Molecular regulation of human hematopoietic stem cells
van Galen, Peter

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 5

Discussion
The road ahead is long and montane. To understand molecular processes that govern stem cell biology to the point where knowledge can be translated to the clinic is a gratifying challenge.
Chapter 5. Discussion

Many important biological processes have been discovered by studying the hematopoietic system, including hierarchical tissue organization and the existence of stem cells. Seminal studies in the mouse often guide the field of hematology and stem cells, but studying human HSC biology is important to gain insight into human disease and translational opportunities. In this chapter, I will discuss technological limitations of studying human HSCs, place our findings in a broader context and highlight areas for future research.

5.1 Modeling mouse and human hematopoiesis

5.1.1 Comparing mouse and human HSCs

Mouse models have shaped our understanding of HSC biology, and provide a framework to investigate human HSC regulation. For example, the majority of work regarding transcriptional programs that direct lymphoid differentiation has been performed in mouse models. The E2A$^{-/-}$, Ebf1$^{-/-}$ or Pax5$^{-/-}$ knockout mouse models have been instrumental in identification of the function of these genes at different stages of B-cell development (Bain et al., 1994; Lin and Grosschedl, 1995; Nutt et al., 1997). It would not have been possible to dissect the function of ID proteins in lymphoid priming and stemness programs without this prior knowledge (Chapter 3). Many molecules have similar effects in both mouse and human systems. For example, mutations in the common IL-2R $\gamma$-chain that is necessary for cytokine signaling lead to severe combined immunodeficiency in both organisms (Cao et al., 1995). An excellent example of conserved molecular mechanisms of HSC regulation also springs from our studies regarding miR-126 (Chapter 2). The observations we made using human cells were mirrored in mouse experiments and vice versa. Conservation through evolution is one of the most reliable indications of the importance of an element for the organism (ENCODE Project Consortium et al., 2012). Therefore, the finding that modulation of miR-126 has similar effects in mouse and human settings indicates that it represents a vital regulatory axis to control the HSC quiescence/activation equilibrium. The knowledge that can be gained by exploiting experimental advantages of mouse models is indispensable and paves the way for investigating human HSCs.

Although many cellular mechanisms are conserved between mice and humans, several striking differences have developed during 75 million years of divergent evolution. The average lifespan of laboratory mice is 2-3 years, at which point 30% have cancer. About 30% of humans will also develop cancer, but that is over a 70-80 year lifespan, indicating that humans have developed distinct antineoplastic mechanisms (Rangarajan and Weinberg, 2003). DNA damage induced by ROS may be 18 times more prevalent in mice than in humans (Adelman et al., 1988; Ames et al., 1993). This could be related to the basal metabolic rate (ml of oxygen consumption per gram of body weight per hour), which is estimated to be 11 times higher in mice.
than in humans. Several aspects of HSC biology also differ. Human HSCs are less proficient in DNA double stranded break repair by non-homologues end joining than hematopoietic progenitor cells, which is opposite in the mouse (Milyavsky et al., 2010; Mohrin et al., 2010; Shao et al., 2012). Different molecules mitigate ionizing radiation between human and mouse hematopoietic progenitor cells (Goff et al., 2013). Cell surface markers expressed by human and mouse HSCs are not conserved (Figure 1) (Larochelle et al., 2011; Sitnicka et al., 2003). Cell cycle characteristics also differ. Estimates of the replication rate for mouse HSC range from 10 to 145 days (Foudi et al., 2009; Takizawa et al., 2011; Wilson et al., 2008), but methods for labeling and tracking cells that were used in these studies cannot be applied to human subjects. Using similar methods for both organisms, the replication rate was estimated to be 2.5 weeks in mice compared to 40 weeks in humans, representing a 16-fold difference (Catlin et al., 2011). Of eighteen genes that were recently identified to enhance mouse HSC function (Deneault et al., 2009), none translated to human HSC (Deneault et al., 2013). In summary, there are extensive differences between mice and humans at an organismal level, as well as at the level of HSC regulation. Therefore, insight into human disease and translational studies will be most relevant when performed using human cells.

5.1.2 Towards a comprehensive understanding of human HSCs

Scientific progress is limited by technological advancements. In recent years, improved methods have opened up new possibilities to study human hematopoietic stem and progenitor cells. First, HSCs and six functionally validated lineage-restricted progenitor cell populations can now be obtained at near clonal purity (Doulatov et al., 2010; Majeti et al., 2007; Notta et al., 2011). As well, improvements to immunodeficient mice for xenografting have resulted in more sensitive and robust models (McDermott et al., 2010). Genome wide expression arrays have provided lists of genes that are enriched in stem cells (Eppert et al., 2011; Ramalho-Santos et al., 2002). As these technologies become available for smaller cell numbers, differential gene expression between HSCs and immediately downstream progenitors have come into focus, revealing complex coordination of self-renewal and differentiation programs (Laurenti et al., 2013). Finally, sophisticated lentiviral vectors are available for gain- and loss-of-function studies to gain insight into gene function (Amendola et al., 2009; 2005). These developments have given us the knowledge and tools to dissect molecular regulation of human hematopoietic stem cells at an unprecedented level of detail.

Several further developments are necessary to fully understand the properties that enable self-renewal and differentiation of human HSCs. Implementation of new technologies to stem cell research is frequently hampered by the difficulty of obtaining large cell numbers. However, the sensitivity of techniques is improving at several levels of cell biology. Intracellular flow cytometry, including phospho-flow, allows for single-cell protein quantification (Perez and Nolan, 2006). The CyTOF mass cytometer, which combines aspects of flow cytometry and mass spectrometry, enables multiplexed analysis of surface and intra-
cellular antigens of single cells (Bodenmiller et al., 2012). Quantitative reverse transcription PCR and genome sequencing is now possible for single cells (Kalisky and Quake, 2011; Navin et al., 2011). Analysis of epigenetic marks combined with RNA-seq enables insight into the dynamics between DNA and histone modification, gene expression and RNA splicing (Gifford et al., 2013). These multiplexed or genome-wide technologies become especially valuable when adapted to work for small cell numbers, such that HSCs and early progenitors can be compared. Similar to the transformative role that RNA expression arrays have had on our understanding of hematopoiesis, such technologies will unveil new genes and processes that regulate human HSCs. Bioinformatic analysis of genome wide arrays will define networks of regulatory elements and generate multiple hypotheses. New insight will then depend on functional validation and detailed follow-up, such as the projects that have been described in this thesis. This brings us to the second limitation of current experimental approaches to study human HSCs, which is genetic manipulation. Over expression of large genes such as BCR-ABL is complicated by lentiviral vector length restrictions, related to the efficiency of RNA encapsidation (Follenzi and Naldini, 2002). Gene knock-down is often inefficient and subjected to hairpin silencing, precluding long-term experiments. These problems could be solved with improved efficiency of genome and epigenome editing technologies. Adapting naturally occurring proteins (such as TAL effector repeats) or protein/RNA complexes (such as CRISPR/Cas) to target endonucleases or epigenetic modifiers to specific DNA sequences are particularly promising developments to open new experimental possibilities. Some of the advantages of genetic mouse models may be made available for human cells (Cong et al., 2013; Mali et al., 2013; Mendenhall et al., 2013). Third, there is room for improvement of assays to study HSC and progenitor cell properties. Although immunodeficient mice provide a more faithful environment for HSC investigation than in vitro methods, there are still several differences between the human and mouse bone marrow niche in which HSCs reside. One of the most evident problems is the lack of cross-reactivity of many mouse cytokines on human cells. Several immunodeficient mice have been genetically engineered to express human cytokines such as SCF, GM-CSF and IL-3 (Ito et al., 2012; 2013). These humanized xenograft models show improved myeloid engraftment. One of the questions that may be answered by these mouse models is whether human HSCs can be divided into different lineage-biased compartments, as has been shown for mouse HSCs (Muller-Sieburg et al., 2012). Mapping heterogeneity within currently defined stem and progenitor compartments using more sensitive in vitro and in vivo methods will elucidate further details of the hematopoietic hierarchy. Ultimately, assays that read out single cell potential will provide the most comprehensive insight, and will also indicate to what extent heterogeneity reflects inherent biological noise, such as variability associated with baseline gene expression levels. In short, the adaptation of multiplexed and genome wide technologies for small cell numbers and sophisticated bioinformatics will generate hypotheses. Improved genetic
Figure 1. Surface marker phenotypes of mouse and human stem and progenitor cell subsets. In the mouse, markers have been identified to separate HSCs into subsets with different repopulating capacity. Gradual differentiation towards the lymphoid lineage proceeds through LMPP and CLP stages. In humans, HSCs and MPPs can be distinguished by expression of CD90 and CD49f. The MLP is the only common lymphoid progenitor that has been identified in humans, and this cell retains some myeloid potential. Myeloid differentiation progresses through similar stages in mouse and human systems. Note that some details remain uncertain, for example, several phenotypes are used to separate mouse HSC subtypes and differences may exist between human MLP-derived...
and epigenetic manipulation of human cells will enable investigators to answer new questions. More sensitive assays and single-cell fate readouts will pinpoint previously unappreciated complexities. Over time, these developments may lead to a comprehensive understanding of the molecular components that regulate HSCs.

5.2 Gene expression control for maintenance of the HSC state

5.2.1 Transcription factor networks in HSCs and early progenitors

Many intrinsic processes must be tightly regulated in HSCs. As well, HSCs need to remain responsive to signals from the environment. The process of differentiation is associated with changes to many cellular properties. Ideally, factors that coordinate these changes should therefore be able to affect multiple regions in the genome. Transcription factors are well equipped to perform various functions, and many of them have been implicated in HSC maintenance, especially by mouse knockout models (Orkin and Zon, 2008; Rossi et al., 2012). The list of transcription factors that have been shown to function in human HSCs is much shorter, but includes BMI-1, AHR, P53 and HIF2a (HOXB4, HES1 and HLF were not assessed by serial transplantation) (Amsellem et al., 2003; Boitano et al., 2010; Buske et al., 2002; Milyavsky et al., 2010; Rizo et al., 2008; Rouault-Pierre et al., 2013; Shojaei et al., 2005). Transcription factors that act downstream of HSCs, during early differentiation, have also been well investigated using mouse models, as described in the introduction of this thesis. Although it is known that many lineage-associated transcription factors are expressed at low levels in HSCs, a process termed lineage priming, the reason for this phenomenon is not well understood. Lineage priming is thought to reflect the multipotent state of HSC, where chromatin is maintained in an accessible configuration to allow for a rapid response to demands for mature blood cell production (Orkin, 2003). However, in Chapter 2 of this thesis, we describe a previously unknown link between stem cell self-renewal and B-lymphoid (hereafter referred to as lymphoid) lineage priming. We used lentiviral over expression of the transcription factor inhibitor ID2 to inhibit the activity of the canonical B-cell factor E47 in HSCs. In addition, we over expressed E47 and reduced the expression of another B-cell factor, EBF1. Each one of these approaches led to the same observation: expression of lymphoid lineage-associated transcription factors in HSCs reduces HSC maintenance in vivo. These results led us to uncover that lymphoid lineage priming negatively influences HSC self-renewal. It is possible that lymphoid transcription factors suppress a self-renewal program, as a major function of E47, Ebf1 and Pax5 is transcriptional suppression of genes associated with alternative lineages (Ikawa et al., 2004; Lukin et al., 2011; Nutt et al., 1999). As well, Ebf1 suppresses Id2 and Id3 expression (Nechanitzky et al., 2013; Pongubala et al., 2008; Thal et al., 2009). Regardless, the insight that ID proteins are highly expressed in HSCs to restrain the transcriptional activity of E proteins is one of the most detailed mechanistic insights we have in the processes that govern self-renewal of human HSCs.

In the mouse, myeloid-biased, balanced and lymphoid-biased HSC subsets
have been identified (Muller-Sieburg et al., 2012). The consequences of heterogeneity within the HSC pool are important to understand as they may influence processes such as aging and clonal expansion preceding leukemia. However, little is known about the stability and regulation of these HSC subsets. The studies we performed with ID genes provide an opportunity to investigate the flexibility of HSCs to convert from a lymphoid-biased to a myeloid-biased state, i.e. to gain the potential of significant myeloid cell production. Upon ID2 overexpression, we observe increased expression of a myeloid program in the human HSC compartment at the population level. Two possibilities could account for this change. First, induction of a myeloid program in HSCs that were originally not biased to the myeloid lineage may increase myeloid lineage commitment. Alternatively, enforced expression of ID2 may cause an expansion of a myeloid-biased subset of HSCs (Figure 2). To distinguish between these possibilities, lentiviral barcoding could be applied to track the fate of individual HSCs over time (Gerrits et al., 2010; Lu et al., 2011; Verovskaya et al., 2013). Performing these experiments in human cells would add significant new knowledge to the field. First, heterogeneity of lineage contribution within the HSC pool has not been established in the human system. Second, ID2 overexpression may skew the fate of individual HSCs towards the myeloid lineage (Figure 2, left). This would demonstrate that protein interactions (ID2 inhibition of E proteins) can alter the bias of single HSCs, whereas this is thought to be largely controlled at the epigene level. This would provide a unique insight into the plasticity of “epigenetically fixed” HSC subsets, and indicate that ID proteins provide a regulatory axis through which HSCs can switch their lineage bias.

5.2.2 MicroRNA control of the HSC state

It is only 13 years ago that microRNAs were recognized as a new class of gene regulatory elements (Pasquinelli et al., 2000). MicroRNAs represent a layer of gene expression control that is superimposed on the transcriptional level, as microRNAs reduce mRNA abundance or

---

Figure 2. Alternative possibilities of ID2 effects on HSCs.

ID2 may alter the bias of individual HSCs (left) or expand a myeloid-biased subset of HSCs (right), both of which would result in increased myeloid output. To distinguish between these possibilities, lineage depleted cord blood could be transduced with lentiviral barcodes. Cells would subsequently be transduced with CTRL or ID2-OE lentivirus and injected into immunodeficient mice. When a graft is established, human lymphoid (CD19⁺) and myeloid (CD33⁺) cells would be sorted and cells sequenced for barcodes to read out clonality. If ID2-OE causes lymphoid-biased HSCs to acquire appreciable myeloid potential (left scenario), clonality of the myeloid lineage would be increased compared to CTRL cells. L, lymphoid-biased; LM, balanced; M, myeloid-biased HSC.
translation post-transcriptionally. Each microRNA typically controls multiple target genes, placing them in an ideal position to coordinate repress or derepress gene expression programs that are related to differentiation, proliferation, apoptosis and other cellular processes. The importance of microRNAs in hematopoiesis is now firmly established, as many studies have revealed their roles in HSC regulation, differentiation and leukemia (Havelange and Garzon, 2010; Lawrie, 2013). However, few microRNAs have been studied in human hematopoiesis and stem cells. In Chapter 4, we describe the most comprehensive functional characterization of a microRNA to date in human and mouse HSCs simultaneously. miR-126 is expressed at high levels in HSCs, and promotes quiescence by attenuating different constituents of the PI3K/AKT signaling pathway. That miR-126 sets a threshold for HSC activation in response to extrinsic stimuli, provides molecular insight into the regulatory mechanisms that are important to maintain human HSCs. Interestingly, most models that increase HSC proliferation, including knockout of the PTEN, which leads to increased AKT activation, lead to short-term expansion of HSCs followed by exhaustion (Li, 2011; Zhang et al., 2006). In contrast, miR-126 knockdown increases HSC proliferation but does not lead to exhaustion in serial transplantation experiments. This difference may be due to the subtle effects of miR-126 on several PI3K/AKT pathway components simultaneously. It is also possible that additional genes are targeted by miR-126 in HSCs. Another striking observation is that miR-126 overexpression leads to increased quiescence in HSCs, but increased cell cycle entry in early progenitors. This effect, which was observed in both mouse and human systems, reflects the cell context-specific effects of microRNAs. In HSCs and early progenitors, different target mRNAs are available, which can underlie the differential effects that miR-126 modulation has in these cell types. The cell-specificity of microRNA function and miR-126 in particular is also evident when comparing the effect on normal hematopoiesis to acute myeloid leukemia. Although miR-126 overexpression leads to a loss of HSC repopulation and miR-126 knockdown leads to increased HSC function, these effects are opposite in the cells that propagate leukemia (Lechman, Gentner, van Galen et al., manuscript in preparation). Thus, knockdown of miR-126 reduces leukemia propagation but expands normal human HSCs. Locked Nucleic Acids (LNA) are available to reduce microRNA expression, and systemic administration has been successful in humans (Elmén et al., 2008; Janssen et al., 2013). The efficiency and biological consequences of LNA-mediated silencing of miR-126 should be tested in human HSCs and leukemia. Our studies suggest that targeting miR-126 represents a promising therapeutic approach for leukemia, as this could lead to depletion of leukemia cells, while simultaneously expanding normal HSCs.

5.3 Maintaining HSC integrity under stress

The HSC pool bears the responsibility of maintaining the blood system throughout life. The maintenance of a healthy HSC pool is vital, but the longevity of HSCs comes with exposure to various sources of stress.
stress. In addition, oncogenic lesions that are acquired by HSCs will persist and increase the chance of malignancy if more lesions arise (Jan et al., 2012). Clonal purity may be of less importance in progenitor cells, since terminal differentiation effectively purges their progeny. Therefore, HSCs have several specialized mechanisms to protect integrity of the HSC pool over time. For example, HSCs show enhanced apoptosis when DNA damage is incurred (Milyavsky et al., 2010). DNA damage accumulation in HSCs leads to reduced self-renewal and increased differentiation (Wang et al., 2012; Yahata et al., 2011). As well, maintenance of redox balance and restriction of reactive oxygen species (ROS) is required for proper HSC function (Ito et al., 2004; Tothova et al., 2007). Thus, damage to the HSC pool is tightly controlled to prevent either loss of function or the initiation of malignancy due to the clonal persistence of oncogenic mutations that arise in HSCs.

Various intrinsic and extrinsic sources of stress can disrupt protein processing in the endoplasmic reticulum (ER). The resulting ER stress causes activation of the unfolded protein response (UPR) that enables the cell to either resolve the stress or initiate apoptosis. Several processes that induce ER stress can pose a danger to the maintenance of HSC pool integrity. First, DNA mutations in protein coding sequences frequently result in protein misfolding; this effect is strong enough to constrain coding sequence evolution (Geiler-Samerotte et al., 2011). Detrimental DNA damage can therefore be detected through misfolded proteins. Second, activation of oncogenes such as mTOR is often accompanied by proteotoxic stress (Wouters and Koritzinsky, 2008). Third, environmental conditions such as severe hypoxia, ROS accumulation, glucose deprivation and nutrient starvation can trigger the UPR (Holtz et al., 2006; Kaufman et al., 2002; Saito et al., 2009). Fourth, viral infection can activate UPR signaling (Zhang and Wang, 2012). In Chapter 4, we show that HSCs have constitutively high PERK activity. This may restrict in their protein translation rate, protect against ROS and promote survival (Harding et al., 1999; Rouschop et al., 2013). However, this can also make HSCs highly sensitive to small increases in additional stress that could exceed the threshold for apoptosis induction. Our findings point to the UPR as a focal point where signals of stress converge, and persistent stress leads to clearance of HSCs. Overall, this supports a paradigm where individual HSCs that sustain damage are purged to protect the integrity of the HSC pool and warrant long-term maintenance of the blood system.

Similar to HSCs, stem cells in other systems may also interpret ER stress as an indication of damage and a potential threat to stem cell pool integrity. For example, UPR activation causes loss of intestinal stem cells through differentiation (Heijmans et al., 2013). The ER stress response in different tissue stem cells should be investigated using approaches we took in Chapter 4, as well as mouse knockout models of UPR genes such as CHOP and GRP78 (Wey et al., 2012; Zinszner et al., 1998). This will lead to a better understanding of the factors that contribute to stem cell longevity and the maintenance of clonal integrity. It will also be interesting to compare UPR signaling in
normal and leukemic hematopoiesis, to see if there is a therapeutic window to target leukemia stem cells.

5.4 Impact on understanding and treatment of disease

5.4.1 The process of aging

Aging and stem cells are inherently connected, since aging requires the passing of time, and stem cells are the most long-lived cells of the tissue they sustain. Aging of the HSC pool is associated with reduced adaptive immunity and increased incidence of hematological disorders including anemia and leukemia (Geiger et al., 2013). Compared to young HSCs, aged HSCs exhibit diminished lymphoid differentiation in favor of myeloid differentiation, which is thought to find its origin in differential persistence of HSC subsets (Figure 3). As well, an expansion of phenotypic HSCs is observed, and all HSC subsets show functional impairment with aging (Dykstra et al., 2011; Pang et al., 2011). Overexpression (OE) of ID2 causes parallels to these aging phenotypes (Chapter 3). Both the myeloid bias and the increase in HSC frequency are similar between ID2-OE and old HSCs (compared to CTRL and young HSCs, respectively), although ID2-OE HSCs have increased serial reconstitution ability whereas old HSCs are functionally impaired. The antagonistic relationship between lymphoid priming and self-renewal that we uncover in Chapter 3 has ramifications for aging research. First, it is possible that lymphoid-biased HSCs are lost during aging because the very presence of lymphoid factors reduces self-renewal. Indeed, myeloid-biased HSCs have a longer lifespan than other types of HSCs (Challen et al., 2010; Muller-Sieburg et al., 2004). This raises the question: is the aging process different for myeloid-biased versus lymphoid-biased HSCs? Should we focus our “rejuvenation” efforts on lymphoid-biased HSCs rather than the bulk? Along these lines, comparing young HSC subsets to old HSC subsets may be more informative than comparing bulk HSCs. This may be within reach now that markers to separate HSC subsets are becoming available (Beerman et al., 2010; Challen et al., 2010; Gekas and Graf, 2013; Morita et al., 2010; Yamamoto et al., 2013). Second, the intricate relationship between lymphoid lineage priming and self-renewal should be further investigated. For example, do the B-cell factors E2A and EBF1, which are known to suppress non-B-lymphoid genes, directly reduce expression of genes associated with self-renewal? Answering these questions will help to better understand the process of aging, and consequently design rational therapies for HSC rejuvenation.

Loss of protein homeostasis (proteostasis) has been classified as a hallmark of aging based on studies in non-hematopoietic tissues (López-Otín et al., 2013), and is an important determinant of stem cell aging (Vilchez et al., 2013). By studying the UPR in Chapter 4 of this thesis, we highlight the detrimental effects of misfolded protein accumulation for HSC survival. By doing so, we extend the importance of proteostasis to HSCs. The link between UPR signaling and HSC aging is reinforced by studies that show UPR activation upon enhanced mTOR signaling by TSC1 or TSC2 deletion (Ozcan et al., 2008), whereas TSC1 deletion in young HSCs mimics the phenotype of old HSCs (Chen et al., 2009). Converse-
Discussion

Inhibition of mTOR by rapamycin can increase lifespan, possibly by inhibition of protein synthesis (Harrison et al., 2009; López-Otín et al., 2013; Selman et al., 2009). Interestingly, rapamycin administration also reverses age-associated HSC phenotypes (Chen et al., 2009). Together, these studies suggest that improved proteostasis may ameliorate HSC aging. As well, genes associated with protein folding are upregulated in aged HSCs, which could indicate accumulation of misfolded proteins (Chambers et al., 2007). To further investigate misfolded protein accumulation with age, expression of chaperones and UPR related proteins could be more extensively assessed in young and old HSCs under basal conditions. As well, the stress response should be compared between young and old HSCs, for example by pharmacological induction of endoplasmic reticulum stress, GRP78 deletion or overexpression of a mutated protein (Geiler-Samerotte et al., 2011; Wey et al., 2012). Overexpression of chaperones that reduce misfolded protein accumulation may prove to be beneficial for HSCs (Morrow et al., 2004; Walker and Lithgow, 2003). If so, small molecule proteostasis regulators may provide a therapeutic approach to slow HSC aging (Calamini et al., 2012). Much remains to be discovered regarding the link between aging and proteostasis, particularly for HSCs. However, the enticing possibility of improving longevity and health of the HSC pool is an inspiring prospect.

5.4.2 Manipulating stem cells for clinical expansion

Umbilical cord blood could be of major clinical utility for allogeneic transplantation due to its rapid availability and less stringent requirements for HLA matching compared to adult bone marrow. However, the shortage of HSCs in each cord blood is associated with delayed engraftment and immune reconstitution as well as graft failure and early mortality. To overcome these obstacles, development of clinically relevant methods for ex vivo HSC expansion is critical (Hofmeister et al., 2007). Several methods for expansion of engrafting HSCs have been published, ranging from cytokine-supplemented culture optimization and genetic manipulation to recombinant protein exposure and small molecule mediated approaches (Dahlberg et al., 2011). Repopulation of mice by human HSCs was improved when standard cytokine conditions were supplemented with IGFBP2 and Angptl5 (Zhang et al., 2008). As well, exposure to the growth factor pleiotrophin increased repopulating HSCs in culture compared to input and cytokine-treated cultures (Himburg et al., 2010). Control of inhibitory feedback signaling by differentiated cells using a
fed-batch culture system enabled a 12-fold expansion of human HSC in liquid culture (Csaszar et al., 2012). As for genetic manipulation, overexpression of HoxB4 leads to 2- to 4-fold expansion of human HSC, although in the mouse it causes a 40-fold expansion (Amsellem et al., 2003; Antonchuk et al., 2002; Buske et al., 2002). NOV over expression was also reported to expand human HSC in vitro and in vivo (Gupta et al., 2007). The aryl hydrocarbon receptor inhibitor SR1 induced a 17-fold increase in HSCs over 21 days of culture (Boitano et al., 2010). Two methods for HSC expansion are in clinical development. Prostaglandin E2 was identified in a zebrafish chemical screen to increase hematopoiesis and short-term exposure of HSCs to PGE₂ showed encouraging results in a phase I clinical trial using double cord blood transplantations (Cutler et al., 2013; Hoggatt et al., 2013; North et al., 2007). An increase in repopulating cells was also accomplished by exposing cord blood to immobilized Notch ligand Delta1 in culture, which improved rapid engraftment of progenitors in patients with hematologic malignancies (Delaney et al., 2010). Despite these promising developments, the low number of stem cells in cord blood samples still limits their use for allogeneic transplantation.

In this thesis, three additional methods for HSC expansion have been described. Reducing lymphoid priming by lentiviral overexpression of the transcription factor inhibitor ID2 led to an 10-fold expansion of repopulating HSCs in secondary transplantations (Chapter 2). This opens up the possibility that exposure to recombinant ID proteins or an E47 inhibitor to attenuate lymphoid priming could be used to transiently increase self-renewal and expand the HSC pool. Derepression of PI3K/AKT signaling by knockdown of miR-126 leads to increased cell cycle entry and expansion of HSCs in vivo (Chapter 3). This data indicates that increased PI3K/AKT signaling, leading to HSC cell cycle entry, does not necessarily cause HSC exhaustion as seen in some mouse knockout models. Possible explanations for HSC proliferation without exhaustion are the modest level by which miR-126 knockdown increases PI3K/AKT signaling or the simultaneous downregulation of multiple miR-126 targets. Finally, we found that overexpression of the chaperone ERDJ4 improves HSC engraftment by limiting the apoptosis-associated effects of the UPR on HSCs (Chapter 4). Therefore, HSC survival during the transplantation procedure may be improved by reducing UPR-mediated apoptosis, for example with PERK inhibitors (Axten et al., 2012). Using genetic manipulation, we uncovered several mechanisms to expand human HSCs. Recapitulation of these effects with small molecules may lead to development of clinically relevant methods to expand cord blood HSCs for transplantation. By increasing our understanding of the molecular regulators that govern HSC self-renewal, we will learn how to harness the therapeutic potential of HSCs.

5.5 References


ional Screen to Identify Novel Effectors of Hematopoietic Stem Cell Activity. Cell 137, 369–379.


Holtz, W.A., Turetzky, J.M., Jong, Y.-J.I., and

108
Discussion


