Somatic stem cells and the origin of cancer

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Most human cancers derive from a single cell targeted by genetic and epigenetic alterations that initiate malignant transformation. Progressively, these early cancer cells give rise to different generations of daughter cells that accumulate additional mutations, acting in concert to drive the full neoplastic phenotype[1,2]. As we have currently deciphered many of the gene pathways disrupted in cancer, our knowledge about the nature of the normal cells susceptible to transformation upon mutation has remained more elusive.

Adult stem cells are those that show long-term replicative potential, together with the capacities of self-renewal and multi-lineage differentiation. These stem cell properties are tightly regulated in normal development, yet their alteration may be a critical issue for tumorigenesis. This concept has arisen from the striking degree of similarity noted between somatic stem cells and cancer cells, including the fundamental abilities to self-renew and differentiate. Given these shared attributes, it has been proposed that cancers are caused by transforming mutations occurring in tissue-specific stem cells[3-9]. This hypothesis has been functionally supported by the observation that among all cancer cells within a particular tumor, only a minute cell fraction has the exclusive potential to regenerate the entire tumor cell population[3,10-13]; these cells with stem-like properties have been termed *cancer stem cells*. Cancer stem cells can originate from mutation in normal somatic stem cells that deregulate their physiological programs. Alternatively, mutations may target more committed progenitor cells or even mature cells, which become reprogrammed to acquire stem-like functions[14,15] In any case, mutated genes should promote expansion of stem/progenitor cells, thus increasing their predisposition to cancer development by expanding self-renewal and pluripotency over their normal tendency towards relative quiescency and proper differentiation.

Key words: cancer stem cells, cancer, leukemia, telomerase, micro-RNAS.

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The ability of the stem cells to produce progeny that expresses different mature phenotypes is called potential or plasticity. Pluripotent embryonic stem cells can give rise to all of the differentiated tissues of the body whereas multipotent somatic stem cells have the capacity to form many but not all different cell types. Some childhood tumors have provided a link between embryonic stem cells and cancer[16-18]. Both neuroblastoma (which arises from fetal neural crest cells of the sympathetic nervous system) and Wilms tumor (which arises from embryonic cells of the kidney) are caused by mutations occurring in embryonic stem cells, giving rise to tumors composed of a mixture of undifferentiated spindle cells, immature epithelial tubules and more differentiated cells. Similarly, teratocarcinomas are made up of a variety of differentiated cell types as well as of embryonic and fetal tissue cells[18,19]. Because these normal embryonic cells physiologically differentiate with age and eventually disappear, neuroblastoma, teratocarcinomas and Wilms tumors only affect to young children[16-18,20]. However, the majority of cancers occurring in adults are derived from multipotent or pluripotent (somatic) stem cells, some of which have been recently discovered.

Here we review the role of the cancer stem cells identified in different hematopoietic and solid tumors, focusing on the molecular networks that govern their homeostatic programs. We will additionally address emerging new concepts and controversial issues of cancer stem cell biology. Last, the potential implica-

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tion of cancer stem cells in the development of lymphoid malignancies will be discussed.

HEMATOPOIETIC STEM CELLS AS TARGETS OF TRANSFORMING MUTATION IN LEUKEMIA

Early bone-marrow reconstitution experiments in immunocompromised mice revealed the existence of the hematopoietic stem cells (HSCs)[21,22]. Recently, many tissue-specific stem cells have been identified[23-27]. Stem cells share the characteristics of unlimited self-renewal, which maintains or expands the stem-cell population, and multi-lineage differentiation, which generates and regenerates tissues[27]. Yet another defining characteristic of stem cells is their limited replication frequency (quiescence) compared to their more proliferative progreny[28]. Although initial evidence for the concept of cancer stem cells also came from early studies[10,29-31]. solid experimental analysis have demonstrated their existence only recently. Two pioneering reports showed that despite most of the tumor cells obtained from patient with acute myeloid leukemia (AML) were unable to proliferate extensively, a small subset were capable of transferring the leukemia to immunocompromised mice. These leukemia-initiating stem cells (LSCs) were prospectively identified as having a CD34+ CD38- phenotype, resembling normal HSCs[11,32,33]. By contrast, CD34+CD38+ leukemic cells were not able to propagate the leukemia. Notably, on each of the morphological and genetic subtypes of AML, only the CD34+CD38- cell population retained the capacity to transfer the leukemia *in vivo*[32]. These data indicate that AMLs exhibiting different stages of differentiation are originated from a common LSC that shares similar cell-surface markers with normal long-term HSCs. Subsequent studies have characterized in detail the phenotypes of normal HSCs and LSCs. Besides a CD34+CD38- phenotype, both lack expression of the lineage markers CD71 and HLA-DR[32,33]. Notably, LSCs also lack expression of CD90 and the stem cell factor receptor CD117 (c-Kit) whereas they had high expression of the interleukin-3-alpha receptor CD123[34,35]. However, despite a common immunophenotype, LSCs are not functionally homogeneous but instead, resembling the normal hematopoiesis, form a hierarchy of cells that differ in their self-renewal potential[36]. The similarities between HSCs and LSCs strongly suggest that HSCs are the source of LSCs when targeted by oncogenic mutations. Nevertheless, these mutations may also target progenitor cells that become de novo leukemia stem-like cells (fig. 1).

Additional evidence supports that AML is a progressive model of cancer resulting from mutations in HSCs and their progeny. The t(8;21)(q22;q22) is the most frequent chromosomal translocation in AML, resulting in a chimeric *AML1-ETO* gene fusion. In HSCs isolated from patients with AML in complete remission, *AML1-ETO* transcripts were found in a fraction of normal bone marrow HSCs[37]. These cells did not show leukemic properties, and normally differentiated to myeloid and erythroid cells. Consequently, the t(8;21) occurred in normal HSCs suggesting that it was the additional mutations in a subset of HSCs or their progeny which led to leukemia development. A different experimental approach has investigated the transformation capacity of a variety of oncogenes involved in human leukemia when ectopically expressed into the different HSC compartments. Short-lived myeloid progenitors transduced with *MLL-ENL* or *MOZ-TIF2* oncogenic fusions generated AML with similar latencies and characteristics than those observed in long-term HSC models[15,38]. Notably, this observation was not expanded to *BCR-ABL* or *MLL-GAS7* gene fusions, which did not transformed committed murine hematopoietic progenitors[38,39]. According to these data, some but not all oncogenes are capable of mediate similar leukemic transformation in both HSCs and committed progenitors. More recently, Eguchi et al expressed the two variants of the TEL-TRKC fusion gene, which are found in leukemias and solid tumors, into the mouse stem cell compartment. Results showed that the leukemia form of *TEL-TRKC* enhanced hematopoietic stem cell renewal and originated leukemia whereas the solid tumor variant of *TEL-TRKC* elicited impairment of hematopoiesis but did not induce cancer[40]. Thus, related oncogenic fusion genes similarly expressed in stem cells may produce diverse cell type-specific developmental impacts. This interesting finding might explain the phenotypic diversity of human leukemia despite having identical chromosomal rearrangements.

Functional studies have also been conducted in chronic myeloid leukemia (CML), a disease of HSCs that undergo hierarchical differentiation, thus resulting in a vast majority of differentiated CML blood cells, whereas a rare cell fraction resembling normal HSCs is responsible for disease maintenance. CML cells also have the potential to generate acute leukemias of myeloid and lymphoid lineage during the blast crisis[41,42]. At the molecular level, CML is characterized by the *BCR-ABL* oncogenic fusion, which can be detected through all lineages of differentiation of CML cells, including the CD34+ stem cell compartment, pointing out to this leukemia as a prototype of stem cell disorder[41-44]. However, recent studies have indicated that the granulocyte-macrophage progenitor cells from patients with CML in blast crisis presented self-renewal capacity, a cell compartment which normally has not this property[45]. These data suggest that despite *BCR-ABL* fusion targets CD34+ stem cells and originate CML in chronic phase, progenitor cells are also responsible for leukemic transformation in the

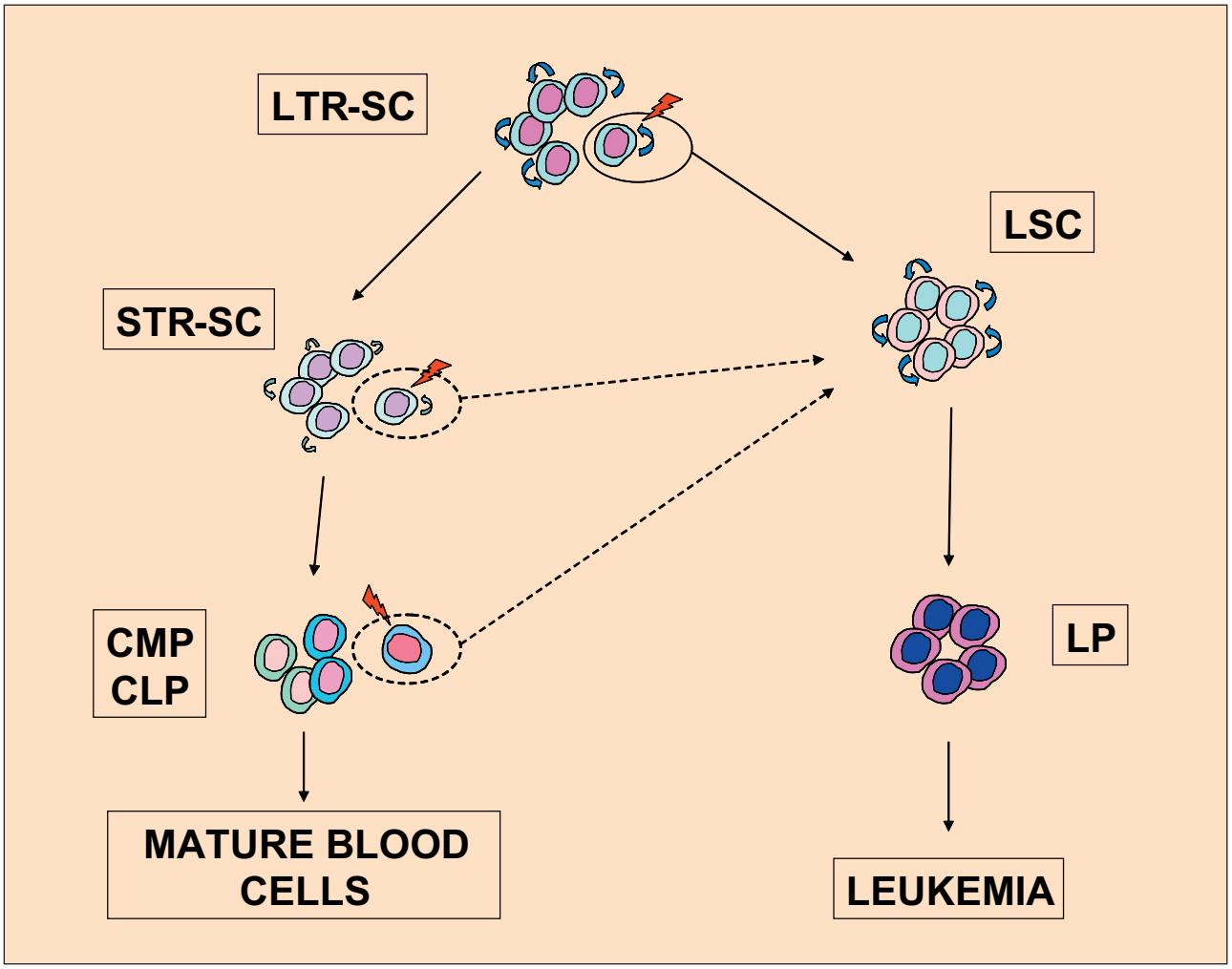

Fig. 1. Stem cells as targets of mutation in leukemia development. LTR-SC, Long-term repopulating stem cell; STR-SC, short-term repopulating stem cell; CMP, common myeloid progenitors; CLP, common lymphoid progenitors; LSC, leukemia stem cell; LP, leukemia progenitors.

blast crisis, demonstrating that the hierarchy of leukemia-initiating stem cells can change during the progression of the disease.

In contrast to myeloid leukemia, progress towards the identification of the stem cell of origin in acute lymphoblastic leukemia (ALL) has been more limited. The best characterized leukemia subtype is ALL with *BCR-ABL* chimeric protein (ALL-Ph+). Functional studies in a mouse model of ALL-Ph+ demonstrated that the only fraction of cells capable of engrafting NOD/SCID mice were CD34+/CD38- cells[46]. These data indicate that primitive progenitor cells rather than more committed lymphoid cells are targeted by *BCR-ABL* gene rearrangement in ALL. A different study used human leukemic cells from patients with other subgroups of ALL[47,48]. ALL cells with a CD34+ CD10- phenotype were capable of transferring leukemia to NOD/SCID mice. The engrafted cells showed the same phenotype of the patients at diagnosis (CD34+, CD10+, CD19+), indicating that CD34+ CD10- progenitor cells differentiated *in vivo*. Further functional characterization studies should determine whether multipotent HSCs or more committed progenitors are commonly targeted in the distinct ALL molecular subgroups or by contrast, specific ALL gene rearrangements occur in different stem cell populations.

CANCER STEM CELLS AS THE ORIGIN OF SOLID TUMORS

The leukemia experimental studies were subsequently extended to include epithelial cancers. Using a model of human breast cancer cells obtained from primary tumors and pleural metastatic effusions that were grown in non-obese diabetic, severe combined immunodeficient (NOD/SCID) mice, investigators distinguished the tumor-initiating cells from the non-tumorigenic cells based on their cell phenotype[12].

Tumor-initiating cells expressed CD44 but little or no CD24. As few as 100 cells with this phenotype were able to form tumors in mice, whereas thousands of tumor cells with other different phenotypes failed. In addition, these cells could be serially passaged from mouse to mouse, and each time the new tumors contained the diverse mixed population of tumorigenic CD44+CD24-/low and non-tumorigenic cells present in the initial tumor[12,49]. The possibility to separate tumorigenic and non-tumorigenic cells will allow elucidating the molecular pathways that account for their different oncogenic potential.

The embryonic features present in several types of brain tumors have suggested that they originate from transformed neural stem or progenitor cells. The best example is childhood medulloblastoma, which is considered a failure of the process of differentiation of the neural primitive pluripotential cerebellum stem cells[18]. In adult brain tumors, similar conclusions have been reached by three different groups[50-52]. Singh and colleagues prospectively identified a cell subpopulation from brain tumors expressing the human neural stem cell marker CD133 that exhibited stem cell properties *in vitro* and *in vivo*. These CD133+ cells were the only cell fraction capable of tumor initiation in NOD-SCID mouse[13]. Injection of as few as 100 cells with this phenotype generated tumors that were serially transplanted, whereas CD133- cells were unable to transfer the neoplasm. These cells were isolated from high and low grade tumors from both children and adults, suggesting that the principles of stem cell leukemogenesis may also be applied to the brain tumor pathogenesis. In contrast, Bachoo et al reported that not only neural stem cells but also mature neural cells (astrocytes) were equally capable of transformation upon *INK4a* and *ARF* synergistic mutations[14]. These data support a different point of view where deregulation of specific genetic pathways, rather than cell-of-origin, dictates the emergence and phenotype of brain cancers[53].

Recently, a mouse model in which expression of oncogenic *K-ras* is spatially and temporally controlled was used to study the stem cell of origin of lung cancer. *Lox-K-ras* mice developed atypical adenomatous hyperplasia that progressed to adenomas and then to adenocarcinomas[54]. In *Lox-K-ras* tumors, a novel cell type expressing both the AT2 cell-specific marker SP-C and the Clara cell-specific marker CCA, termed double-positive cells, were identified in adenomas, particularly in lesions continuous with bronchiolar hyperplasia. The study of their counterpart cells in normal lung led to the identification of a regional pulmonary stem cell population, termed bronchioalveolar stem cells (BASCs), at the bronchioalveolar duct junction. BASCs exhibited self-renewal potential, were multipotent in clonal assays and most remarkably, expanded in response to oncogenic *K-ras* in culture and in precursors of lung tumors *in vivo*[55]. These data point to BASCs as the putative cells of origin for lung adenocarcinoma.

In 2004, Houghton et al published a controversial study supporting that gastric cancer is originated not from epithelial cells of the stomach but from bone marrow-derived cells that are recruited after *Helicobcter* infection to repair the microbe-induced damage[56]. These bone marrow cells progressed through metaplasia and dysplasia to ultimately form epithelial cancer. Although this report further supports the well-accepted concept that chronic inflammation fosters cancer development, the results indicate that gastric tumors might arise from visiting bone marrow cells rather than from the transformation of local tissue-specific cells. In fact, *Helicobacter pylori* infection causes malignant transformation of the gastric mucosa-associated lymphoid tissue (MALT), leading to the generation of MALT lymphoma through activation of NF-κB pathway[57-61]. However, because bone marrow cells can adopt the phenotype of other cells by spontaneous fusion, this might have been the originating cause of this model of gastric cancer[62]. More evidence is needed to prove any of these hypotheses in gastric cancer and in other inflammatory neoplasias.

ONCOGENE AND TUMOR SUPPRESSOR GENE NETWORKS REGULATE SOMATIC AND CANCER STEM CELLS

Disruption of critical self-renewal pathways

Cancer can be primarily considered as an alteration of the stem cell self-renewal pathway. Thus, it is not surprising that several genes that control normal stem-cell self-renewal can cause cancer when deregulated by mutations. WNT signaling pathway regulates proliferation and self-renewal of different organs whereas activating mutations of WNT proteins have been identified in a variety of cancers[63-65]. Other signaling pathways involved in stem cell self-renewal include Notch, Sonic hedgehog (Shh), phosphatidylinositol-3 kinase (PIK3), and Homeobox (HOX) and Polycomb families, all of which are deregulated in hematopoietic and epithelial tumors[64,66-72]. A crucial task for investigators is to search for genetic and functional similarities and differences between normal and cancer stem cell self-renewal pathways.

BMI1 is a gene member of the Polycomb Group family that has been involved in cancer[72]. In mouse models, forced expression of *BMI1* in lymphocytes provokes lymphoma[73] whereas *BMI1* is amplified and over-expressed in human mantle cell lymphoma[74] specially in Ki67+ proliferating cells[75]. *BMI1* deletion in mice causes defects in hematopoiesis, neurological

development and body growth[76]. *BMI1* acts through repressing two cyclin-dependent kinase inhibitors, *P16/INK4a* and *P14/ARF*[77]. Two simultaneous reports showed that *BMI1* has an essential role in regulating the proliferation of both normal and leukemic stem cells[78,79]. In addition, *BMI1* is also required for the self-renewal of stem cells in the peripheral and central nervous system[80]. These studies demonstrate that *BMI1* presents similar functions in cancer and normal stem cells, yet its altered expression in stem cells may lead to tumorigenesis through disruption of genes (*INK4a* and *ARF* among others) that regulate cell cycle control, apoptosis and DNA repair pathways.

However, a recent study challenges this concept and supports the existence of genes or pathways that have different effects on normal and cancer stem cells. The tumor suppressor *PTEN* is a phosphatase that negatively regulates signaling through the PI3K pathway, inhibiting survival and proliferation[81]. Inactivation of *PTEN* has been reported in many cancer types, including lymphoid and myeloid leukemias[81-84]. Mice with conditional deletion of *PTEN* gene in adult HSCs developed myeloproliferative disease within days that evolved to transplantable leukemia within weeks. In contrast, normal HSCs without *PTEN* were progressively depleted and were unable to stably reconstitute irradiated mice after transplantation[85,86]. Thus, *PTEN* inactivation leads to depletion of normal HSCs but promotes the generation and expansion of leukemia-initiating cells. Most importantly, authors also demonstrated the potential therapeutic application of the differences of *PTEN* function in normal and cancer stem cells[85]. In *PTEN* deficient mouse, the PI3K pathway is constitutively activated, which in turn activates the mammalian target of rapamycin (mTOR). The drug rapamycin inhibits mTOR kinase activity[87-89]. In the *PTEN*-deficient mice, rapamycin depleted leukemia-initiating cells and rescued the capacity of normal HSCs to provide long-term multilineage reconstitution to irradiated mice.

Telomerase activity and telomere length regulate cancer stem cells

Normal human cells have two critical points that regulate their life span, the senescence and crisis phases. Senescence is associated with telomere shortening and cell cycle arrest. If cells *bypass* this stage, they keep growing until telomeres become critically short and cells enter crisis phase, characterized by chromosome instability and apoptosis[90]. To investigate the role of stem cells as targets for neoplastic transformation, adult human mesenchymal stem cells (hMSC) were transduced with the telomerase *hTERT* gene[91]. Long-term cultures of hMSC-TERT+ cells were characterized by progressive loss of contact inhibition, anchorage independence and formation of tumors in immunodeficient mice. This transformation was associated with deletion of *INK4a/ARF* locus and *KRAS* activating mutation[91,92]. More recently, two independent groups have intriguingly reported the spontaneous malignant transformation of similar mesenchymal stem cells *in vitro*[93,94]. In one report, hMSCs isolated from adipose tissue bypassed senescence and crisis phases, after which 50% of the cells kept proliferating, acquired chromosome instability and were able of generating tumors in immunodeficient mice[93]. The spontaneous transformation was accompanied by loss of *INK4a* gene expression, *MYC* alteration and high telomerase activity. Similar findings were reported by Miura et al. using murine bone marrow-derived mesenchymal stem cells, which resulted in spontaneous fibrosarcoma formation[94]. The transformation process was also associated with chromosomal abnormalities involving *MYC* and upregulation of telomerase activity. The potential spontaneous transformation of somatic stem cells should deserve further attention because of their potential clinical use in tissue regenerative medicine.

In light of these previous reports, increased telomerase activity may be responsible, at least in part, for the malignant transformation of human and murine mesenchymal stem cells[91-94]. This finding is not totally unexpected, as alterations in telomere length and telomerase activity may facilitate cancer development by regulating genomic stability and cell lifespan[95]. In fact, a combination of shortened telomeres and increased telomerase activity is seen in most hematological and solid tumors[96] and *mTERT* transgenic mice with increased telomerase activity spontaneously develop cancer[97]. The best model to study telomere functions in stem cells has been the hematopoietic cell compartment[95,98]. With age, HSCs lose telomeric DNA while maintaining detectable telomerase activity, what seems a mechanism to limit progressively the hematopoietic regeneration[99]. In fact, long-term repopulating capacity of telomerase-deficient murine HSCs is remarkably deficient[100]. Notably, these defects are not repaired by Tert overexpression in HSCs, indicating additional telomere-independent factors that regulate HSC regeneration[101]. Together, these data point out to a role of telomeres in the regulation of HSC homeostasis[102]. Two recent studies investigated the effects of telomerase and telomere length on epidermal stem cell behaviour[103,104]. Using different constitutive and inducible transgenic mouse models, authors demonstrated that telomere length and telomerase activity are independent determinants of the mobilization efficiency and proliferative capacity of epidermal stem cells. Thus, telomere shortening inhibited mobilization of stem cells out of their niche and resulted in suppression of stem cell proliferation. In contrast,

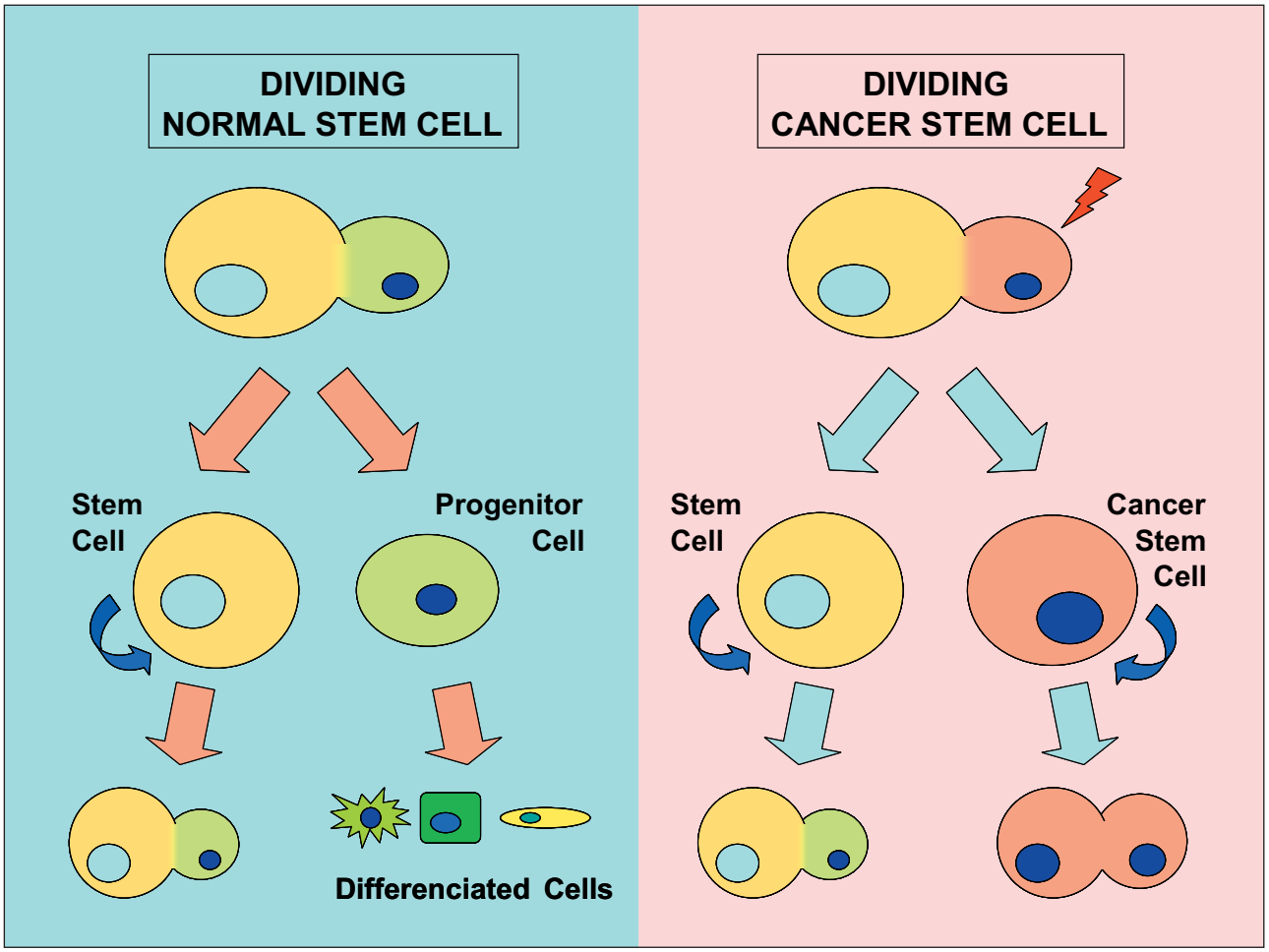

Fig. 2. Asymmetric and symmetric stem cell division modes occurring stem cells.

telomerase activation promoted stem cell mobilization and proliferation. These findings may explain the increased susceptibility of *mTERT* mice to develop skin tumors and anticipate a critical role of telomerase and telomere length in the regulation of cancer stem cells[97,98,102].

Asymmetric division of stem cells and cancer

Stem cells fulfil their biological functions over the lifespan of the organism, thus requiring tight regulation of the self-renewal process. During early embryonic growth, stem cells divide symmetrically and each daughter cell retains the properties of the parental cell. The adult stem cells normally divide asymmetrically, whereby one daughter cell retains the properties of the parental stem cell and the other daughter cell begins differentiation[105]. But how can impairment of asymmetric division in stem cells lead to tumorigenesis? *Drosophila melanogaster* is an excellent model organism for the study of cancer development upon gene mutation[71]. One of the best characterized examples is neuroblastoma, a tumor originated from neuroblast stem cells. Normally, Drosophila neuroblasts divide asymmetrically, a process which is controlled by genes regulating cell polarity and cell fate[106,107]. Using *Drosophila* as a model, three recent studies have shown that the loss of polarity and impairment of asymmetric division in stem cells leads to tumorigenesis[108-110]. In these reports, neuroblasts containing mutations in various genes that control cell division *(Raps, Mira, Numb, Brat* and *Pros)* developed aggressive tumors that could be retransplanted into new hosts. Importantly, asymmetric division was disrupted in mutant stem cells, whereby both daughter cells grow and behave like neuroblasts leading to the formation of tumors[108-110]. This transformation was associated with genome instability and centrosome alterations (fig. 2). Together, these data indicate that invertebrate cells rapidly develop cancer upon mutation of the asymmetric division machinery, by adopting an aberrant mode of symmetric division[111].

Although there is no solid proof to link asymmetric cell division deregulation and human cancer, there are at least three examples that suggest this possibili-

ty. The adenomatous polyposis coli *(APC)* gene is required for the asymmetric division of *Drosophila* spermatogonial stem cells and it is also a critical tumour suppressor in human colon cancer[112-114]. A human homologue of the *Drosophila* gene *LgL* is *HUGL1*, which controls cell polarity and is commonly inactivated in cancer in humans and in mice[115-117]. Last, inactivation of Numb may in turn activate the Notch signalling pathway, commonly observed in human solid and hematopoietic tumors[118,119]. It may be hypothesized that APC mutations occurring in intestinal epithelial stem cells, HUGL1 or Numb alterations may lead to cancer by altering asymmetric cell division. The study of these and other human homologs of the *Drosophila* genes involved in the asymmetric/symmetric cell division switch may provide novel insights into human cancer development.

Micro-RNAs as regulatory elements of cancer stem cells

One of the key features of stem cells is their capacity to divide for long periods of time while being in a more quiescent state than their more proliferative progeny. For that, cell cycle must be critically controlled in stem cells. Recent studies have suggested a role of the microRNA pathway in the regulation of stem cell division[120,121]. MicroRNAs (miRNAs) are small noncoding RNAs that regulate expression of coding genes involved in the control of development, proliferation and apoptosis, primarily through translational repression[122,123]. Remarkably, miRNAs are deregulated by genomic alterations in cancer cells[124,125], thus targeting oncogenes and suppressor genes in an aberrant manner[126-129]. One of the best characterized examples is the miRNA cluster *miR-17-92* in the chromosome 13q31 amplicon, commonly observed in B-cell lymphoma and different carcinomas[123,130,131]. *miR-17-92* over-expression appears to collaborate with *MYC* in promoting tumorigenesis[127,128].

Initial evidence connecting miRNAs, stem cells and cancer was provided by Lu et al by classifying miRNA expression patterns in 334 primary tumors of different types and in normal tissues[132]. Notably, tumors displayed a miRNA profile that was reminiscent of that in the stem cells from which they were derived, providing a better indicator of tissue lineage that the conventional mRNA profiles. Overall, the expression level for most miRNAs was significantly reduced in the tumors while retaining expression of stem cell miRNAs, including the *miR-17-92* cluster. One of the components of the *miR-17-92* cluster, *miR-19*, has been shown to down-regulate the tumor suppressor PTEN[133]. which is directly implicated in stem cell development[85,86]. It may be speculated that silencing of PTEN as a consequence of the miR-19 over-expression observed in many lymphomas and carcinomas may take place in tissue-specific stem cells, leading to cancer development. Another example was provided by Chen et al by showing that *miR-181*, which is preferentially expressed in bone marrow B-lymphoid cells, when ectopically expressed in hematopoietic stem or progenitor cells leads to an increased fraction of B-lineage that may ultimate cause malignancy[134]. A recent study provided additional links between miRNAs and the regulation of stem cells. The miR-221 and *miR-222* genes were shown to regulate CD34+ stem cell proliferation and differentiation by unblocking kit protein production at mRNA level, thus leading to eryhtroleukemia[135]. More direct evidence was provided by Hatfield et al, who deonstrated the necessity of the miRNA pathway for proper control of germline stem cell division in *Drosophila*[120]. Analysis of these stem cells mutant for dicer-1, the double-stranded RNaseIII essential for miRNA biogenesis, revealed a marked reduction in the rate of germline cyst production. These mutant stem cells were defective in cell cycle control by delaying the G1 to S transition, thus making stem cells insensitive to stop signals[120]. If this miRNA machinery is mutated, it might contribute to tumor formation by promoting cell cycling and proliferation. Because silencing of miRNAs *in vivo* with «antagomirs» have been successfully used to treat disease in mouse models[136]. understanding the role of the miRNA pathway in the regulation of stem cell division may provide new opportunities for cancer treatment.

Tumor progenitor genes and epigenetic origin of cancer

It is widely accepted that cancer includes a heterogeneous group of disorders caused by a series of clonally selected genetic and epigenetic changes in oncogenes and tumor suppressor genes[1,2]. However, a recent theory suggests that cancer originates from a polyclonal epigenetic alteration of stem and progenitor cells within specific tissues, initially producing a pre-neoplastic epigenetically aberrant state[137]. Subsequently, an initiating mutation (specific activation of oncogenes in leukemia and sarcoma or inactivation of tumor suppressor genes in most solid tumors) targets a subpopulation of the epigenetically disrupted progenitor cells, thus leading to cancer development. A third stage would involve the acquisition of additional genetic and epigenetic changes that promote tumor progression and metastasis[137]. The epigenetic progenitor origin of human cancer hypothesis is based on several observations. First, epigenetic changes (global hypomethylation and specific gene promoter methylation) are detected early in tumorigenesis and what is more relevant, even in normal tissues before tumors arise[138-140]. Second, loss of genomic imprinting (parent-of-origin-specific gene si-

lencing of a specific gene) is a different epigenetic mechanism that causes reduction or absence of expression of a specific allele of a gene in somatic cells of the offspring. Loss of imprinting (LOI) of the insuline-like growth factor 2 *(IGF2)* gene is observed in 50% of Wilms tumors and is associated with fivefold increased frequency of colorectal neoplasias[141-144]. Consequently, LOI of *IGF2* might cause cancer by increasing the progenitor cell population in the kidney in Wilms tumor and in the gastrointestinal tract in colorectal cancer. According to this theory, authors define tumor-progenitor genes as those that suffer epigenetic modification in stem cells thus promoting malignant transformation[137]. Examples of these tumor progenitor genes are, in addition to *IGF2*, the POU transcription factor OCT4 or the forkhead transcription factor FOXD3, whose inappropriate expression could augment proliferation and block differentiation in stem cells[145,146]. One additional example may be represented by two DNA repair genes, ataxia teleangiectasia mutated *(ATM)* and checkpoint kinase 2 *(CHK2)*, frequently inactivated in mantle cell lymphoma. This B-cell lymphoma is characterized by the chromosomal translocation t(11;14)(q32;q21) that causes deregulation of cyclin D1 oncoprotein and is thought to be the earliest mutation in the disease[147]. In more than 50% of cases, genomic deletion or mutation of *ATM* or *CHK2* genes accumulate to the initial t(11;14)[75,148,149]. Notably, in a small subset of patients with mantle cell lymphoma, germline mutations of *ATM* or *CHK2* have been observed, indicating that this alterations preceded and may have conditioned the acquisition of the t(11;14)[148,149]. According to the epigenetics theory, *ATM* or *CHK2* (and perhaps other DNA repair genes) might become silenced by epigenetic mechanisms (promoter hypermethylation, LOI) in a polyclonal stem or progenitor cell population. These stem/progenitor cells with damage of the DNA repair machinery would not control properly the physiological process of immunoglobulin gene rearrangement in early B-cells properly, thus causing aberrant chromosomal translocations that will promote lymphoma formation. Because the evidences supporting the epigenetic origin of cancer are still circumstantial, further experimental work will determine whether this interesting model is, at least in part, implicated in cancer development.

THE STEM CELL NICHE AS A MASTER REGULATOR OF CANCER FORMATION

Normal somatic and cancer stem cells not only share the capacities of self-renewal and multi-lineage differentiation, but are also similarly surrounded by a specialized cell microenvironment, termed the *stem cell niche*. The niche is the physical anchoring site for stem cells composed of cells that function to maintain and regulate stem cells homeostasis[150]. Adhesion molecules are also involved in the interaction between stem cells in their niche and the extracellular matrix. The niche regulates stem cell behaviour through providing extrinsic signals that balance stem cell proliferation, differentiation and symmetric/asymmetric cell division. In addition, it has been proposed that the stem cell niche functions to prevent tumorigenesis by regulating self-renewal and proliferation rate in stem cells. Thus, intrinsic mutation in the niche may promote cancer development[5,151]. Furthermore, the molecular machinery used by normal stem cells for homing to or mobilizing from the niche may be utilized by cancer stem cells for invasion and metastasis[151].

Still, the idea of cancer as a consequence of stem cell niche mutations is hypothetical, although recent data support the role of the vascular niche in initiating metastasis[152]. In addition, skin tumors can be caused by telomerase activation, which promotes epidermal stem cell mobilization out of their niche together with increased cell proliferation[97,103]. The study of signalling pathways that control mobilization and homing of stem cells in the niche will enhance our understanding of cancer development, invasiveness, and metastasis. Furthermore, if cancer stem cells depend on their niche to survive, targeting the cell components of the niche may result in therapies that complement those targeting directly the cancer stem cells[150].

DO LYMPHOID MALIGNANCIES ARISE FROM MUTATIONS THAT DEREGULATE SOMATIC STEM CELLS?

In contrast to myeloid neoplasias, the identity of the stem cells responsible for the initiation and maintenance of lymphoid malignancies has not been resolved. Yet, lymphocyte lineage development, which follows a tightly regulated, hierarchical order which also permits a great flexibility, has been well characterized during the last years[153-156]. HSCs early differentiate to multipotent progenitors (MMP), which have lost long-term self-renewal capacity and are committed to either the lymphoid (common lymphoid progenitor or CLP) or myeloid (common myeloid progenitor or CMP) lineages. CLPs are restricted to lymphoid development, giving rise to B cells, T cells, dendritic cells and natural killer cells. These developmental steps are controlled by diverse cytokines and transcription factors cooperating to achieve efficient generation of mature lymphocytes[154]. In early stages, both PU.1 and Ikaros control the balance between myeloid or lymphoid commitment of MMPs through regulation of different signaling receptors (FLT3, c-KIT and IL-7R)[157-159]. More committed, earliest B cell progenitor transition to pro-B cell depends on addi-

tional transcription factors (E2A, EBF, PAX5 and BCL11A), which coordinately activate appropriate B-cell expression programs and immunoglobulin heavy-chain gene rearrangements[160-165]. (fig. 3). Alternatively, CLPs can differentiate to T-cells through Notch1 activation, which is negatively regulated by PAX5[166-168]. Recent studies have shown the role of FOXP1 in the transition of pro-B cells to pre-B cells, a step which is also controlled by IRF4/MUM1 and IRF8[169,170]. More differentiated B-cells are also tightly programmed by transcription factors. OBF1 and Aiolos are crucial for the transition of pre-B cells to immature B cells expressing BCR, as well as for the mobilization of immature B-cells from bone marrow to the spleen, whereas NF-κB activation has essential roles in the transition of immature to mature B-cells[171-173]. Additionally, BCL6, IRF4, BLIMP1, XBP1 and PAX5 are involved in the terminal differentiation of B-cells into plasma cells[174-182].

Chromosomal translocations in lymphoid malignancies usually juxtapose the immunoglobulin or T-cell receptor genes with a variety of oncogenes which result deregulated[183,184]. Notably, many of the genes involved in the lymphocyte developmental network are targeted in such chromosomal translocations, namely *PAX5, OBF1, BCL11A, BCL6, IRF4/MUM1, Ikaros, NOTCH1* and *FOXP1* (table 1)[155,164,183,185-191]. Other regulatory genes are also altered through alternative genetic mechanisms, such as gene amplification of the NF-κB family gene REL, activating mutation of NOTCH1 and inactivating mutation of BLIMP1[119,192-194]. Although these genetic rearrangements are thought to be early events in the lymphoma pathogenesis, transgenic mouse models for the lymphoma oncogenes have shown a great heterogeneity in driving malignancy in vivo. Thus, while mice with deregulated expression of *MYC* develop tumors displaying features typical of human Burkitt lymphoma, other models did not generate lymphoma spontaneously or developed tumors that did not resemble human disease[195-201]. A possible explanation for the failure of this mouse models could be the necessity to express the aberrant oncogene into the adequate stem or progenitor cells to generate human-like lymphoma. Recent evidence supports this hypothesis. A transgenic mouse model where expression of *BCL6* oncogene was restricted to the somatic stem cell compartment developed diffuse large B cell lymphoma that mimicked human disease (Pérez-Caro and Sánchez-García, abstract AACR 2006). This lymphoma could only be propagated to secondary recipients by stem cells expressing *BCL6*. Moreover, gene expression patterns of stem cells expressing *BCL6* showed a *BCL6* signature and established a B-cell differentiation program. These results indicate that *BCL6* oncogene is capable of driving a specific genetic program in stem cells that mediate their transformation into lymphoma cells. This concept is reinforced by NOTCH1, an essential regulator of T cell lineage development. More than 50% of human T-cell acute lymphoblastic leukemias, including all major molecular oncogenic subtypes, have activating mutations of NOTCH1 gene[119]. Notably, constitutively activated forms of the NOTCH1 receptor are potent inducers of T cell acute lymphoblastic leukemia/lymphoma when expressed in murine bone marrow stem cells[202]. The mechanism by which NOTCH1 induces T-cell malignancy in stem cells appears to be an increase in their self-renewal and proliferative properties[202,203]. Theoretically, other transcription factors controlling lymphocyte fate could, like *BCL6* and NOTCH1, drive malignancy when mutated in stem cells.

Lineage commitment of lymphoid cells has been generally considered an irreversible process, but a recent challenging report by Xie et al. showed that enforced expression of C/EBPα/β transcription factors in differentiated B-cells leads to their reprogramming into macrophages by inhibiting PAX5 and synergizing with PU.1[204]. This exceptional lymphoid plasticity might be also observed in lymphoma, as mutations in differentiated B or T cell lymphocytes may reprogram them into *de novo* stem-like cells. In support of this hypothesis, a recent report describes the mechanisms for B-cell lymphocyte reprogramming in malignancy[205]. Hodgkin and Reed-Sternberg (HRS) cells, the tumor cells in classical Hodgkin lymphoma, derive from mature B cells but have largely lost their B-cell phenotype. This extensive reprogramming is experimentally caused by the inhibition of the transcription factor E2A in HRS cells[205]. These data conclude that the plasticity of mature human lymphoid cells upon mutation may contribute to cancer development. A different approach was used to investigate the cells of origin of multiple myeloma, a clonal B-cell neoplasm that affects terminally differentiated B cells (plasma cells)[206]. Among the CD138+ myeloma plasma cells, a more immature and rare cell subpopulation that lacked CD138 expression was responsible for initiation and maintenance of the disease[29,207]. These cells, which resembled post-germinal center B cells rather than fully differentiated plasma cells, may represent the cancer stem cells of origin of myeloma.

CANCER STEM CELLS FROM A THERAPEUTIC PERSPECTIVE

Hypothetically, conventional chemotherapy kills the rapidly proliferating tumor cells whereas cancer stem cells remain viable during treatment because of their relative quiescence[208]. Thus, when treatment ceases, cancer stem cells regenerate the tumor entirely, explaining why cancer can relapse in patients in complete remission[3,4,49]. Consequently, destruction of the stem cell population capable of initiating and main-

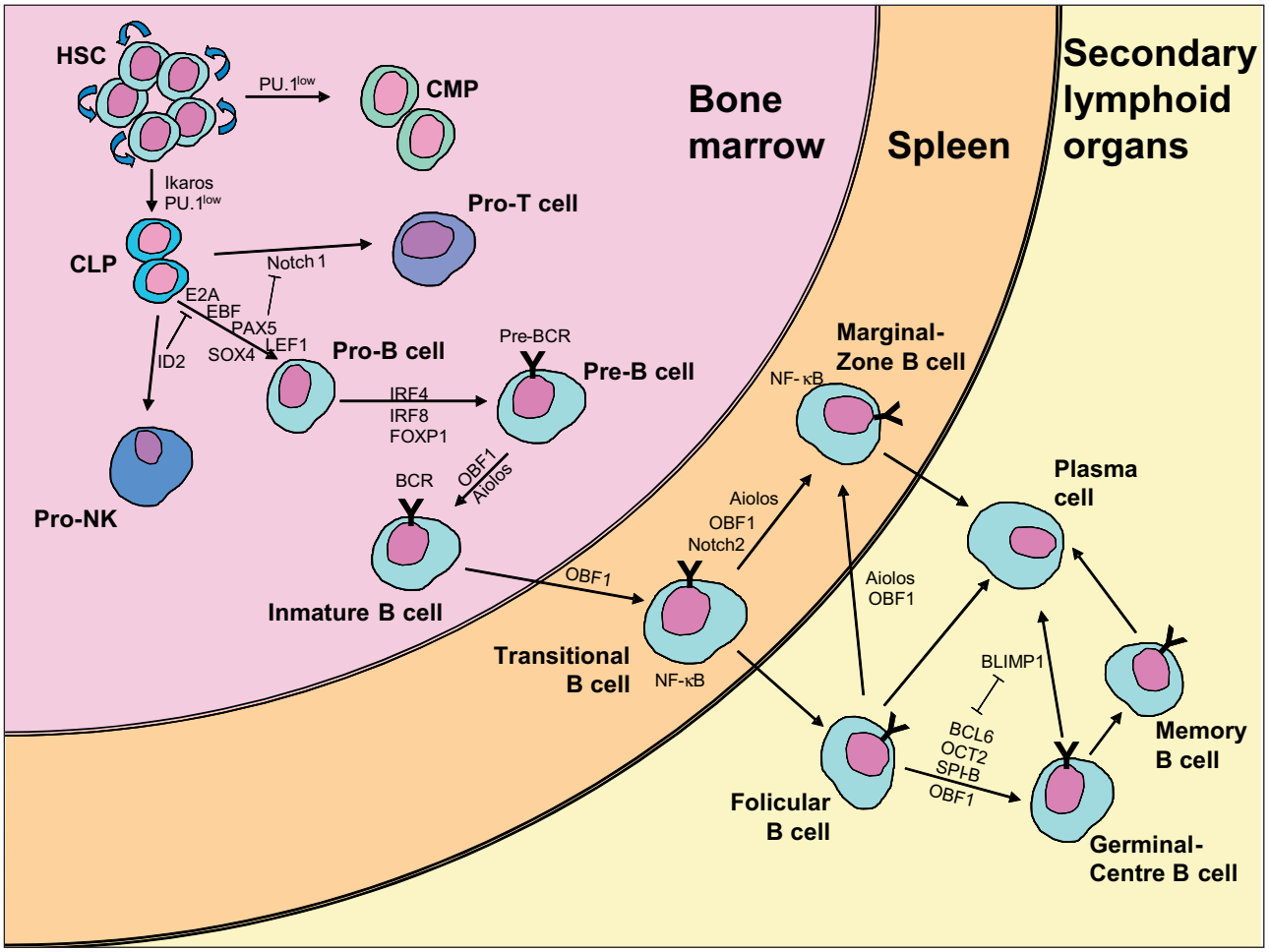

Fig. 3. Genetic control of B-cell lymphoid lineage developmental network in bone marrow, spleen and secondary lymphoid organs. Specific transcription factors that regulate each developmental stem are shown (for details, see text). HSC, hematopoietic stem cell; BCR, B-cell receptor; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; pro-NK, pro-natural killer cell.

taining tumors challenges the treatment of human malignancies. But to date, there is only circumstantial evidence, and even contradictory, of this selective resistance of cancer stem cells to chemo- or radiotherapy. Normal tissue-specific stem cells, including HSCs, are more resistant to chemotherapy than mature cells from the same tissues. It can relate to the higher expression of drug-resistance proteins such as MDR1 and ABC transporters in stem cells, which might make them less sensitive to chemotherapy and apoptosis induction[209,210]. Supporting this concept, forced expression of the anti-apoptotic gene *BCL2* in mouse hematopoietic stem cells makes them more resistant to radiotherapy[211]. A number of studies have evaluated that cancer stem cells, resembling normal stem cells, may be highly resistant to standard chemotherapy. Costello et al reported that CD34+CD38- AML stem cells presented reduced *in vitro* sensitivity to the chemotherapeutic agent daunorubicin compared to other AML cell populations and to normal HSCs as well[212]. In contrast, Guzman and colleagues demonstrated that idarubicine (a daunorubicin analog) combined with the drug MG-132 selectively killed CD34+CD38- AML stem cells but not normal HSCs[213]. This apparently contradictory results may simply indicate that cancer stem cells are either sensitive or resistant to the different chemotherapeutic combinations. In fact, cancer stem cell sensitivity can also vary even with specific molecular-directed therapies. Imatinib, a potent tyrosine kinase inhibitor of the *BCR-ABL* protein, has replaced interferon-alpha as the standard therapy of patients with CML[214,215]. However, ongoing clinical trials have not shown a clear survival benefit for the use of imatinib vs interpheron-alpha[216]. Indeed, recent data suggest that imatinib may not achieve complete ablation of CML cells[217], and even patients with CML who present complete molecular response almost invariably relapse when the drug is discontinued[216,218]. These observations may be explained by in vitro findings suggesting that ima-

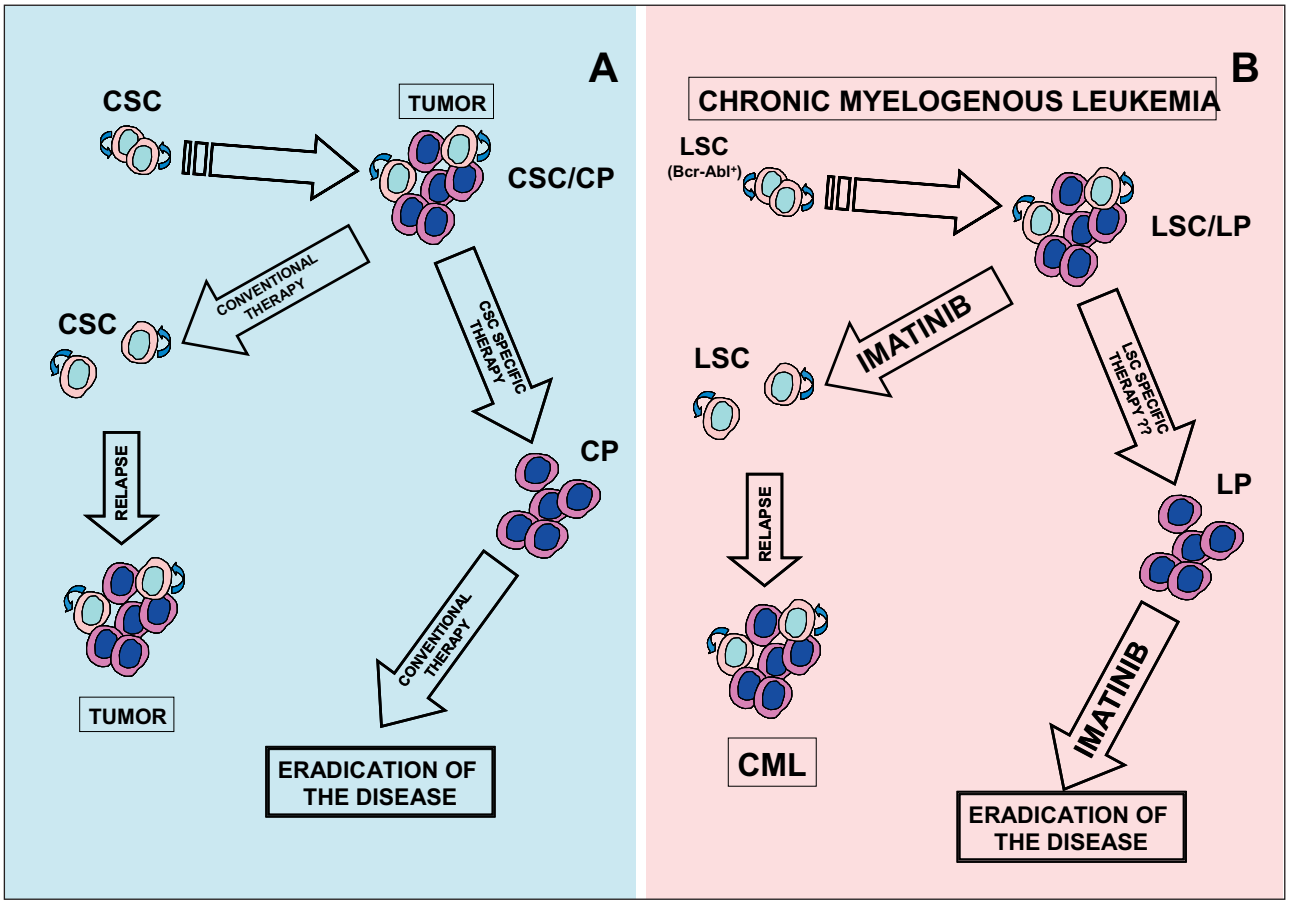

Fig. 4. Cancer stem cells and cancer treatment. A) Conventional therapies such as chemo or radiotherapy eradicate most tumor cells, including more committed cancer progenitor cells (CP) and differentiated cells, but may leave untouched the cancer stem cells (CSC). Thus, when therapy ceases, tumor arises from these remaining CSC. B) In chronic myeloid leukemia (CML), BCR-ABL+ hematopoietic stem cell generates the disease, which is composed of leukemic stem ells (LSC) and more committed leukemia progenitor cells (LP). Imatimib therapy seems to eradicate LP but not LSC, thus allowing leukemia relapse when treatment stops. Novel specific therapies that target BCR-ABL+ LSC are needed to ultimately eradicate the disease.

tinib, which is very active against differentiated CML progenitors, shows limited activity against CML stem cells[219-221]. Unequivocal *in vivo* proof of the resistance of leukemic stem cells to imatinib was recently provided by Sanchez-Garcia et al[222]. A transgenic mouse model where expression of *BCR-ABL*-p210 was restricted to the somatic stem cell compartment led to development of CML that spontaneously evolved to blast crisis, thus mimicking human disease. Notably, imatinib treatment of these mice was not associated with clinical survival benefit, whereas CML stem cells expressing BCR-ABL were shown to be resistant *in vivo* to the drug (fig. 4). This mouse model may be useful not only to investigate imatinib responses in CML but also to test novel more potent *BCR-ABL* tyrosine-kinase inhibitors[223,224].

On the contrary, there are examples of successful eradication of cancer stem cells after targeting appropriate transforming mutated pathways[85]. As discussed above, PTEN inactivation in mice leads to depletion of normal HSCs but promotes the expansion of leukemia-initiating cells, thus leading to myeloid leukemia[85,86]. PTEN deficiency activates PI3K pathway, which in turn activates mTOR. Rapamycin is an active drug that inhibits mTOR kinase activity, and patients with acute leukemia respond to this drug[87,88]. In the PTEN-deficient mice, rapamycin successfully eradicated leukemia by depleting leukemia-initiating cells and rescuing the capacity of normal HSCs to provide long-term multilineage reconstitution. A second molecular therapeutic target in leukemia is NOTCH1, which induces T-cell malignancy in stem cells by increasing their self-renewal and proliferative capacities, and is activated in more than half of the patients with T-cell ALL[119,202,203]. Interestingly, inhibitors of the proteolytic enzyme γ-secretase are active in NOTCH1-mutated T-cell leukemia/lymphoma, and therapeutic clinical trials using this drug are now under development. Like these therapeutic agents (rapamycin, inhibitors of γ-secre-

TABLE 1. **Oncogenes and tumor suppressor genes that regulate lymphoid cell differentiation and are involved in lymphoma oncogenesis by genetic alteration**

Protein	Genetic alteration	Molecular consequence	Leukemia/lymphoma	Gene function	References
APAX5	t(9;14)(p13;q32), SHH	PAX-IGH fusion → PAX5 deregulated expression	LPL, DLBCL, SMZL	Homeodomain protein that directs early B cell lineage commitment	185,225
E2A	t(1;19)(q23;p13)	E2A-PBX fusion	B-cell precursor ALL	Regulation of pro-B cell development and survival in coordination with EBF	160,226
IKAROS	t(3;7)(q27;p12)	BCL6-IKAROS fusion	DLBCL	Essential for B, T, NK and DC cell development	159,190
NOTCH1	t(7;9)(q34;q34.3), mutations	NOTCH1-TCRB fusion; extracellular domain and C-terminal gain-of-function mutations	T-cell ALL	Control of T-cell lineage development	119,202
IRF4	t(6;14)(p25;q32)	IRF4-IGH fusion, → IRF4 deregulated expression	Multiple myeloma	Regulation of pre-BCR signalling, plasma cell differentiation and class-switch recombination	170,180,188
OBF1	t(3;11)(q27;q23)	BCL6-OBF1 fusion	DLBCL	Essential for B cell immune response and germinal center formation	171,172,191
BCL11A	t(2;14)(p13;q32), gene amplification	BCL11A-IGH fusion → BCL11A deregulated expression	CLL, B-cell lymphoma, Hodgkin lymphoma	Essential for postnatal development and normal B and T cell lymphopoiesis	164,165
BCL6	t(3;14)(q27;q32), others, SHH	BCL6 deregulated expression	DLBCL	Transcriptional repressor required for germinal center formation	174,181,182,225
FOXP1	t(3;14)(p14;q32), gene amplification	FOXP1-IGH fusion → FOXP1 deregulated expression	MALT lymphoma, DLBCL	Regulation of pro-B cell to pre-B cell transition and and of IGH locus V(D)J recombination	169,187
BLIMP1	Mutation, gene deletion	Bi-allelic loss-of-function mutations	Activated DLBCL	Regulation of terminal B-cell differentiation; blocks BCL6	176,177,193,194
BCL9	t(1;14)q21;q32)	BCL9-IGH fusion → BCL9 deregulated expression	B-cell precursor ALL	Activation of Wnt signalling pathway	227,228
REL	Gene amplification	Gene and protein over-expression	DLBCL, PMBCL, Hodgkin lymphoma	NF-κB activation; regulation of immature B cell transition to mature B cells	192,229
c-KIT	Mutation, gene amplification	Activating mutations → ligand independent tyrosine kinase activity	Solid tumors; chronic myeloid leukemia	Receptor for stem cell factor with tyrosine-kinase activity; essential for B and T cell development	230,231
FLT3	Activating mutation	Internal tandem duplications	AML, hyperdiploid ALL	Tyrosine-kinase receptor essential for B-cell lineage commitment; repressed by PAX5	232,233

LPL: lymphoplasmacytic lymphoma; DLBCL: diffuse large B cell lymphoma; SMZL: splenic marginal zone lymphoma; ALL: acute lymphoblastic leukemia; CLL: B-cell chronic lymphocytic leukemia; PMBCL: primary mediastinal B cell lymphoma; AML: acute myeloid leukemia; SHH: somatic hypermutation.

tase), to find other drugs that specifically target the defective pathways in cancer stem cells without damaging normal stem cells is a major goal in the battle against cancer.

CONCLUSIONS

The cancer stem cell hypothesis proposes that most cancers derive from tissue-specific stem cells or their immediate progeny through mutations that deregulate their self-renewal and differentiation pathways. Because stem cells are exposed to various tumorigenic agents throughout their lifetime, the ageing stem cell may represent a major target of malignant transformation. Solid demonstration of the existence of cancer stem cells has been shown in myeloid leukemia and in a subset of solid tumors, which represent a minority of human cancers. In addition, cancer stem cells have been defined by their functional capacity to form tumors in immunocompromised mice rather than by specific genetic studies. Detailed isolation and molecular characterization of all tissue-specific cancer stem cells should be a major task not only to better understand cancer biology but also to improve current treatments. Novel therapies should be designed to specifically target molecules or pathways damaged in cancer stem cells but not in normal cells. These efforts might result in higher cure rates for patients with cancer.

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