Developmental changes in hematopoietic stem cell properties

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Hematopoietic stem cells (HSCs) comprise a rare population of cells that can regenerate and maintain lifelong blood cell production. This functionality is achieved through their ability to undergo many divisions without activating a poised, but latent, capacity for differentiation into multiple blood cell types. Throughout life, HSCs undergo sequential changes in several key properties. These affect mechanisms that regulate the self-renewal, turnover and differentiation of HSCs as well as the properties of the committed progenitors and terminally differentiated cells derived from them. Recent findings point to the Lin28b-let-7 pathway as a master regulator of many of these changes with important implications for the clinical use of HSCs for marrow rescue and gene therapy, as well as furthering our understanding of the different pathogenesis of childhood and adult-onset leukemia.

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INTRODUCTION

The mammalian hematopoietic system produces a collection of morphologically and functionally diverse mature cell types, which together perform key physiological processes including solute and O₂ transport, immunity and hemostasis. All of these mature blood cells are ultimately derived by a continuous, multi-step process of lineage restriction and activation of specific differentiation programs in rare self-maintaining hematopoietic stem cells (HSCs).¹

Much progress has been made recently in identifying cell-surface markers that correlate closely with the possession of HSC functionality, thus permitting the isolation of HSCs from human and mouse sources at very high purities (10–50%).² These advances, augmented by gene knockout studies, have allowed the identification of many genes that are required for HSCs to be generated and/or maintained at normal levels.³ Nevertheless, a molecular or phenotypic signature that permits the direct, specific and sensitive detection of HSCs remains elusive. Thus, the measurement and characterization of HSCs continues to rely on clonal tracking methods that use retrospective approaches to detect the ability of single input cells to produce mature short-lived blood cells in vivo for prolonged periods.⁴ The durability of this output potential and the demonstration of an accompanying ability to produce undifferentiated progeny with the same long-term blood cell producing capacity, is now recognized to be a key aspect of the definition of HSCs. This is particularly critical given the extensive but inevitably constrained self-maintaining ability of other types of long-term multipotent hematopoietic cells that can be prospectively isolated and shown to have different molecular features.⁵–⁷

An intriguing feature of HSCs is their high degree of heterogeneity.⁸ A long recognized source of such variability in HSC properties is their developmental stage. This has been historically apparent in comparisons of hematopoietic cells present in fetal and adult tissues,⁹–¹¹ but also found to continue into old age.¹² Early evidence of differences between the biological properties of primitive hematopoietic cells from fetal and adult tissues were revealed from investigations of mouse hematopoietic cells that form macroscopically visible clones of differentiating blood cells in the spleen of irradiated mice injected 9–14 days previously.¹³ These so-called colony-forming units-spleen (CFU-S) were initially thought to detect a fraction of HSCs that colonize the spleen because they could generate all myeloid cell types (erythroid, megakaryopoietic and granulopoietic) as well as progeny CFU-S with similar potentialities demonstrable in secondary transplants.¹⁴,¹⁵ However, it is now known that most CFU-S do not overlap

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with HSCs that possess lifelong repopulating ability. Rather, they represent a type of multipotent, transient amplifying cell.16,17 Nevertheless, the quantitative nature of the CFU-S assay made it possible to demonstrate the high cycling activity of fetal CFU-S, which contrasts with the very low cycling activity characteristic of CFU-S in the unperturbed adult.9 Similarly, the rate and ultimate level of regeneration of CFU-S from mouse fetal liver (FL) cells transplanted into irradiated recipients was found to be much greater than that obtained from a comparable transplant of adult bone marrow (BM) cells.10 More recently, evidence of a similar superiority in the regenerative pace and output of human FL and adult BM transplants in irradiated immunodeficient (NOD/SCID) mice has been noted.18,19

In this review, we summarize salient examples of differences in the properties of fetal and adult hematopoietic cells with a particular focus on those that affect or are initiated within the HSC compartment. We also discuss recent findings pertinent to understanding the underlying molecular mechanisms regulating these changes.

ORIGIN OF HSCS
HSCs arise in different sites and change their site of activity during development
The first hematopoietic cells to be produced during embryogenesis are nucleated erythrocytes and macrophages. These cells appear within the extra-embryonic blood islands of the yolk sac between embryonic day 7–7.5 in the mouse20 and around day 18 of gestation in humans.21 Soon after, erythroid, granulopoietic and multipotent progenitors with in vitro clonogenic activity and limited in vivo repopulating activity appear, but all before cells with long-term transplatable HSC activity can be detected.18,22,23 In both species, current evidence suggests that the latter arise within the aorta-gonado-mesonephros region of the embryo proper, at E10.524–27 in mice and after 5–6 weeks of gestation in humans.23,28 Subsequently, HSCs can also be found in other tissues including the placenta29–31 as well as the FL.32 There is some evidence that HSCs may arise independently in the yolk sac33 and placenta30 but those found in the FL are thought to be derived entirely from HSCs that originate elsewhere.34,35 HSCs then expand rapidly in the FL, which becomes the major site of hematopoiesis until birth.11,25,36,37 Colonization of the BM begins in the mouse on E17 and in both species, the BM becomes the major hematopoietic organ soon after birth and throughout adulthood.38

Given the importance of environmental signals in eliciting responses to changing body needs for different blood cell types throughout the growth of the embryo and after birth, it seems likely that the activity of HSCs (and their differentiating progeny) is influenced by the dramatic changes in their locations that take place during this period. Although the role of such extrinsic factors have been difficult to interrogate, evidence of intrinsic changes have been easier to infer from comparisons of their behaviour when assessed in the same environment either in vitro or in vivo (that is, after transplantation into adult-irradiated recipients).

Do adult HSCs arise from their fetal counterparts?
A fundamental question pertaining to the developmental origin of HSCs is the extent to which adult BM HSCs are lineally related to those that first appear in the embryo. The strongest evidence supporting this lineal relationship comes from studies using inducible recombination-based marking strategies. The first of these studies made use of floxed yellow fluorescent protein transgenic mice that express a tamoxifen-inducible Cre-ER T recombinase under the control of the stem cell enhancer of the Scl gene (which encodes a transcription factor required for the establishment of hematopoiesis).39,40 When recombination was induced at E10.5 and E11.5 by tamoxifen injections, 17% of the Lin- Sca1 + Kit + cells became yellow fluorescent protein plus at E14.5 and subsequent transplantation of these marked cells into irradiated adult recipients resulted in an 8–10% contribution of their yellow fluorescent protein plus progeny to the donor-derived hematopoietic cells detected 5 months post transplant—directly demonstrating the derivation of adult HSCs from fetal cells that express Scl. Notably, the contribution of E10.5/E11.5 marked cells to adult hematopoiesis was far from complete. Thus some adult HSCs may be derived later in development in a de novo fashion or from fetal cells expressing Scl that are not HSCs, or from fetal HSCs in which Cre is not successfully activated. Using a similar approach, but instead employing a Runx1-dependent marking system, it has been shown that E7.5 Runx1-expressing cells, which are presumably primitive precursors of erythrocytes, give rise to a significant proportion of fetal and adult hematopoietic cells.41 Evidence of a linear relationship between fetal and adult hematopoietic cells is further provided by the finding that single transplanted E14.5 FL HSCs, can give rise to long-term stable hematopoiesis following their transplantation into adult recipients, a property that would not be expected if fetal HSCs had only transient output capacities.7

Early developmental changes manifested in the differentiating progeny of HSCs
The erythroid lineage. The primitive erythrocytes that appear first in the developing mammalian embryo are a transient population distinguished from the later definitive erythrocytes in their retention of a nucleus, expression of embryonic globin genes and exclusive origin from the yolk sac.42 In contrast to this primitive-to-definitive erythrocyte switch that occurs in all mammals, a second type of hemoglobin switching, referred to as the fetal-to-adult switch, occurs exclusively in humans and old world monkeys. This latter transition involves a change from γ- to β-globin gene expression and is initiated in the mid-gestation fetus and completed within the first year after birth. It is thought to facilitate increased oxygen scavenging from maternal blood, as the HbE molecule (α2γ2) has a higher oxygen affinity than the adult-type hemoglobin (α2β2).

Differences between fetal and adult erythropoiesis. The erythropoiesis restricted progenitors (colony-forming unit erythroid; CFU-E) in the mouse have also been reported. For example, fetal CFU-E are more sensitive to erythropoietin
dose-response analyses of their ability to express their clono-
genic potential in vitro. The change in erythropoietin sensitivity of CFU-E was reported to occur gradually between fetal and adult life.

Megakaryocyte progenitors. In the BM of adult humans, many megakaryocytes undergo multiple endomitotic divisions to produce large cells of high ploidy before platelets are released from them by a process of fragmentation of their cytoplasm. In contrast, the megakaryocytes produced in human fetal hematopoietic tissues initiate a similar process of platelet release when the cells are smaller and lower ploidy but at an equivalent stage of cytoplasmic maturity. Interestingly, this difference is replicated in vitro when the outputs of clonogenic precursors of megakaryocytes present in human umbilical cord blood (CB) and mobilized adult peripheral blood are compared, suggesting the operation of an underlying cell-intrinsic mechanism.

Granulopoietic cells. Human FL monocyctic cells are distinct from their adult counterparts with respect to their ability to differentiate directly from a CD14-CD16- precursor to CD14+CD16+ cells, without producing a CD14 highCD16- intermediate. Fetal monocyctic cells also differ from adult monocytes in their display of properties characteristic of adult M2 macrophages, including increased expression of chemokines, scavenger receptors and tissue-degrading enzymes.

Lymphopoiesis. It was first demonstrated in 1985 that a subset of mouse B-cells, called B-2 B-cells, can be produced upon transplantation of either neonatal liver or adult BM; however, another subset, called B-1 B-cells, can be efficiently generated only by transplants of neonatal liver. More recently, it was shown that this difference applies specifically to B-1a cells. Emerging evidence also suggests that splenic marginal zone B-cells are of uniquely fetal origin, a notion that is supported by a recent report of a marginal zone-restricted embryonic progenitor.

During mouse development, the first CD4-CD8- cells that appear in the thymus express the Vγ3 Vδ1 T-cell receptor, followed by a second wave of T-cell receptor Vγ4 Vδ1 cells. Like B-1a B-cells, the production of these fetal-type T-cells is assumed to be mediated at the HSC level, as adult HSCs produce only Vγ2, Vγ5 and T-cell receptor zβ T-cells. Similarly, invariant natural killer T cells are only produced during a short developmental window, with a peak at 2 weeks after birth in mice. Recent evidence suggests that this phenomenon is a consequence of a higher invariant natural killer T cell potential for neonatal double positive (CD4+CD8+) thymocytes.

Developmental changes in the properties of HSCs

Lineage output potentialities. Historically, HSCs have been assumed to be primed epigenetically to generate all blood cell lineages with equal probabilities, the heterogeneity in observed mature cell outputs being determined by poorly understood stochastic events. Indeed, in textbook descriptions of HSCs, self-renewal of multi-potency is typically the key defining phraseology used. In addition, evidence exists for self-amplifying and cross-suppressive transcription factor-mediated mechanisms of ‘commitment’ leading to the ultimate activation of singular differentiation ‘programs’ within initially multipotent cells, Pu.1 and Gata1 being the classic examples. The more recent discovery of self-perpetuating unique patterns of differentiation in serially transplanted clones derived from single mouse HSCs, and a change in their relative prevalence during ontogeny/aging, was thus unanticipated.

We have introduced the terms γ-, β-, γ- and δ-HSCs to distinguish four subsets of mouse HSCs based on their relative individual contributions to the myeloid (granulocyte–monocyte) as compared with the lymphoid (B + T) cells present in the peripheral blood 16 weeks post-transplant. The α-HSCs and β-HSCs are of particular interest because both of these subsets are uniquely and equivalently endowed with durable self-renewal ability (serial transplantability). All four subtypes are present in the mouse FL, however, the lymphoid-deficient α-HSCs are barely detectable in contrast to the prevalent β-HSCs. In contrast, adult BM contains approximately half as many α-HSCs as β-HSCs. Interestingly, by 3 weeks of age, the α-HSC content of the BM is already very similar to that of the adult BM. However, this developmental increase in the proportion of α-HSCs does not appear to be intrinsically determined, as an adult-like distribution of their numbers is seen in the newly colonized E18.5 BM, even though the distribution in the FL is the same as at E14.5. These findings suggest that the BM microenvironment, as compared with that of the FL, is more stimulatory to α-versus β-HSC expansion, or selectively favors seeding of the BM with α- as compared with β-HSCs.

Transplants of FL HSCs produce, on average, an ~twofold higher contribution of granulocytes and monocytes (Ly6g+ and/or Mac1+) to their total leukocyte progeny, as compared with adult BM HSCs. Intriguingly, this higher myeloid output of fetal mouse HSCs switches to an adult pattern between 3 and 4 weeks of age and also appears to be mediated at the HSC level, as shown by serial transplant experiments.

Proliferative activity. HSCs in fetal tissues show the same high cycling activity as CFU-S and, similarly, become a largely quiescent population in the adult (changing from an estimated fraction of cycling cells of close to 100% in the E14.5 FL of mice to ~10% in adult BM). Interestingly, the cycling status of HSCs in mice appears to be site-independent as it remains unchanged as the HSCs move from the FL to the BM until 3 weeks after birth when it is abruptly altered within a 1-week period. These features again point strongly to an intrinsically determined mechanism. In humans, an analogous change in HSC turnover rate is inferred from the abrupt change in the rate of leukocyte telomere shortening that appears to occur between 1 and 3 years after birth and more clearly evident between 1 and 2 years after birth in individually tracked baboons. A recent study suggests that a
Developmentally regulated decrease in expression of the transcription factor, C/EBPα, is responsible for this in vivo switch in HSC cycling activity in the mouse.72

A number of cell-surface markers and dye-efflux phenotypes used to isolate HSCs from adult mouse BM, show an altered pattern of expression when cycling HSCs are studied (for example, fetal mouse HSCs or stimulated adult mouse BM HSCs).73 As a result, different phenotypes had to be used to enable fetal and adult HSCs to be isolated at high purities.65,74

More recently, new mouse HSC markers have been discovered that are not affected by the cycling status or different developmental origin of the HSCs.73 Their use allows mouse HSCs to be isolated at near purity from every in vivo source, thus far studied65,75 thereby bypassing many caveats in historical comparisons of HSCs purified using different phenotypes.

**HSC self-renewal.** In mice, it has been shown that E14.5 FL HSCs differ from adult BM HSCs in the rate at which they regenerate hematopoiesis, including their own numbers, in transplanted irradiated recipients.1,68,67,76,77 This difference is thought to be at least partly due to a greater in vivo self-renewal of the FL HSCs, as their superior regenerative activity is seen regardless of the number of HSCs initially transplanted (over a 100-fold range of 10–1000).78 Intriguingly, like the higher myeloid output of FL HSCs (noted above), this property of HSCs, in mice, changes concomitantly with the decrease in their proliferative activity between 3 and 4 weeks after birth.65

**Responsiveness to growth factors.** Steel factor (SF) is a transmembrane growth factor that is encoded by the *Sl* locus. SF binds to Kit (CD117), a type III receptor tyrosine kinase encoded by a gene in the W locus. Even before the products encoded by the W and Sl loci were known to represent a receptor-ligand pair mediating HSC responses, studies of the defects caused by mutations at both loci had pointed to their complementary involvement in HSC regulation. For example, both fetal and adult hematopoietic tissues from mice carrying mutations within the kinase domain of Kit show reduced colony formation by CFU-S.79 Mice with a *W*ΔI/*W*ΔI genotype are of particular interest because they are viable and fertile (in contrast to those with more severe *W* mutations),80 but still have significantly reduced HSC functionality as assessed in limiting dilution transplant assays (10- to 20-fold). As a result, sublethally irradiated adult *W*ΔI/*W*ΔI mice can be used as hosts to enable the detection of transplanted (wild-type) HSCs with the same sensitivity as lethally irradiated wild-type hosts given a minimal radioprotective transplant.81 In contrast, Sl-mutant mice, which have deletions in the SF genomic sequence,82 have a microenvironmental niche defect that prevents the formation of spleen colonies by CFU-S, as well as the regeneration and maintenance of their numbers throughout adult life.79

In the mouse, HSCs from all stages of development express the same levels of Kit on the cell surface regardless of their cycling status or position in the cell cycle.58,83–85 However, several lines of evidence point to differences in the requirements and responsiveness of fetal and adult HSCs to SF stimulation. *Sl/Sl* mice do not express any form of SF and die around E15–16 due to the dependence of fetal as well as adult erythropoiesis on this growth factor. However, their CFU-S and Thy1.2Lin–Sca-1+ hematopoietic stem and progenitor cells increase at the same rate as their wild-type counterparts, three- to fivefold between E13–15, thus demonstrating that fetal HSC expansion can occur ‘naturally’ in the absence of SF.83 In vitro, fetal mouse HSCs can be shown to be SF responsive, but stimulation of their self-renewal is sixfold more sensitive to SF than adult HSCs.84,86–88

Fetal B-cell progenitors in the mouse also differ from their adult counterparts with respect to their responsiveness to growth factors. In this case, they are able to give rise to mature progeny without interleukin-7 and thymic stromal-derived lymphopoietin, factors that are necessary for maturation of adult BM-derived progenitors.89 Interestingly, the timing of this developmental transition (that is, from interleukin-7 and thymic stromal-derived lymphopoietin independence to dependence) occurs 1–2 weeks after birth, a time point strikingly similar to postnatal changes affecting several other HSC properties.65,68

**Regulation of developmental changes in HSC properties**

The evidence that most, if not all, adult HSCs and the blood cells they subsequently generate, are derived from fetal HSCs implies that HSCs undergo changes that influence the molecular programs active within them and their differentiating progeny. This possibility is further supported by the recent discovery of specific pathways regulating the different behavior of fetal and adult HSCs, and the associated differences in the transcriptome of prospectively isolated fetal and adult HSCs.75,90,91

The concept that some genes may only be required for HSC functioning at certain developmental stages was first demonstrated for *Bmi1*, a member of the polycomb-repressive complex. When this gene is deleted, a normal HSC population is generated in the FL; however, BM failure secondary to HSC depletion occurs 1–2 months after birth,92 a time point which directly follows the fetal-to-adult transition in several HSC properties.65,89 Similarly, *Gfi1*−/− mice develop normally, but when their BM cells are transplanted they have a reduced ability to repopulate irradiated recipients suggesting their adult HSCs are impaired.93 *Tel/Etv6* has also been shown to be an essential and selective regulator of adult HSC survival.94 More recently, the transcription factor C/EBPα was shown to be required for acquisition of quiescence of mouse adult HSCs but not by fetal HSCs, which are naturally cycling.72 Conversely, several gene products have recently been demonstrated to be important for fetal but not adult HSCs. The first of these identified was *Sox17*, which is expressed at a higher level in fetal compared with adult HSCs, using both direct measurements of gene expression and an analysis of HSCs isolated from a *Sox17* knock-in reporter mouse.95 Inducible deletion of the *Sox17* gene was then used to
demonstrate an essential role for Sox17 in maintaining HSCs in fetal/neonatal mice. Importantly, Sox17 deletion in adult mice had no effect on HSC numbers or functionality, however, forced expression of Sox17 in adult BM was found to enhance the HSC-derived output of myeloid cells (a fetal HSC property).

Another central question regarding developmental changes in HSC properties is whether the timing, onset and maintenance of such changes are governed by extrinsic factors (that is, hormones, growth factors or paracrine ‘niche’ signals) or intrinsic factors (that is, cell-intrinsic timers and epigenetics). While it is known that FL stromal cells express factors such as insulin-like growth factor 2 and angiopoietin-like factors that would be anticipated to supply a unique set of signals to HSCs in the FL as compared with the adult BM microenvironment, there is also evidence that HSC developmental states and transitions are intrinsically regulated—likely a complex interplay of both is operative.

Direct evidence for a cell-intrinsic mechanism underlying the developmentally coordinated changes in properties of fetal and adult HSCs has been obtained from several recent studies. Our own work has implicated the Lin28b-let-7-Hmga2 pathway. In a comparison of E14.5 FL and adult BM HSCs purified from mice, we found that Lin28b is expressed at higher levels in the fetal HSCs and lentivirus-mediated overexpression of Lin28b in adult HSCs led to heightened Hmga2 levels through inhibition of the let-7 family of messenger RNAs (miRNAs). Moreover, lentivirus-mediated overexpression of either Lin28b (or its homologue Lin28) in adult HSCs enhanced the self-renewal activity of the HSCs to mimic that typical of FL HSCs when transplanted into irradiated hosts. In addition, this result was phenocopied by overexpression of Hmga2 in adult HSCs. Conversely, fetal HSCs from Hmga2-null mice display an adult-like, reduced self-renewal activity in the same type of transplantation experiment. Together, these findings demonstrate that Lin28b is a master determinant of the high self-renewal of fetal HSCs and acts by permitting a high-level of Hmga2 expression in these cells.

Similarly to HSCs, Lin28b is expressed at higher levels in B-cell precursors in the mouse E14.5 FL as compared with similar subsets in the adult BM. Moreover, ectopic overexpression of Lin28 in adult BM hematopoietic stem and progenitor cells can activate many of the fetal lymphoid phenotypes summarized above. Strikingly, Lin28-mediated fetal-like reprogramming of adult BM cells also induce a fetal-like interleukin-7 and thymic stromal-derived lymphopoietin independence upon B progenitors. However, Lin28-mediated activation of several of these fetal lymphoid phenotypes is not mediated by Hmga2 derepression, as direct overexpression of this gene cannot activate them.

Let-7 miRNAs also regulate developmental differences in megakaryopoiesis and control human fetal hemoglobin expression. This latter let-7-mediated effect was also shown to be a consequence of LIN28B expression, which leads to reduced levels of BCL11A, a known repressor of β-globin expression. Together, these findings strongly support a role for the Lin28b-let-7 axis as a ‘master regulator’ of the fetal HSC state (Figure 1). Intriguingly, while the Lin28b-let-7 pairing seems to be a common feature of many fetal-specific properties of HSCs, the downstream target of this pairing appears to be property-dependent. For example, in mice, Hmga2 is the downstream mediator of developmental differences in HSC self-renewal, with BCL11A being identified as downstream of LIN28B-induced HbF activation in human erythroid cells.

Hmga2 and Lin28b and let-7 miRNAs change their levels gradually between fetal and adult life. This suggests that these genes may be components of a developmental clock, in which the timing is determined by accumulation and/or titration of

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**Figure 1** Lin28b is a master regulator of fetal hematopoietic stem cell (HSC) identity. The higher level of Lin28b expression in fetal (as compared with adult) HSCs leads to a correspondingly lower level of let-7 messenger RNAs (miRNAs) and thus permits a higher expression of Hmga2. This elevated level of Hmga2 is responsible for the enhanced self-renewal activity of fetal as compared with adult HSCs in the mouse. Lin28b also regulates other differences seen between fetal and adult hematopoiesis; for example, the activation of fetal-type lymphopoiesis and the expression (in human erythroid cells) of fetal hemoglobin. These properties are not a consequence of Lin28b-mediated derepression of Hmga2 expression. This figure is modified with permission from a previously published version.
these molecules. The Lin28–let-7 pairing has been previously implicated in the regulation of such a developmental transition in Caenorhabditis elegans. This larval-to-adult transition, which involves a switch from cycling to quiescence of a stem-like population known as seam cells, is regulated by the downregulation of Lin28, which in turn triggers an upregulation of let-7.101 In let-7-mutant animals, the seam cells do not exit the cell cycle but instead reiterate a larval fate.101 Thus, the timing mechanism of this transition is thought to involve the accumulation of let-7 miRNAs. The findings that let-7 levels increase in HSCs between fetal and adult life60 bear a striking resemblance to this larval-to-adult transition of C. elegans. Thus, the developmental down-regulation of Lin28b, which regulates the differences in the self-renewal activities and lymphoid differentiation potential of fetal compared with adult HSCs, may also be responsible for the timing of this transition that occurs between 3 and 4 weeks of age. This also raises the possibility that such cell-intrinsic development timing mechanisms represent highly evolutionarily conserved processes.

As mentioned, developmental transitions of hematopoietic cells not only occur in HSCs, but also in megakaryocyte progenitors,49,50 myeloid progenitors,9,10 CFU-E,43 B-cell progenitors52,89 and T-cells.57 Furthermore, as the differential expression pattern of Lin28b, let-7 miRNAs and Hmga2 between fetal and adult HSCs is also apparent in populations composed principally of hematopoietic progenitors (that is, Lin² Sca1¹ Kit¹ cells),91 this suggests that developmental programs active within HSCs may remain active within more differentiated hematopoietic subsets. This is supported by the recent evidence that let-7 miRNAs also regulate developmental differences in B-cells, T-cells90 and megakaryocytes.99

Clinical implications
Pathogenesis of pediatric malignancies. Approximately 2% of all cancer cases in western countries occur in children before they reach 15 years of age.102 These include, in descending order of prevalence, leukemia, lymphoma, CNS tumors, neuroblastomas, soft-tissue sarcomas, nephroblastomas (Wilms’ tumor), bone tumors, retinoblastomas, hepatoblastomas and germ cell tumors.102 In contrast to adult leukemias, which are most frequently myeloid diseases, the majority of those occurring in children are acute lymphoblastic leukemias.103 Interestingly, the incidence of acute lymphoblastic leukemias does not increase gradually throughout childhood, but rather shows a peak at approximately 5 years of age.102 It may, therefore, be speculated that the microenvironment and/or leukemia-initiating cells undergo a transition in their susceptibility to oncogenic transformation during early childhood.

This concept is strengthened by the observation of pediatric-exclusive mutations and susceptibility syndromes, and examples of spontaneous age-associated tumor regression in children. The TEL-AML1 fusion oncogene is the most frequent mutation found in pediatric acute lymphoblastic leukemias, present in ~25% of all cases104 but is rarely, if ever, found in adult acute lymphoblastic leukemias.105 While the reasons for this remain unknown, a possible explanation could be the absence or presence of different protective or cooperating factors in the hematopoietic cells of children but not adults. This explanation could also apply to the window of early childhood in which patients with Noonan syndrome appear to be at an increased risk of developing leukemia, a risk that subsequently decreases to a baseline level despite the persistence of the responsible inherited genetic lesions.106 Additional evidence of age-specific cooperating oncogenic factors can be inferred from the observations of occasional pediatric leukemias that resolve spontaneously. One example, although rare, are cases of juvenile myelomonocytic leukemia that may regress, even in the absence of treatment.107 Similarly, a megakaryoblastic leukemia that occurs exclusively in children with Down syndrome, known as the transient myeloproliferative disorder, can also spontaneously resolve within the first 3 months of life.108 Notably, even some pediatric solid tumors undergo a spontaneous regression.102

Lin28b and Hmga2 are oncogenes,109,110 whereas let-7 miRNAs are tumor suppressors.111 Thus, the expression pattern of these genes in fetal and neonatal hematopoietic cells would be anticipated to create a ‘primed’ background for the generation of leukemia. Furthermore, these genes are cell intrinsically programmed to switch their expression pattern during the transition to adulthood, which might serve as a mechanism responsible for the occasionally observed spontaneous regression of certain fetal/neonatal onset leukemic disorders. In these cases, the ‘developmental hits,’ which are cooperating with acquired or genetic lesions, would be predicted to be downregulated during early life, thus leading to a collapse of the molecular program driving the progression of these diseases. The recently elucidated role of the HMGA2–IGF2BP2 axis in the pathogenesis of NRAS-mutant embryonic rhabdomyosarcoma112 lends strong support for this idea.

HSC transplantation. HSC transplantation is a well-established technique that has been used to treat over 30 different types of familial and acquired hematologic diseases.113 A major barrier to patient access to allogeneic HSC transplantation is the requirement to have a human leukocyte antigen-matched donor. Currently, ~30% of patients requiring allogeneic HSC transplantation are not candidates for this life-saving procedure due to lack of a suitable donor.113 Three general strategies have been proposed to improve the supply of HSC transplantation sources in order to permit more patients access to this treatment. The first of these, sometimes referred to as the ‘holy grail’ of translational HSC research, is the ex vivo expansion of HSCs. As this occurs readily in vitro (that is, following HSC transplantation or during growth and development), it should theoretically be possible to replicate these conditions in vitro, and thereby enable the generation of a large supply of HSCs even using autologous sources when these may be limiting. However, in spite of substantial efforts to identify such conditions, the most recent protocols offer only modest, if any, net increases in HSC numbers.114 Another
The approach has been to direct the differentiation of embryonic stem cells or induced pluripotent stem cells to a hematopoietic fate. This has also been met with limited success to date and has netted only convincing evidence for the generation of hematopoietic progenitors. The third of such strategies has been the use of alternate sources of HSCs, most notably CB. CB transplants are now routinely used in the pediatric setting; but their utility in adults is more problematic due to the limiting numbers of repopulating cells in individual CB collections.

An additional strategy is to manipulate HSCs *ex vivo* in a manner that will enhance their rate of expansion in transplant recipients. This could theoretically increase the effective cell dose, and in turn broaden the utility of CB and/or *ex vivo*-expanded HSCs. Evidence of such an effect is found in the dominant clones containing HMGA2 insertional mutations observed in several clinical gene therapy trials. Using new sequencing technologies, the clonal dynamics of HSC and progenitor cells within patients undergoing gene therapy for hematologic conditions can be studied. In a recent report of eight patients who were being monitored following a transplantation of lentivirus-corrected autologous BM cells for treatment of their X-linked severe combined immunodeficiency, two patients were found to harbor clones where the viral cassette had inserted within the large third intron of the HMGA2 gene. In both of these cases, the HMGA2 insertional mutant clone demonstrated a dominant behaviour, with one clone representing 6% of all transduced cells. In both of these cases, the mutation generated a truncated transcript lacking the let-7 mRNA binding sites, thus leading to derepression (that is overexpression) of HMGA2. A similar mutation and case of clonal dominance were recently reported in a patient undergoing gene therapy treatment for β-thalassemia. Interestingly, in this case, the clonal dominance was responsible for the therapeutic effect, as it permitted the pool of corrected HSCs to reach a therapeutic threshold. This finding suggests that deliberate overexpression of HMGA2 in parallel with the transgene of interest might similarly enhance the efficacy of such a therapy and be particularly useful in cases like β-thalassemia where a large portion of HSCs must be corrected in order to achieve a benefit.

**Conclusions and future directions**

As summarized in this review, HSCs in mice and humans undergo a multitude of developmental changes that alter the properties of themselves and the more committed progeny they produce. Molecular regulators of some of these processes have recently been identified, most notably the Lin28b-let-7 axis, which regulates multiple developmentally determined changes. Importantly, this pathway and its downstream targets, including Hmga2 and Igf2bp2, are differentially expressed across development and/or responsible for developmental differences in stem cell properties of other tissue-specific stem cells among different species. Thus, we predict that this pathway will be found to be responsible for modulating many core properties of diverse stem cell types that must change to support organismal growth and its eventual arrest.

Future studies focused on the relevance of this pathway to human HSC biology are also anticipated. Observations of dominant HMGA2-activated clones in patients undergoing gene therapy treatments has provided convincing evidence that the let-7-HMGA2 pairing is active in human HSCs, and that HMGA2 may activate a fetal-like self-renewal potential in these cells. It is also established that Lin28b is expressed at significantly higher levels in human FL and CB as compared with adult hematopoietic tissues (BM, thymus and lymph nodes). It will thus be particularly interesting to discover whether LIN28 expression can influence the susceptibility of naive human HSCs to transformation and whether fetal-specific pathways are aberrantly activated in adult malignancies in which LIN28, LIN28B or HMGA2 mutations are present.

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