Donaher, Joana L., Murphy, Danielle A., Chau, S. & Yang, J. Spatiotemporal Regulation of Epithelial-Mesenchymal Transition Is Essential for Squamous Cell Carcinoma Metastasis. *Cancer Cell* **22**, 725-736.
70. Ocaña, Oscar H.*, et al.* Metastatic Colonization Requires the Repression of the Epithelial-Mesenchymal Transition Inducer Prrx1. *Cancer Cell* **22**, 709-724.
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. *Nature communications* **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. Rodriguez-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-1alpha Balance Determines the Metabolic Phenotype and Plasticity of Pancreatic Cancer Stem Cells. *Cell metabolism* **22**, 590-605 (2015).
84. Peiris-Pages, M., Martinez-Outschoorn, U.E., Pestell, R.G., Sotgia, F. & Lisanti, M.P. Cancer stem cell metabolism. *Breast cancer research : BCR* **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 U S A* **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).

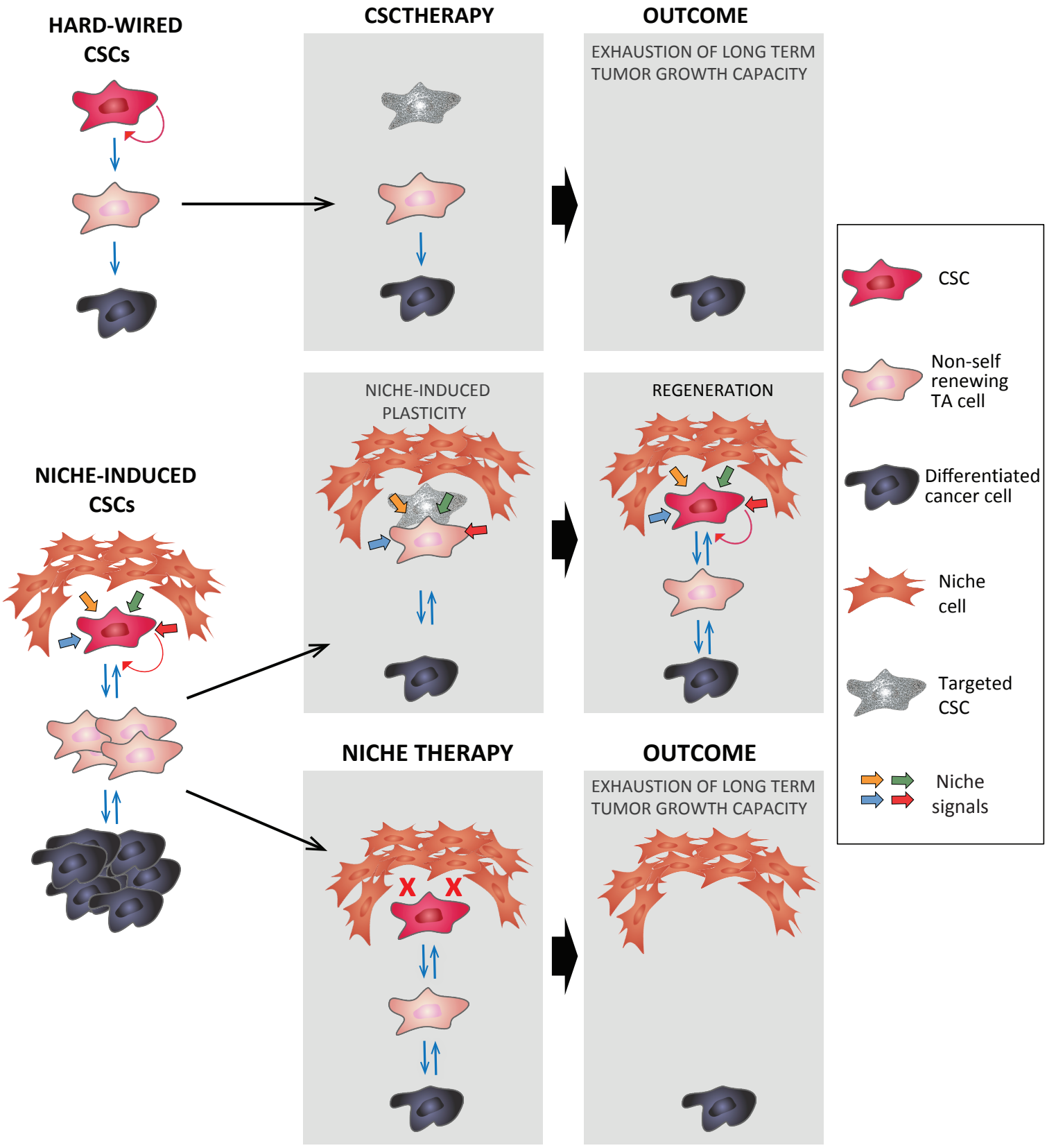
89. Krebs, A.M., *et al.* The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer. *Nat Cell Biol* **19**, 518-529 (2017).
90. Dupuy, F., *et al.* PDK1-Dependent Metabolic Reprogramming Dictates Metastatic Potential in Breast Cancer. *Cell metabolism* **22**, 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 biology* **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 3 Adults with Acute Leukemia Given Continuous Infusions of H-3-Thymidine for 8 or 10 Days. *Cancer* **25**, 1237-& (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 I Monogr*, 81-& (1969).
101. Clarkson, B. The survival value of the dormant state in neoplastic and normal populations. In: Control of Proliferation in Animal Cells. *(Clarkson B and Baserga R, eds). Cold Spring Harbor Laboratory. New York*, 945-972 (1974).
102. Clarkson, B.D. & Fried, J. Changing Concepts of Treatment in Acute Leukemia. *Med Clin N Am* **55**, 561-& (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. Massague, J. TGFbeta signalling in context. *Nat Rev Mol Cell Biol* **13**, 616-630 (2012).
106. Bragado, P., *et al.* TGF-beta2 dictates disseminated tumour cell fate in target organs through TGF-beta-RIII and p38alpha/beta 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* (2016).
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 U S A* **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 genetics* **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. *Nature medicine* **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. *Science translational medicine* **7**, 314ra186 (2015).
120. Gong, X.*, et al.* LGR5-Targeted Antibody-Drug Conjugate Eradicates Gastrointestinal Tumors and Prevents Recurrence. *Molecular cancer therapeutics* **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. *Science translational medicine* **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. *Nature chemical biology* **12**, 60-69 (2016).
127. Jiang, X.*, et al.* Inactivating mutations of RNF43 confer Wnt dependency in pancreatic ductal adenocarcinoma. *Proc Natl Acad Sci U S A* **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 U S A* **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. *Nature medicine* **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 U S A* **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**, C511-521 (2015).
136. Kahn, M. Can we safely target the WNT pathway? *Nature reviews. Drug discovery* **13**, 513-532 (2014).
137. Matano, M.*, et al.* Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. *Nature medicine* **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 U S A* **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. *Nature medicine* **17**, 867-874 (2011).
142. Malanchi, I.*, et al.* Interactions between cancer stem cells and their niche govern metastaic olonization *Nature* **480**, 151-284 (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. *Nature reviews. Clinical oncology* **12**, 445-464 (2015).
145. Andersson, E.R. & Lendahl, U. Therapeutic modulation of Notch signalling--are we there yet? *Nature reviews. Drug discovery* **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 reviews* **28**, 143-153 (2014).
147. de Goeij, B.E. & Lambert, J.M. New developments for antibody-drug conjugate-based therapeutic approaches. *Current opinion in immunology* **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. *Current opinion in cell biology* **43**, 46-54 (2016).
152. Llorens-Bobadilla, E. & Martin-Villalba, A. Adult NSC diversity and plasticity: the role of the niche. *Current opinion in neurobiology* **42**, 68-74 (2016).

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. *Nature biotechnology* **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. *Nature medicine* **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, 941-947 (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).


A
Classical SC/CSC view
Updated SC/CSC view
CSC / SC
Non-self renewing TA cell
Differentiated cell
Niche cell
Niche signals
Limited niche size
B
SC Asymmetric Division
Neutral Competition Outcomes
1 TA
1 Stem Cell
2 Stem Cells
OR
1 Stem Cell
1 TA
OR
2 TAs
C
NICHE DEPENDENCY
MALIGNANCY


HARD-WIRED CSCs
CSCTHERAPY
OUTCOME
EXHAUSTION OF LONG TERM TUMOR GROWTH CAPACITY
NICHE-INDUCED CSCs
NICHE-INDUCED PLASTICITY
REGENERATION
NICHE THERAPY
OUTCOME
EXHAUSTION OF LONG TERM TUMOR GROWTH CAPACITY
X X
CSC
Non-self renewing TA cell
Differentiated cancer cell
Niche cell
Targeted CSC
Niche signals