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Oncogenesis and cancer stem cells: current opinions and future directions

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Abstract

There is increasing evidence to show that only a subset of cancer cells drives the growth and progression of a tumour. These cells share similar properties with normal stem cells and are termed 'cancer stem cells'. Cancer stem cells have been identified in acute myeloid leukaemia and in some solid tumours by their distinct expression of cell surface antigens. Their long-term, self-renewing capacity is thought to be a determining factor in the maintenance and regrowth of the tumour. Studies on haematopoietic cancers show that important signalling pathways and genes for normal haematopoiesis, such as Wnt, NF-κB, Notch, hedgehog (Hh) and Bmi1, are oncogenic, thereby potentially involved in cancer stem cell regulation. Elimination of cancer stem cells in tumours could result in the degeneration of downstream cells, which makes them potential targets for new cancer therapies.

Keywords: cancer stem cell • haematopoietic stem cell • acute myeloid leukaemia • oncogenesis • signalling pathways • niche

Introduction

Stem cell research is a promising and rapidly growing field of biomedicine. Stem cells are defined as cells with capacity to undergo extended self-renewal through mitotic division, and can differentiate into mature cells forming the tissues. There are two broad types of stem cells in mammalian organisms: embryonic stem cells that are found in blastocysts, and adult stem cells found in adult tissues. In adult organisms, stem cells are responsible for tissue renewal, and recovery of damaged or aged tissues. Recently, there has been increasing evidence to support a cancer stem cell hypothesis, which states that tumours are initiated and maintained by a subset of cancer cells, 'the cancer stem cells'. Cancer stem cells share many similar properties with normal stem cells, such as prolonged self-renewal capacity, resistance to radiation and many cytotoxic agents. They have been identified in various cancers such as leukaemias and some well studied solid tumours, like tumours of the central nervous system (CNS) and the mammary gland, by their distinct presentation of cell surface markers common to normal stem cells. The role of cancer stem cells in oncogenesis is considered here, and therapeutic implications are highlighted. Current evidence supporting the existence of cancer stem cells is addressed, including recent findings from leukaemias and solid tumours. Disputed arguments regarding the origin of cancer stem cells are reviewed in terms of whether their origin lies in stem cells or progenitor cells. Relevant discussion in this review concentrates on these two possibilities, with specific emphasis on the role of the niche in cancer stem cell formation. Several molecular pathways and transcription factors, such as Wnt, NF-κB, Notch, hedgehog (Hh) and Bmi1 seem to play essential roles in the self-renewing behaviour of normal stem cells. Here, mechanisms of self-renewal are critically assessed, showing how disruption of these pathways can eliminate cancer stem cells and eventually lead to novel cancer therapies.

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Stem cells and haematopoiesis

Stem cells were initially identified in bone marrow by two Canadian scientists, Till and McCulloch, studying radiation sensitivity of marrow cells in murine models [1]. Classically, a stem cell is defined by two important characteristics. First, it undergoes self-renewal division. This process can be asymmetric, in which a daughter cell is produced with developmental potential identical to the undifferentiated parental cell [2]. Secondly, it undergoes differentiation to give progenitor cells. Strictly, this requires stem cells to be totipotent or pluripotent cells that can give rise to all mature cell types. However, multipotent progenitor cells may be referred to as stem cells in some contexts [2]. Emphasis is given here to the difference between 'self-renewal' and 'proliferation'. Proliferation of cells refers to the growth of a cell population through mitotic divisions that only generate daughter cells with limited differentiative potential [3]. On the other hand, self-renewing cell division can be either asymmetric or symmetric. Stem cells have the ability to undergo a symmetric self-renewing division, causing two identical daughters cells that retain self-renewal capacity, or an asymmetric division that produces one differentiated progenitor cell and one daughter stem cell [3]. In addition, the symmetric division of a stem cell may also result in two progenitor cells that will further differentiate into different lineages of cells [3].

Haematopoiesis is the formation of blood cellular components from stem cells, and this process is strictly regulated. It first occurs in the yolk sac, and later in foetal liver, spleen and bone marrow. In adults, this process takes place in the bone marrow [4]. Haematopoiesis involves a multipotent haematopoietic stem cell (HSC) that gives rise to the two main blood cell lineages: the lymphoid and myeloid lineages. In humans, HSC show a $CD34^{+/lo}CD38^{-}$ phenotype, whereas in mouse a $CD34^{-/lo}CD117^{+}Sca\text{-}1^{+}$ surface marker expression is normally observed [5]. In the bone marrow, most HSC remain in a quiescent (G_0) state, thereby preserving their capacity to self-renew. In particular, the quiescent state is thought to be an essential means to protect HSC from stress, and so sustain their long-term self-renewing capacity [4]. Haematopoiesis involves the production of blood cells from HSC through mitotic division. HSCs produce a large number of slightly differentiated progeny, referred to as transit amplifying progenitor cells [6]. These progenitors undergo a limited number of mitotic cycles before entering a post-mitotic, fully differentiated state, so the activity of a relatively small number of stem cells can be amplified to produce a large number of differentiated progeny. Early committed progenitors express low levels of transcription factors that may commit cells to discrete lineages [6]. The number of HSC is strictly regulated by two mechanisms: the balance between self-renewal and differentiation, and the balance between cell survival and apoptosis. Differentiation of HSC to give cells of different lineages depends on external signals received by progenitors. The exact mechanism by which these external signals are coordinated remains unclear. Nevertheless several cell-intrinsic and cell-extrinsic regulators showing a vital role in cell fate decisions have been isolated. For instance, PU.1 commits cells to the myeloid lineage [7]. Interleukin-7 receptor (IL-7R) is necessary for the development of early lymphocytes [8], while GATA-1 plays an essential role in erythrocytic and megakaryocytic differentiation [9]. An important role for apoptosis in haematopoiesis is supported by studies on the *Bcl-2* gene. Bcl-2 prevents apoptosis by blocking cytochrome C release from mitochondria. The *Bcl-2* gene encodes an integral membrane protein located on the outer membrane of mitochondria [10]. Cytosolic cytochrome C is necessary for the initiation of the apoptotic program, and inhibition of Bcl-2 results in elevation of cytochrome C in the cytosol and a corresponding decrease in the mitochondria [10]. In the haematopoietic system, overexpression of Bcl-2 prevents cells from undergoing apoptosis in response to a variety of stimuli and results in increased numbers of HSC in the bone marrow [11, 12].

The cancer stem cell hypothesis

The cancer stem cell hypothesis states that only a small subset of cancer stem cells exists within a tumour. These cancer stem cells have the capacity to self-renew and to form the heterogeneous lineages of cancer cells that comprise the tumour. In the literature, cancer stem cells have also been referred to as tumour initiating cells, or tumour stem cells. Cancer stem cells are not defined solely by their surface markers, since none of the markers used to isolate stem cells in various normal and cancerous tissues are expressed exclusively by cancer stem cells [5]. For example, CD34 is present both on HSC and on acute myeloid leukaemia (AML) stem cells [13], and CD133 has been detected both on normal and tumorigenic brain stem cells [14]. Hence, cancer stem cells can only be defined experimentally by their ability to recapitulate the formation of a growing tumour.

The cancer stem cell hypothesis contrasts with the working theory for oncogenesis, that differentiated cells become tumorigenic through accumulation of mutations in proto-oncogenes or tumour suppressor genes [15]. These genes act to regulate cell growth through regulation of growth-related factors such that overactivation can lead to uncontrolled growth and development of cancers [15]. Current models of tumorigenic progression usually relate oncogenesis to the accumulation of a series of molecular events within the cell, such as gene mutations and chromosomal translocations. These models do not consider the cell which is target to these molecular events, which is the focus of the cancer stem cell hypothesis. Thus, the hypothesis is a supplement, rather than an alternative to the current oncogenic theory.

It has long been known that solid tumours comprise a heterogeneous collection of cell types, and that only a small

Table 1 Phenotypic distinction between normal HSC and AML stem cells

Normal HSC	AML stem cells
Self-renew	Self-renew
High differentiative potential	High differentiative potential
Differentiation highly regulated	Differentiation poorly regulated
Long life span, resistant to apoptosis	Long life span, resistant to apoptosis
$CD34^+CD38^-$ $CD90^+CD117^+CD123^-$	$CD34^+CD38^-CD90^-$ $CD117^-CD123^+$

proportion of cells in a tumour are able to form new colonies *in vitro* and *in vivo*. For instance, less than 0.1% of cells in lung, ovarian or brain cancers are able to form colonies in soft agar [16]. In general, there are two models to explain such a low success rate for tumour regrowth. Firstly, the conventional stochastic model states that most cancer cells of different phenotype are able to proliferate extensively, but the possibility that each cancer cell shows this proliferative potential is now known to be low [16]. Secondly, the cancer stem cell model predicts that most cancer cells have only limited proliferative potential, while a small subset of cancer stem cells constantly self-renews and differentiates into the various downstream cell types within a tumour [16]. While both models limit 'tumour initiating' cells to a small number, their underlying biological principles are different. According to the alternative stochastic model, cells within a tumour are relatively homogeneous, and the genetic changes leading to malignancy are operative in all cells. The cancer stem cell model, however, hypothesizes that the tumorigenic pathway in a small population of cancer cells may operate differently in cancer stem cells compared with the majority of cells in the tumour. The poor prognosis of some therapies, which primarily target the majority population of cells within a cancer, could have a basis in the heterogeneity amongst cells in a tumour and their different sensitivity to treatment regimes. Recent evidence now points to the treatment resistance of stem cells in breast cancer [17] and colon cancer [18]. However, the argument that tumours are sustained by a small number of cancer stem cells has been challenged [19]. One issue of contention is that tumorigenicity of cancer cells is measured by engraftment in mice. The outcome of grafting is influenced by the high death rate of cells in the selective environment of sometimes a xenogeneic host [20]. In fact, solid tumours are heterogeneous and may contain a population of fully transformed cells which are transplantable into syngeneic hosts. However, the experimental protocols required to demonstrate cancer development from small numbers of stem cells present within a tumour will understandably reflect a very selective environment.

Evidence from leukaemias and solid tumours

The first definitive evidence favouring the cancer stem cell hypothesis was obtained with the discovery of cells with stem cell attributes in AML patients [14]. AML is a cancer of the myeloid lineage of white blood cells characterized by rapid proliferation of white blood cells in the bone marrow along with interference in the production of normal blood cells [21]. The heterogeneous population of cells within AML can be separated on the basis of cell surface antigen expression by flow cytometry [22]. Studies have shown that only a small population of human AML cells is able to transfer leukaemia when transplanted into non-obese diabetic/severe combined immunodeficiency disease (NOD/SCID) mice [14]. These cells make up approximately 0.2% of the total AML cells, and have a $CD34^+CD38^-$ phenotype similar to that of stem cells in normal NOD/SCID mice [14]. In contrast, other AML cells were unable to induce leukaemia even in large numbers. [13, 14, 16, 23]. A recent study of identical twins has now confirmed the cancer stem cell origin of a related disease, common acute lymphoblastic leukaemia (cALL). In this study, a rare population of $CD34^+CD38^-CD19^+$ cells was isolated from each of two identical twins, one with cALL, and the other showing a healthy phenotype [24]. The isolated cells were phenotypically similar to, but distinct from normal HSC, and acted as self-renewing cancer stem cells upon secondary engraftment [24]. This finding, together with previous studies, showed that pre-leukemic stem cells arise as a result of a chromosomal translocation which occurs in stem cells *in utero* to create a hybrid protein 'TEL-AML1'. This genetic mistake can set in motion a cascade of events that eventually leads to the initiation of cALL [24].

Cancer stem cells have also been identified in solid tumours such as cancers of the CNS, as well as breast and colon cancers. *In vitro* and *in vivo* experiments on human glioblastoma and medulloblastoma have shown that a small subset of tumour cells can account for all the proliferative activity of the tumour [25]. These cells express CD133 surface antigens, showing a similar phenotype to normal neural stem cells, and are resistant to ionizing radiation because they are more efficient at inducing the repair of damaged DNA than the majority of the tumour cells [25–27]. The formation of a tumour in NOD/SCID mouse brains requires as few as 100 $CD133^+$ cells, whereas the transplantation of 100,000 $CD133^-$ cells does not result in a CNS tumour [25, 28]. Another study demonstrating the existence of cancer stem cells in the brain involves the use of bone morphogenetic proteins (BMPs), soluble factors that normally induce neural progenitors to differentiate into mature astrocytes. Treatment of $CD133^+$ glioblastoma progenitor cells *in vitro* with BMP reduces the size of the tumour, which later develops in the animal upon engraftment, and prolongs the animals' lifespan [29]. The BMP-treated tumour cells engrafted into mice were more mature and less invasive. $CD133^+$ cells could not be identified in the small tumours that formed in mice, and were incapable of forming new cancers upon serial

engraftment [29]. However, the exact mechanism by which BMP controls oncogenic progression remains unclear, and the true stem cells are probably a subpopulation of the $CD133^+$ fraction. Nevertheless, both findings support the hypothesis for a cancer stem cell origin of CNS tumours. Similarly in breast cancer, a minor, phenotypically distinct tumour cell population has been isolated that is able to form mammary tumours in NOD/SCID mice, whereas cells of other phenotype are non-tumorigenic [15]. Cancer stem cells show $CD44^+CD24^-$ surface marker expression, and 100 cells can consistently form new breast cancers upon engraftment into mice [15, 30]. Within colon cancers, a small proportion of $CD133^+$ cells with similar attributes has also been observed and cancer stem cells have been identified as cells with the $CD44^+EpCAM^{hi}CD166^+$ phenotype [31–33]. This evidence extends the involvement of cancer stem cells to multiple types of leukaemias and tumours. While the direct development of stem cells into cancer cells is the main tenet of the cancer stem cell hypothesis, a further interpretation encompasses the dedifferentiation of progenitor cells into cancer cells *via* acquisition of self-renewal capacity and studies on glioma cells provide support for this model [33].

Cellular origin and the role of niche

If the cancer stem cell hypothesis is correct, and cancer stem cells do exist, one needs to ask where these cells come from. Cancer stem cells, including leukemic stem cells, are thought to arise by two possible pathways: (1) a normal stem cell acquires one or more mutations that disables the growth regulating mechanism of the cell, making it tumorigenic; (2) a progenitor cell acquires the properties of self-renewal by mutation, and becomes a cancer stem cell [3]. Two arguments supporting model A have been proposed. Firstly, the self-renewing machinery in stem cells becomes activated, and maintaining this activation may be simpler than turning it on *de novo* in a more differentiated cell [16]; secondly, stem cells persist for a long time due to self-renewal, giving a considerable opportunity for mutations to accumulate in these cells [16]. The similarity in cell surface marker expression between leukemic stem cells and normal HSC also suggests that this might be the case (Table 1).

Model B focuses on progenitor cells and their acquisition of self-renewal potential. By this model, since progenitor cells proliferate for a much shorter period of time, in most situations only days or weeks [5]. There are now numerous examples where leukemic stem cells reflect committed granulocyte-macrophage progenitors [34, 35] including blast crisis in CML [36]. The possibility that initial mutations occur in long-lived stem cells, but be manifest in more committed progenitors resulting in their acquisition of stem cell properties should also be considered [37]. Progenitors would first need to acquire the complete self-renewal potential of stem cells in order to obtain long-term persistence, and to allow further mutations to accumulate within the cell. In fact, stem cells can reflect both committed progenitors and multipotential HSC, which have self-renewal capacity [38].

An important issue to address is the role of niches in the regulation of cancer stem cell self-renewal and differentiation. A niche is defined as the microenvironment which supports stem cell systems. Studies on melanocytes have shown that normal neural stem cells are often concentrated in regions rich in blood vessels, namely vascular niches [39]. It has been suggested that the regulatory potential of a niche should persist when stem cells are absent, and that a niche should be able to reprogram newly introduced cells to become stem cells [40]. Niches are thought to be responsible for maintaining a proper balance between self-renewal and differentiation of stem cells [39]. In a study of brain tumours, a niche model has been proposed whereby cancer stem cells receive signals secreted by niche cells that allow them to self-renew and to generate 'transit amplifying cells', which then rapidly proliferate and make up the bulk of the tumour. The continued generation of transit amplifying cells from cancer stem cells therefore allows the tumour to keep growing [41].

Molecular basis of self-renewal and differentiation

Self-renewal is a defining property of both normal and tumorigenic stem cells. It has been proposed that the Wnt, Bmi1, Notch and sonic hedgehog (Shh) pathways are critically involved in the regulation of self-renewal in both normal and cancer stem cells [42]. Differential expression of several transcription factors determines the fate of stem cells and plays an essential role in control of stem cell self-renewal, differentiation and lineage commitment. Here, current knowledge of these pathways is discussed in relation to their role in the haematopoietic system. Several examples from solid tumours are also discussed.

Wnt signalling

Wnt signalling is one of the most well studied molecular pathways regulating stem cell self-renewal and proliferation. There are two major categories of Wnt signalling: the canonical pathway in which a cytoplasmic protein β-catenin is a key mediator, and the β-catenin independent, non-canonical pathway. Canonical Wnt signals are vitally involved in cell fate determination, whereas non-canonical signals are essential for control of cell movement and tissue polarity [43]. In the canonical Wnt pathway, signalling is initiated by the binding of Wnt ligands to a receptor complex comprising a receptor of the Frizzled family and a co-receptor, Lrp5/6, that belongs to the low-density lipoprotein receptor family [44]. In the absence of Wnt signalling, β-catenin binds to axin, adenomatous polyyposis coli and glycogen synthase kinase 3β. The resulting complex is then phosphorylated and degraded, which keeps

β-catenin at a low level [45, 46]. However, when the *Wnt* gene is activated and signalling is present, β-catenin is uncoupled from the degradation complex, thus accumulating in the nucleus where it binds Lef/Tcf transcription factors to activate target genes like *Fgf20*, *Dkk1*, *Wist1*, *Myc* and *Ccnd1* [44]. Signalling is accompanied by increased expression of specification and/or self-renewal related genes such as *HoxB4*, which promotes the expansion of HSC without losing their ability to differentiate into normal lymphoid and myeloid cells.

In the haematopoietic system, the ligands of Wnt proteins are produced by HSC as well as by niche cells, suggesting autocrine or paracrine Wnt usage [47]. Indeed, the Wnt pathway may contribute to the induction of proliferation of early haematopoietic cells [44]. In particular, NOD/SCID mice treated with Wnt5A-conditioned medium show a higher repopulation rate for transplanted human HSC [48]. On the other hand, differentiation of murine haematopoietic progenitor cells is inhibited in the presence of Wnt proteins. *In vitro*, soluble Wnt proteins can cooperate with steel factor (also known as stem cell factor) to inhibit the differentiation of progenitors, resulting in increased frequency of $CD34^+$ cells in culture [49]. In addition, β-catenin as well as purified Wnt3A proteins are able to enhance the self-renewal of murine HSC *in vitro* and promote HSC reconstitution of the haematopoietic system of irradiated mice [50].

Dysregulation of Wnt signalling is critical to the initiation of epithelial carcinomas like colon cancer [51, 52]. Here, emphasis is given to its emerging role in leukaemia. The AML-associated translocation products AML1-ETO, promyelocytic leukaemia retinoic acid receptor-α (PML-RARα) and promyelocytic zinc finger protein (PLZF-RARα) are associated with Wnt signalling [53]. A co-activator of Wnt Tcf and Lef transcription factors is plakoglobin, whose presence in leukaemia positively regulates the production of all three fusion proteins mentioned, inducing expression of β-catenin and hence enhancing the replating capacity of leukemic cells *in vitro* [53]. Wnt signalling may also be involved in lymphoid leukaemia. For instance, Wnt16 was initially cloned due to over-expression in pre-B-cell leukaemia lines having E2A-PbX translocation, suggesting an oncogenesis model involving autocrine Wnt usage [53]. Recently loss of β-catenin was shown to impair the self-renewal capacity of both normal and CML stem cells *in vivo* [54]. The cumulative evidence now suggests that Wnt signalling may promote stem cell self-renewal in normal haematopoiesis. While dysregulation of the pathway is implicated in various haematopoietic malignancies, the exact mechanism of Wnt regulation remains unclear. It is also important to investigate how the Wnt pathway interacts with other pathways such as Shh and Notch to regulate stem cell self-renewal.

Notch and NF-κB signalling

The Notch pathway is another signal transduction pathway that functions as a major regulator of cell fate in the haematopoietic system. It is also thought to interact with NF-κB pathway during this process. Similar to *Wnt* genes, *Notch* genes encode evolutionarily conserved transmembrane bound receptors and signalling is mediated *via* cell-to-cell contact [55]. The pathway is initiated when Notch ligands bind to the epidermal growth factor (EGF)-like receptors Notch1–4, and signalling is processed by the enzyme γ-secretase [56, 57]. An *in vitro* mouse model has shown that Notch signalling is highly active in HSC and is down-regulated as differentiation proceeds [58]. However, inhibition of the pathway significantly accelerates cell differentiation [58]. This suggests a crucial role for the Notch pathway in the maintenance of HSC capable of self-renewal in an undifferentiated state. Importantly, identified target genes of the Notch pathway include some well studied proto-oncogenes and cell cycle regulators, including *myc*, *Cdkn1a* and *cyclinD1* [57]. Dysregulation of the Notch pathway is implicated in various malignancies such as breast cancer, medulloblastoma, colorectal cancer, pancreatic cancer and leukaemia [59, 60]. In leukaemia, a causative role of the Notch1 protein (N1ICD) in disease induction was first described when it was found that overexpression of N1ICD led to immature T-cell neoplasm in mice [57]. Subsequent studies went further to show a link between N1ICD dysfunction and various other lymphomas, like anaplastic large cell lymphoma [61], in which oncogenesis is due to an important role for the N1ICD in HSC self-renewal [62, 63]. In addition, recent studies suggest that Notch3 may be a co-contributor in regulating the self-renewal mechanism of early T-cell precursors [57], and thus the development of T-cell leukaemia. However, direct evidence illustrating the cellular targets of Notch-related malignant transformations is rare. This finding indicates an essential role for the Notch pathway in the self-renewal of cancer stem cells.

NF-κB signalling is involved in many cellular processes including response to immune or inflammatory stimuli, cell growth and apoptosis [64]. In HSC and haematopoietic progenitors derived from umbilical cord blood, NF-κB regulatory proteins and transcription factors for genes like *tumour necrosis factor* (*Tnf*), *fibroblast growth factor* (*Fgf*) and *Notch1* are overexpressed [55]. The pivotal role of NF-κB in the control of apoptosis [42] links this transcription factor to the development of haematopoietic cancers. Dysfunction of NF-κB is observed in multiple myeloma, chronic lymphocytic leukaemia and several other lymphomas, in which the *NF-κB* gene is constantly activated [65]. Manipulation of the NF-κB pathway could be used to drive stem cells to a desired fate. There has been increasing evidence to show that Notch and NF-κB interact with each other in disease pathogenesis. A direct link between Notch and NF-κB was provided by a recent study using N1ICD to induce the activity of *NF-κB* reporters. In this study, Aifantis and colleagues [66] identified NF-κB as one of the major mediators of Notch1-induced transformation. They reported that constitutively active Notch1 mediates the NF-κB pathway transcriptionally *via* the IκB kinase complex, which consequently results in the expression of several well characterized target genes of NF-κB in HSC [66, 67].

Hedgehog signalling and Bmi1

The Hh pathway, in which Bmi1is a key regulator, also plays a crucial role in stem cell self-renewal and cancer cell proliferation. Hh is a known cell cycle regulator of HSC. Under homeostatic conditions, activation of the Hh pathway induces cell cycling and expands the pool of stem cells in bone marrow [68, 69]. However, Hh activation modulates specific cell cycle regulators, such as CyclinD1, which can result in HSC exhaustion [69] such that HSC lose their ability to regenerate haematopoiesis upon secondary engraftments [69]. The proliferation of primitive haematopoietic cells induced by Hh activation is thought to involve BMPs [69]. In particular, cytokine-induced proliferation of HSC can be inhibited by antibodies to Hh, and a similar effect can be obtained using an inhibitor of BMP4 [68]. Furthermore, studies based on multiple myeloma, a plasma cell malignancy of the bone marrow, suggest that Hh activation is heterogeneous across the spectrum of multiple myeloma stem cells and their more differentiated progeny [68]. For HSC, Polycomb group genes have been shown to play a key role in preventing premature senescence, and hence prolong the life span of the stem cell [70]. Recent studies have shown that Bmi1 is a key regulator in this process. For example, Bmi-1 determines the proliferative capacity of normal and leukemic stem cells [71, 72]. There has been evidence to suggest that Bmi1 regulates stem cell self-renewal by modulating genes in the Shh pathway, one of three homologous Hh pathways, As a gene downstream of Shh, it regulates proliferation, survival and lineage commitment [73, 74]. In this process, Bmi1 affects the activities of p53 and CDK4/CDK6 cyclin-dependent kinases by repressing two corresponding tumour suppressors, $p16^{Ink4a}$ and $p19^{Arf}$ [70]. Bmi1 is highly expressed in purified HSC and its expression declines as differentiation progresses. Conversely, inactivation of Bmi1 in the mouse leads to a decrease in the number of haematopoietic cells and impaired proliferative response of these cells to mitogens [75]. Also, it has been shown that Bmi1 is implicated in self-renewal of neural stem cells [76], although not for neural progenitor proliferation [77].

Concluding remarks: cancer stem cells as a target for new cancer therapy

This review advances several propositions. Firstly, the ability to undergo continuous self-renewal is a distinct property of stem cells. Self-renewal plays a key role in both normal and malignant tissue formation. In normal haematopoietic systems, HSC are the only cells that have long-term self-renewing capacity, and their numbers are strictly controlled by the balance between self-renewal and differentiation, as well as by cell survival and apoptosis. Secondly, the cancer stem cell model of tumour pathogenesis predicts that only a small proportion of cancer cells are able to undergo prolonged self-renewal, and are thereby responsible for the initiation and maintenance of the tumour. Evidence for the existence of self-renewing cell populations comes from studies on leukaemias and some solid tumours, including cancers of the CNS, the mammary gland and the colorectal tract. Thirdly, current opinions on the origin of cancer stem cells are primarily twofold: cancer stem cells could arise by mutation of stem cells, or could be derived from a mutated progenitor cell that retains self-renewing capacity. The niche, or microenvironment of a stem cell, seems to play a pivotal role in regulating self-renewal and differentiation of normal stem cells. Thus it is also possible that dysfunction of the niche may instruct a normal stem cell or progenitor to gain malignant transformation. However, there has been little research into the role of the niche in oncogenesis. Fourthly, several molecular pathways are important regulators of stem cell self-renewal and differentiation. The Wnt, Hh-Bmi1, NF-κB and Notch pathways, in which small molecules are key players, are the most studied of these. They are critically important in normal self-renewal and differentiation. Dysregulation of these pathways has been implicated in various types of cancers. A thorough understanding of the mechanism of these pathways and how they interact with each other is essential for elucidation of the biology of normal and malignant stem cells, and hence the overall behaviour of tumours.

The concept of cancer stem cells in cancer pathogenesis has profound significance for our understanding and treatment of disease. Existing therapeutic approaches to cancer treatment, including chemotherapy and radiotherapy, primarily aim to eliminate the bulk of rapidly dividing but terminally differentiated cells of the tumour. Like normal stem cells, cancer stem cells are highly resistant to commonly used cytotoxic agents, such as cytarabine, adriamycin [78], and γ-radiation [79]. Therefore cancer stem cells probably survive after treatment has finished, which could explain the high refractory rate of cancer to many of these treatments. For new therapies, a reasonable approach would be to target critical components of the self-renewal pathways described here. Strategies may include interventions in receptor/ligand binding, and blocking of critical pathway regulators. For instance, in the Notch pathway, a promising strategy is to block receptor ligand binding using inhibitory antibodies directed against Jagged1 or DLL4 [25]. Similarly, the use of small molecular inhibitors of the γ-secretase complex prevents the release of N1ICD, showing an immediate therapeutic effect on Kaposi sarcoma found in HIV1-infected individuals, glioblastomas and lung adenocarcinomas [57, 80, 81]. In addition, interruption of niche function can slow the growth of some brain tumours. It has been shown that disruption of vascular niches in medulloblastomas has resulted in significant reduction in the number of cancer stem cells and the growth rate of the tumour [82]. Similar results were seen with glioblastoma cells, raising the possibility that depletion of niche cells may be a promising way for eliminating cancer stem cells in many situations [83].

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