APPLYING THE PRINCIPLES OF STEM-CELL BIOLOGY TO CANCER

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Why are tumours heterogeneous, in terms of cell phenotype and proliferative potential, even in cases in which all cells are derived from a single clone? Ongoing mutagenesis can partially explain this heterogeneity, but it also seems that some tumours arise from small populations of 'cancer stem cells' that give rise to phenotypically diverse cancer cells, with less proliferative potential. These cancer stem cells are likely to arise from mutations that dysregulate normal stem-cell self-renewal. Using this information, it might be possible to devise more effective therapies.

STEM CELL
A self-renewing, typically multipotent, progenitor with the broadest developmental potential in a particular tissue at a particular time.

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Parallels have long been drawn between somatic STEM CELLS and cancer cells. Both types of cells self-renew, although somatic stem cells do so in a highly regulated manner, whereas cancer cells self-renew in a poorly controlled manner. Both types of cells also differentiate, although somatic stem cells generate normal, mature cells, whereas cancer cells often differentiate abnormally[1]. For example, teratocarcinomas give rise to diverse types of differentiated cells, such as cartilage and bone[1]; medulloblastomas often contain cancer cells that resemble neurons and glia[2]; and myeloid leukaemia cells seem to differentiate into several lineages of blood cells[3–5]. So, somatic stem cells and cancer cells both have organogenic capacity, but somatic stem cells generate normal tissues and cancer cells generate abnormal tissues. These parallels raise the question of whether we can improve cancer therapy by applying the principles of stem-cell biology to understanding tumour development and progression.

For the principles of stem-cell biology to apply to tumorigenesis, cancers would have to be organized hierarchically into clonally derived populations of cells with different proliferative potentials — just like cells within normal tissues. Decades ago, it was found that when cancer cells of many different types were assayed for their proliferative potential in various *in vitro* or *in vivo* assays, only a small minority of cells were able to proliferate extensively (reviewed in REF. 6). This gave rise to the idea that malignant tumours are comprised of both CANCER STEM CELLS, which have great proliferative potential, as well as more differentiated cancer cells, with limited proliferative potential[7] (BOX 1). This model is reflected in the biology of teratocarcinomas, which contain both malignant undifferentiated cells as well as benign, post-mitotic mature cells[1]. But it was not clear whether it also applied to more common cancers, for which there is less obvious evidence for the coexistence of undifferentiated and differentiated cells.

The existence of cancer stem cells was first proven in the context of acute myeloid leukaemia (AML). In this case, surface markers were used to distinguish AML stem cells from the remaining AML cells, which had limited proliferative potential[5,8]. More recently, this principle has also been extended to breast cancer[9] and glioblastoma[10]. These recent advances indicate that many types of cancer cells can be organized into hierarchies, leading from malignant cancer stem cells, which have extensive proliferative potential, to differentiated cancer cells, which have limited proliferative potential.

Another implication of the cancer-stem-cell hypothesis is that there should be mechanistic similarities between the SELF-RENEWAL of normal stem cells and the proliferation of cancer cells. Indeed, mutations that dysregulate the pathways that control normal stem-cell self-renewal cause a diverse range of cancers[6,11,12] (FIG. 1; TABLE 1). This indicates that cancer can be considered a disease of unregulated self-renewal in which mutations convert normal stem-cell self-renewal pathways into engines for neoplastic proliferation. Recent studies have supported this concept, showing that specific gene products regulate both the self-renewal of normal somatic stem cells and the proliferation of cancer stem cells[13,14].

Summary

- Not all cancer cells are created equal. There are intrinsic differences among cancer cells from the same patient in terms of their ability to proliferate and form tumours *in vivo*.
- A subset of cancer cells have the properties of cancer stem cells, which self-renew to generate additional cancer stem cells and differentiate to generate phenotypically diverse cancer cells with limited proliferative potential. Cancer stem cells are highly enriched for the ability to form tumours following transplantation relative to bulk tumour cells or non-tumorigenic cancer cells.
- Cancer stem cells have been characterized in the context of human acute myeloid leukaemia, breast cancer and glioblastoma. In each case, surface markers have been identified that distinguish cancer stem cells from cancer cells with more limited proliferative potential, allowing the prospective identification of cancer stem cells.
- In some cases, cancer stem cells might arise from the mutational transformation of normal stem cells, whereas in other cases mutations might cause restricted progenitors or differentiated cells to acquire properties of cancer stem cells such as self-renewal potential.
- The neoplastic proliferation of cancer stem cells is likely to be driven by mutations that inappropriately activate pathways that promote the self-renewal of normal stem cells. Examples of these pathways include the WNT, and BMI1-dependent pathways that regulate the self-renewal of haematopoietic stem cells and neural stem cells.
- Further characterization of cancer stem cells might lead to improved diagnostics and therapies by allowing us to better identify and target cancer stem cells. To cure cancer it is necessary to kill, differentiate or prevent the metastasis of cancer stem cells.

If cancer stem cells arise from mutations that dysregulate stem-cell self-renewal pathways, and tumours then arise from the self-renewal and differentiation of cancer stem cells, we might need to make fundamental changes to the way in which we treat cancer. Cancer stem cells might be more resistant to chemotherapy than other cancer cells. Stem cells are more likely to express drug resistance and anti-apoptotic genes than differentiated cells. This could make cancer stem cells more resistant to chemotherapy than more differentiated cancer cells. If so, a small population of cancer stem cells could preferentially survive treatment, even in cases in which chemotherapy causes an apparently complete regression of the primary tumour. This would be consistent with the observation that chemotherapies that cause primary tumour regression rarely prevent metastasis.

Therefore, to cure cancer it is imperative to devise therapies that effectively target the cancer stem cells (FIG. 2). Screens to identify agents with the ability to kill this subset of cancer cells might lead to more effective therapies. The model of the cancer stem cell makes specific predictions about how this might be accomplished (BOX 2).

Identification of cancer stem cells

Similarities between cancer cells and normal stem cells led to the notion of cancer stem cells[16–19]. The existence of such cells was first clearly documented in the context of leukaemia. Early studies had shown that only a few percent of leukaemia cells proliferated extensively *in vitro* or *in vivo*[20,21]. But it was not clear whether every leukaemia cell had the same small chance of proliferating, or whether there were intrinsic differences among leukaemia cells from the same patient in terms of their ability to proliferate. That is, perhaps only a small subset of leukaemia cells were able to proliferate extensively, whereas most leukaemia cells had only a limited ability to proliferate. John Dick and colleagues resolved this question by showing that only a small subset of human AML cells that were phenotypically similar to normal haematopoietic stem cells could transfer AML when transplanted into immunodeficient mice. Other AML cells were unable to induce leukaemia[5,8]. This indicates that AML cells are intrinsically heterogeneous in their proliferative potential, and that AML stem cells give rise to a much larger population of leukaemia cells that lack the ability to proliferate extensively.

Recent experiments have extended this model to include epithelial cancers[9]. Uncultured specimens of human breast cancer cells from nine patients were separated into fractions that expressed different surface molecules, and then injected into immunodeficient mice. Again, only a small population of the tumour cells were able to induce tumour formation in the mice. These cells were found to express Cd44 (an adhesion molecule that binds hyaluronate), but little or no Cd24 (an adhesion molecule that binds P-selectin). As few as 200 $Cd44^{+}Cd24^{-/low}$ cancer cells were able to consistently form tumours, whereas injection of thousands of cancer cells that had other phenotypes failed to form tumours. These tumorigenic cells behaved like cancer stem cells in that they not only gave rise to additional $Cd44^{+}Cd24^{-/low}$ cells, which could be serially passaged from one mouse to another, but they also gave rise to diverse populations of non-tumorigenic breast cancer cells with other phenotypes. These findings indicate that, like teratocarcinoma cells and AML cells, breast cancer cells intrinsically differ in their tumorigenic potential. The ability to isolate tumorigenic breast cancer cells will allow researchers to more precisely study the genes that are required for malignancy and neoplastic proliferation.

Similar results have been observed for cancers of the central nervous system (CNS). Three groups have cultured cells with characteristics of CNS stem cells from various human brain tumours[10,15,22]. Peter Dirks and colleagues showed that a small subset of cells that express the human neural-stem-cell marker CD133 accounted for almost all *in vitro* proliferative activity[10]. In culture, these $CD133^{+}$ cells gave rise to cells that expressed neuronal and/or glial markers in proportions that mirrored the phenotypes of cells within the original tumours. As these tumour-derived PROGENITORS gave rise to karyotypically abnormal cells in culture, they are likely to be cancer cells, rather than normal CNS stem cells that contaminated the tumour specimens. Nonetheless, it will also be important to perform experiments to show that the tumour-derived $CD133^{+}$ cells, but not the $CD133^{-}$ cells, form tumours in immunodeficient mice.

Furthermore, it will be important to show that single cells from a PROSPECTIVELY IDENTIFIED population of cancer stem cells can self-renew to generate phenotypically similar tumorigenic daughter cells, as well as differentiate into phenotypically diverse non-tumorigenic daughter

CANCER STEM CELL
A cancer cell that has the potential to transfer disease or to form tumours following transplantation. Cancer stem cells have the potential to self-renew, forming additional tumorigenic cancer cells of similar phenotype, and to give rise to phenotypically diverse cancer cells with more limited proliferative potential.

SELF-RENEWAL
The process by which a progenitor gives rise to daughter progenitors of equivalent developmental potential. For example, multipotent stem cells self-renew by dividing to generate one or two multipotent daughter cells.

PROGENITOR
Any cell that divides to give rise to other cells. Progenitors include both stem cells and restricted progenitors.

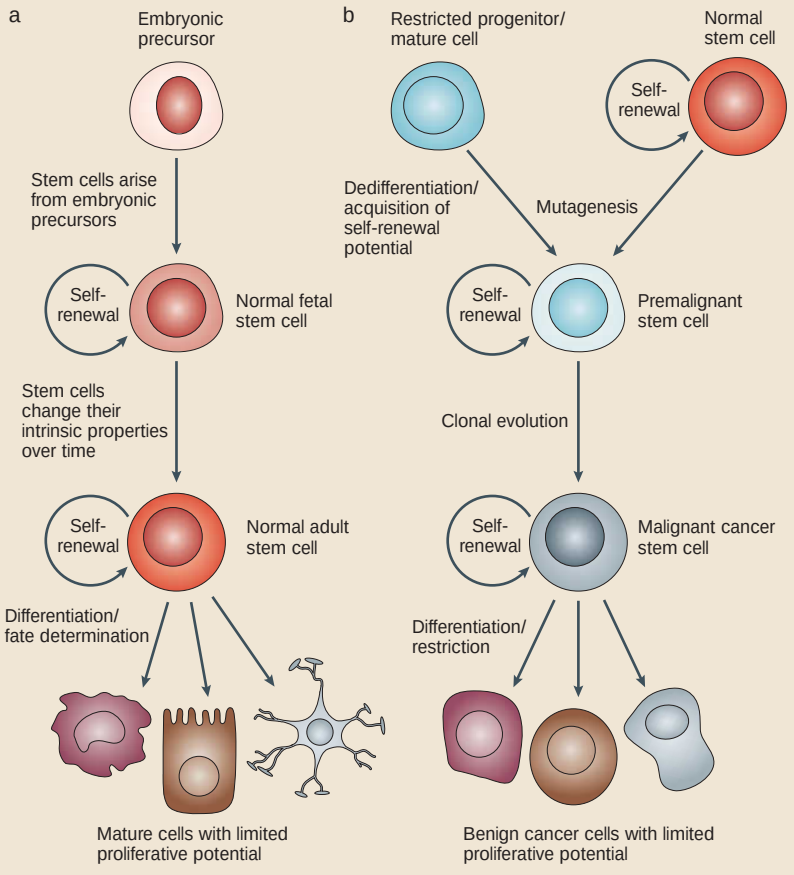
PROSPECTIVE IDENTIFICATION
The ability to reliably predict which cells are stem cells and which are not *in vivo* or among freshly dissociated cells that have not yet been cultured. This is typically done based on surface-marker expression, such as by isolating highly purified populations of uncultured stem cells by flow cytometry.

RESTRICTED PROGENITOR
A cell that divides to give rise to other cells, but which has a more limited developmental potential than the stem cells in the same tissue from which it arises.

cells during tumorigenesis *in vivo* (BOX 1). Although this could demonstrate the self-renewal and differentiation of single cancer stem cells, the efficiency with which single cancer stem cells are able to form tumours *in vivo* is another issue. It is possible that tumorigenesis by single cancer stem cells is inherently inefficient. Microenvironments that are permissive for tumorigenesis could be rare under many circumstances, or difficult for tumorigenic cells to access following transplantation or metastasis.

Box 1 | Parallels between normal stem cells and cancer stem cells

Normal somatic stem cells arise from embryonic precursors during fetal development (see **a**). These fetal stem cells self-renew to form daughter stem cells and differentiate to generate diverse mature progeny. Fetal stem cells often give rise to adult stem cells in the same tissues, but the properties of the adult stem cells differ from the properties of the fetal stem cells. Nonetheless, the adult stem cells often continue to self-renew and undergo multilineage differentiation to maintain the adult tissues. In some cases, cancer stem cells can arise from the mutational transformation of normal stem cells, whereas in other cases mutations might cause restricted progenitors or differentiated cells to acquire properties of cancer stem cells, such as self-renewal potential[1,6,99] (see **b**). These pre-malignant stem cells would be subject to genomic instability and clonal evolution, but they would be distinguished from other cancer cells by their tumorigenic potential, their ability to generate additional cancer stem cells (self-renewal) and their ability to generate phenotypically diverse non-tumorigenic cancer cells (with more limited proliferative potential). In some cancers, like teratocarcinoma, undifferentiated and differentiated cancer cells can clearly be identified histologically. In other cancers, like breast cancer, undifferentiated and differentiated cancer cells often cannot be distinguished by histology, although studies have shown that only a subset of breast cancer cells can form tumours following transplantation into immunocompromised mice[9]. So, the growth and progression of many cancers can be driven by a minority population of cancer stem cells, just as the growth of most normal tissues is driven by small populations of somatic stem cells in those tissues.



Cellular origin of cancer stem cells

The cellular origin of cancer stem cells has not been definitively determined. The fact that several mutations are necessary for a cell to become cancerous[23] indicates that in many tissues the mutations must accumulate in stem cells. This is because in many tissues in which cancers commonly arise (for example, blood, gut epithelium and skin), the RESTRICTED PROGENITORS and differentiated cells tend to have a short life-span. So, in contrast to the stem cells that might persist throughout life in these tissues, there is little opportunity for mutations to accumulate in the restricted progenitors/differentiated cells.

As cancer stem cells must self-renew, it follows that they are derived either from self-renewing normal stem cells (which could be transformed by dysregulating a self-renewal pathway that they already express) or from more differentiated cells that acquire the ability to self-renew as a result of oncogenic mutations (BOX 1). The fact that leukaemic stem cells have a surface-marker phenotype that is similar to normal haematopoietic stem cells[5,24] supports the idea that they arise from haematopoietic stem cells. Indeed, several groups have shown that leukaemogenic mutations increase the proliferation and block the differentiation of normal haematopoietic stem/progenitor cells[25,26]. However, there also seem to be some phenotypic differences between leukaemic stem cells and haematopoietic stem cells, including differences in THY1 and interleukin-3-receptor-α expression[27,28]. This supports the possibility that early mutations occur in haematopoietic stem cells and the final transforming events either alter the phenotype of the stem cells or occur in early downstream progenitors. We should continue to gain new insights into the biology of neoplasms by examining the effects of oncogene expression on the phenotype and function of normal stem/progenitor cells, rather than depending exclusively on cell lines for such studies.

Stem-cell self-renewal and cancer proliferation

Several signalling pathways that regulate normal stem-cell self-renewal cause neoplastic proliferation when dysregulated by mutations. For example, the WNT[29–32], sonic hedgehog (SHH)[2,33–36], Notch[37–39], PTEN[40,41] and, most recently, the BMI1 (REFS 13,14) pathways have all been shown to promote the self-renewal of somatic stem cells, as well as neoplastic proliferation in the same tissues when dysregulated (TABLE 1). Recent reviews have addressed the roles of PTEN, Notch and SHH in stem-cell self-renewal and tumorigenesis[2,6,11,42]. We will focus on recent results that link the WNT and BMI1 pathways of stem-cell self-renewal with neoplastic proliferation.

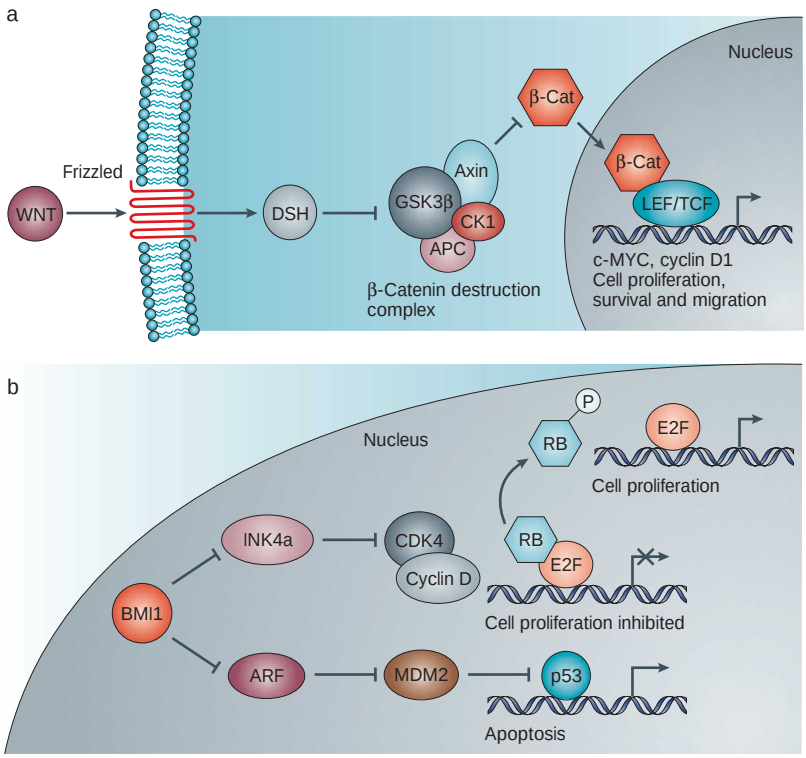


Figure 1 | **Control of stem-cell self-renewal by the WNT and BMI1 pathways. a** | WNT binding to the Frizzled receptors activates Dishevelled (DSH), which disrupts a complex of glycogen synthase kinase 3β (GSK3β), casein kinase 1 (CK1), axin and adenomatosis polyposis coli (APC). When present in this complex, CK1 and GSK3β phosphorylate β-catenin, leading to its degradation[100]. By disrupting the GSK3β complex, DSH allows β-catenin to accumulate and translocate to the nucleus, where it binds LEF/TCF family members and activates the expression of target genes. These include genes encoding proteins that promote proliferation (such as c-MYC and cyclin D1), survival or migration. **b** | BMI1 promotes cell proliferation by directly or indirectly inhibiting the transcription of *CDKN2A*, which encodes two cyclin-dependent kinase inhibitors, INK4A (also known as p16) and ARF (also known as p14 in humans and p19 in mice), which block proliferation by indirectly promoting the activation of RB and p53, respectively[70,101]. In the absence of INK4A, RB is phosphorylated and inactivated by a complex of CDK4 and cyclin D. This phosphorylation allows E2F-dependent expression of cell-cycle genes, and allows the cell to enter the cell cycle. In the absence of ARF, MDM2 inhibits the p53-dependent expression of pro-apoptotic genes. BMI1 therefore promotes cell proliferation and inhibits cell death.

WNT signalling epithelial stem cells and cancer

WNT proteins are secreted molecules that regulate proliferation and patterning during development. WNTs bind to receptors called Frizzleds, which cause β-catenin to accumulate and translocate into the nucleus, where it binds to the LEF/TCF transcription factors and activates the transcription of genes that promote proliferation (FIG. 1). Mutations that activate the WNT pathway have been implicated in a wide variety of cancers, including those of the colon, prostate and ovary[29]. Expression of stabilized β-catenin promotes the self-renewal of CNS stem cells[43] and keratinocyte stem cells[30] and leads to tumorigenesis in the CNS[44] and skin[45,46]. This raises the question of whether ectopic activation of WNT signalling causes the neoplastic proliferation of normal stem cells by over-activating their normal self-renewal programme.

Intestinal epithelial stem cells reside at the bottom of the intestinal crypts, where they proliferate and give rise to progenitors that differentiate as they migrate towards the intestinal lumen. So, the bottom of the crypt is thought to be a stem-cell niche, in which the environment maintains stem cells, such that migration out of the crypt leads to differentiation. Wnt signalling is required for the self-renewal of normal intestinal epithelial stem cells, as *Tcf4*-deficient mice lack proliferating cells in the intestinal crypts[47]. Presumably, there are several downstream pathways that mediate the effect of WNT signalling on intestinal epithelial stem-cell self-renewal. Two key downstream pathways that are WNT-regulated are EPH-family adhesion molecules, which control migration out of the crypt (and therefore differentiation)[48], and c-MYC, which promotes proliferation[49]. WNT signalling therefore maintains the integrity of the stem-cell niche and promotes self-renewal by regulating both migration and proliferation.

WNT signalling activates the same downstream pathways in colorectal cancer cells[49]. This prompted Hans Clevers and colleagues to conclude that β-catenin/TCF signalling confers a crypt progenitor phenotype on colorectal cancer cells. Mutations that activate WNT signalling cause the hyperproliferation of crypt progenitors, generating benign polyps[50] in which multilineage differentiation is evident[51]. A dominant-negative form of TCF4 induces cell-cycle arrest and the expression of intestinal epithelial differentiation markers in colorectal cancer cells *in vitro*. So, tumorigenesis in the intestinal epithelium seems to be caused, at least initially, by the hyper-self-renewal of intestinal-crypt stem cells, followed by the accumulation of additional mutations that confer malignancy and allow cancer progression[52,53] (BOX 1).

Intestinal epithelial stem cells, however, cannot be prospectively identified, assayed for self-renewal potential or transplanted *in vivo*. It also remains to be determined whether colorectal cancer cells are intrinsically heterogeneous in their proliferative potential as has been shown for breast cancer and AML cells. Ultimately, it will be important to find out whether colorectal cancer cells that express markers of intestinal epithelial stem cells are able to self-renew and then differentiate into phenotypically distinct colorectal cancer cells with more limited proliferative potential. These issues aside, studies of the WNT pathway have provided a strong association between the self-renewal capacity of normal intestinal epithelial stem cells and the proliferation of colorectal cancer cells.

WNT, haematopoietic stem cells and leukaemia

The self-renewal of haematopoietic stem cells is also promoted by WNT signalling[31,32,54–56]. Overexpression of stabilized β-catenin in cultured bone-marrow haematopoietic stem cells from mice increased the numbers of these cells by at least 100-fold in long-term culture, as measured by their ability to reconstitute the haematopoietic systems of irradiated mice following transplantation[32]. A companion paper from the same authors showed that purified Wnt3a promoted the

Table 1 | Signalling pathways, stem cells and cancer

Pathway	Stem cell	Cancer
WNT	Haematopoietic stem cells[31,32] Intestinal epithelial stem cells[47] Keratinocyte stem cells[30,85] Cerebellar granule-cell progenitors[86*] CNS stem cells[43]	Lymphoblastic leukaemia[60,61] Colorectal cancer[49] Pilomatricoma[45,46] Medulloblastoma[44] Gliomas?
SHH	Hair-follicle progenitors[87,88*] Cerebellar granule-cell progenitors[33*] CNS stem cells[35]	Basal-cell carcinoma[89–91] Medulloblastoma[34,92-94] Gliomas[95]
BMI1	Haematopoietic stem cells[13]	B-cell lymphomas[71] AML[14]
Notch	Haematopoietic stem cells[38] Mammary epithelial stem cells[96]	Lymphoblastic leukaemia[37] Breast cancer[97]
PTEN	Neural stem cells[41]	Gliomas[98]

In all cases it is unknown whether the cancers arise from the transformation of stem cells or other cells in their tissues of origin. *It is uncertain whether these WNT/SHH-responsive progenitor populations are multipotent stem cells or restricted progenitors. AML, acute myeloid leukaemia; CNS, central nervous system; PTEN, phosphatase and tensin homologue deleted from chromosome 10; SHH, Sonic hedgehog.

self-renewal and inhibited the differentiation of haematopoietic stem cells in culture[31]. In cultures that were supplemented with purified Wnt3a, Thy1^{low}Sca1^{+}lineage^{-}c-Kit^{+} cells (which are highly enriched for haematopoietic stem cells) were sixfold more likely to proliferate, were less likely to express differentiation markers and self-renewed enough that they were estimated to be 5–25-fold more likely to reconstitute irradiated mice. Together, these findings indicate that Wnt-pathway activation promotes the self-renewal of haematopoietic stem cells in culture. Although these experiments used haematopoietic stem cells that overexpressed Bcl2 to prevent them from prematurely dying or differentiating in culture, control experiments showed that wild-type haematopoietic stem cells responded similarly to Wnt3a[32]. So, consistent with other reports[54–56], Bcl2 overexpression was not necessary for haematopoietic stem-cell self-renewal in response to Wnt3a.

Ectopic expression of *axin*, a negative regulator of Wnt signalling (FIG. 1), inhibited proliferation of haematopoietic stem cells, promoted apoptosis *in vitro* and reduced the ability of these cells to reconstitute irradiated mice[32]. Although this indicates that Wnt signalling is required for self-renewal of haematopoietic stem cells, it remains to be determined whether this is the case *in vivo*. For example, it is possible that other factors are present in the more complex growth-factor environment *in vivo* that can support self-renewal of haematopoietic stem cells independent of the Wnt pathway. *β-Catenin*-deficient mice die during gastrulation[57], before the onset of haematopoiesis, so it will be necessary to examine mice that are conditionally deficient for *β-catenin*[58] to determine whether β-catenin is required for self-renewal of haematopoietic stem cells *in vivo*. *Wnt3a*-deficient mice die with severe patterning defects at E12.5 (REF. 59). Although this is after the initiation of fetal-liver haematopoiesis, the effect of *Wnt3a* deficiency on the function of fetal haematopoietic stem cells has not yet been examined. So, it remains to be determined whether Wnt-pathway signalling is required for self-renewal of haematopoietic stem cells *in vivo*.

Recent studies have also implicated Wnt signalling in haematopoietic malignancies[60,61], although it is unknown whether mutations in Wnt-pathway components are required for the genesis or progression of these malignancies.

POLYCOMB FAMILY
Polycomb family members repress gene expression by assembling into multimeric protein complexes that alter chromatin structure. Polycomb family members regulate the expression of cell-cycle genes as well as *HOX* genes, and are known to regulate proliferation and patterning.

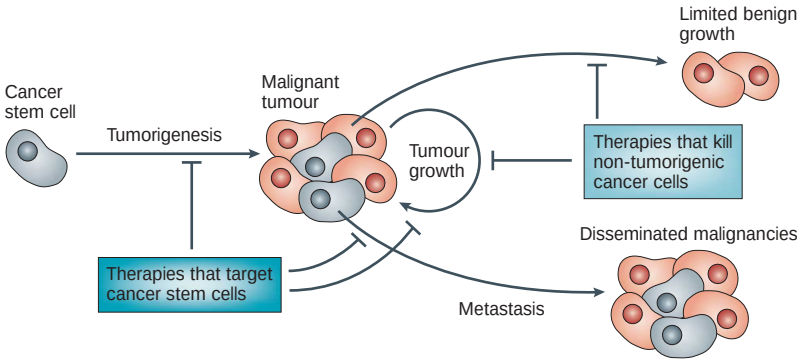


Figure 2 | **Therapeutic implications of cancer stem cells.** Cancer stem cells (grey) self-renew and differentiate within tumours to form additional cancer stem cells as well as non-tumorigenic cancer cells (orange), which have limited proliferative potential. As the tumour grows, these cells can either undergo limited benign growth or form disseminated malignancies. Therapies that kill, induce differentiation or prevent the metastasis of cancer stem cells represent potential cures. Therapies that kill primarily non-tumorigenic cancer cells can shrink tumours, but will not cure the patient because the cancer stem cells will regenerate the tumour. By prospectively identifying and characterizing cancer stem cells it might be possible to identify more effective therapies. The intrinsic differences in tumorigenic potential among cancer cells might also explain why it is possible to detect disseminated solid cancer cells in patients that never develop metastatic disease. The identification and characterization of cancer stem cells should therefore also lead to diagnostic methods that can distinguish between disseminated tumorigenic and non-tumorigenic cells, as well as provide a better understanding of the mechanisms that regulate migration of cancer stem cells.

BMI1, self-renewal and leukaemia

Members of the POLYCOMB FAMILY, including RAE28, BMI1 and EZH2, regulate chromatin remodelling, act as transcriptional repressors and have been implicated in normal stem-cell function and cancer[62–64]. In humans, high-level expression of EZH2 by prostate cancer cells has been linked to poor prognosis[65], and *BMI1* is amplified in many cases of Mantle-cell lymphoma[66]. In mice, the *Bmi1* proto-oncogene causes lymphoma when overexpressed in lymphocytes[67,68]. *Bmi1* deletion in mice leads to defects in axial-skeleton patterning, haematopoiesis and neurological function, as well as to progressive retardation of postnatal growth[69]. Although *Bmi1*-deficient mice survive to adulthood, they ultimately die as a result of haematopoietic and neurological defects[69]. Bmi1 promotes the overall growth of mice and the proliferation of lymphocytes and embryonic fibroblasts partly by directly or indirectly repressing the expression of *Cdkn2a*, which encodes two cyclin-dependent kinase inhibitors, Ink4a (also known as p16) and Arf (also known as p14 in humans and p19 in mice)[70,71] (FIG. 1). However, deletion of *Ink4a* and *Arf* only partially rescues the growth and lymphocyte counts of *Bmi1*-deficient mice, so there must also be other downstream pathways that mediate the effect of Bmi1 on proliferation.

Box 2 | Targeting cancer-stem-cell self-renewal

Genes that regulate the self-renewal of normal stem cells must promote proliferation and maintain multipotentiality. To the extent that cancer stem cells seem to self-renew and differentiate as well[5,8,9], the self-renewal pathway in these cells must also promote proliferation and the maintenance of the cancer stem-cell state. Therapies that induce the differentiation of cancer stem cells[102], or that even transiently inhibit the maintenance of the stem-cell state, should lead to the exhaustion of the pool of cancer stem cells and to the conversion of malignant cancers into benign tumours. For example, transient inactivation of MYC leads to the differentiation of sarcoma cells into osteocytes, and a loss of neoplastic phenotype that cannot be restored even by reactivation of MYC[103]. This raises the possibility that MYC is required for maintenance of identity of sarcoma stem cells, such that in its absence the cells differentiate to benign osteocytes.

Recent studies have shown that *Bmi1* is required for the self-renewal of haematopoietic stem cells, as well as leukaemic stem cells. Although haematopoietic stem cells are present in normal numbers in the fetal liver of *Bmi1*$^{-/-}$ mice, they are depleted in the postnatal bone marrow[13]. Reconstitution experiments indicate that *Bmi1*$^{-/-}$ haematopoietic stem cells have only limited self-renewal potential, as *Bmi1*$^{-/-}$ fetal liver cells were able to reconstitute primary recipient mice for less than 8 weeks, and cells that were derived from these mice were unable to reconstitute secondary recipient mice. So, *Bmi1*$^{-/-}$ mice seem to die of haematopoietic failure because their haematopoietic stem cells have insufficient self-renewal potential to persist into adulthood.

The proliferation of leukaemic stem cells in a mouse model of AML was also promoted by *Bmi1* (REF. 14). *Bmi1*-expressing leukaemic cells were able to induce leukaemia when transplanted into irradiated mice, but leukaemic *Bmi1*$^{-/-}$ cells had only limited proliferative potential and were unable to induce disease. Bmi1 activity is therefore necessary for the self-renewal of both haematopoietic stem cells and leukaemic stem cells. As discussed above, the similarities in marker expression between haematopoietic stem cells and AML stem cells[5] make it tempting to speculate that haematopoietic stem cells are transformed into leukaemic stem cells by oncogenic mutations. However, it is also possible that transforming mutations occur in restricted progenitors or differentiated cells, causing these cells to de-differentiate or to otherwise acquire the properties of cancer stem cells[1,6] (BOX 1).

Important questions remain with regard to the roles of WNT signalling and BMI1 in regulating the self-renewal of normal and cancer stem cells. Is BMI1 expression or function activated in response to WNT signalling, or are these independent pathways that control self-renewal? Will gain-of-function mutations in these pathways have similar or different effects on self-renewal/neoplastic proliferation? Can mutations in differentiated cells re-activate the expression of stem-cell self-renewal pathways that involve genes such as*BMI1*, which are often not expressed in differentiated cells[72,73]? Future work in these areas is likely to yield additional important insights into the mechanistic links between normal stem-cell self-renewal and the neoplastic proliferation of cancer stem cells.

Therapeutic implications

Various stem cells, including haematopoietic stem cells, characteristically express drug-resistance proteins, such as the MDR1 and ABC transporters[74,75], which might make them less sensitive to chemotherapy and apoptosis induction[76,77]. If cancer stem cells also tend to express these proteins at higher levels than differentiated cancer cells, then cancer stem cells might also be more resistant to chemotherapy. This could explain the frequent failure of chemotherapy to cure metastatic cancer, despite its ability to shrink tumours. That is, the ability of chemotherapy to kill more differentiated cancer cells with limited proliferative potential could lead to tumour shrinkage, but if cancer stem cells survive, they will continue the process of tumour growth and progression. Screens that are designed to identify agents that efficiently kill cancer stem cells (FIG. 2) might lead to more effective treatments for metastatic cancer.

Microarray analysis has been used to identify subtypes of cancers that have not been distinguished by pathological criteria[78–80]. This could lead to improved diagnosis and treatment by allowing physicians to better recognize and predict the ways in which distinct subtypes of cancer respond to therapies[81,82]. But the identification of stem cells in breast cancer[9] and AML[5,8] raises the question of the extent to which these cells have a different gene-expression profile from cancer cells with limited proliferative potential. If a minority population of cancer stem cells drives the growth, progression and metastasis of some tumours, then the gene-expression profile of the cancer stem cells should most precisely predict or reflect treatment responses. This raises the possibility that cancers could be classified and outcomes could be predicted more accurately, at least in the case of some cancers, by comparing the gene-expression profiles of tumorigenic cancer cells from different patients, rather than using whole-tumour RNA. By comparing the gene-expression profiles of cancer stem cells, cancer cells with limited proliferative potential, normal stem cells and normal tissue, it might be possible to identify therapeutic targets that are preferentially expressed in cancer stem cells.

If the survival and neoplastic proliferation of cancer stem cells depend on the same pathways that maintain normal somatic stem-cell populations, then will therapies directed against these pathways have toxic side effects? After all, adults retain normal stem-cell populations in the bone marrow, gut, muscle, liver, skin, brain and other locations that are involved in the maintenance of those tissues. Fortunately, there are reasons to believe that it will be possible to identify agents that kill cancer stem cells without being unacceptably toxic for normal stem cells, even if normal stem cells express the same targets. The extent to which a stem-cell population acutely depends on a particular signalling pathway depends on the mitotic activity of the cells, the amount of regenerative activity in the tissue and the stage of development, among other things. Therefore, cancer stem cells are likely to be more dependent on some pathways than normal stem cells, even if the pathways are active in both. We also might be able to do without

some types of normal stem cells. For example, the loss of mammary epithelial stem cells would be an acceptable side effect of breast cancer therapy in many patients, as the mammary epithelium is already lost in patients that undergo mastectomy. As long as severe damage to stem cells in crucial tissues (such as the blood, gut epithelium and skin) is avoided, toxicities against some other types of stem cells might be well tolerated.

Although only limited data are available, there are already some examples of agents that are selectively toxic to cancer stem cells. It was recently shown that the combination of idarubicin with a proteasome inhibitor killed leukaemic stem cells, but not normal haematopoietic stem cells[83]. In patients with testicular germ-cell cancers, common chemotherapeutics like cisplatin, etoposide and bleomycin can efficiently kill the undifferentiated cancer cells and spare enough spermatogonial stem cells that a substantial fraction of patients remain fertile[84]. Together, these observations provide proof of principle that therapies that are directed against cancer stem cells will not necessarily be unduly toxic to normal stem cells. However, it remains to be determined whether agents that kill cancer stem cells without killing normal stem cells will be readily identified, or whether such agents will be rare relative to agents that are toxic to both normal and cancer stem cells.

Future directions

It will be important to extend the approaches that have been used to show the existence of cancer stem cells in AML and breast cancer to other cancers. It remains unclear whether most cancers will be organized in a hierarchy of cells with different proliferative potentials or whether AML, breast cancer and teratocarcinoma will be unusual in that regard. Cancers that include tumorigenic cancer stem cells as well as cancer cells with more limited proliferative potential will have to be studied in a way that accounts for this heterogeneity.

Gene-expression profiling studies, efforts to develop new diagnostics and efforts to develop or test therapeutics should be performed on primary cancer cells, rather than cell lines, whenever possible. It is also important to consider the possibility of gene-expression differences between tumorigenic cancer cells and cancer cells with limited proliferative potential. By doing so, it might be possible to uncover previously unrecognized differences between cancer cells that will allow us to more effectively classify, diagnose and treat cancers.

If therapeutics are developed that can effectively target cancer stem cells, it will be important to determine the frequency with which non-tumorigenic cancer cells (those with limited proliferative potential) might evolve to acquire properties of cancer stem cells. This might occur more readily in some cancer types than in others, depending on the tissue involved, and could confound the effectiveness of agents that efficiently target cancer stem cells. Nonetheless, by targeting pathways that are necessary for the maintenance of cancer-stem-cell identity (BOX 2), it might be possible to develop therapies that are effective against metastatic disease.

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Even a transient loss of MYC function can lead to an irreversible loss of neoplastic cells. This is consistent with the idea that MYC is required to maintain the state of cancer stem cells, much as it would be expected to be required for normal stem-cell self-renewal. So, targeting pathways that are required for maintenance of stem-cell identity might be used to convert malignancies into benign tumours.

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Conflict of interests statement

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

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