Unraveling the molecular components and genetic blueprints of stem cells

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Remarkable progress in stem cell biology research over the past few years has provoked a promise for the future of tissue regeneration and gene therapies; so much so, that the use of stem cells in clinical therapy seemed to be just around the corner. However, we now realize there is still a huge task before us to improve our understanding of the nature of stem cells before utilizing them to benefit human health. Stem cell behavior is determined by specific gene products; thus, unraveling the molecular components and genetic blueprints of stem cells will provide important insight into understanding stem cell properties. Here we summarize the research of various groups using microarray technology and other approaches to determine the gene expression profiles in stem cells, particularly in hematopoietic stem cells (HSCs). These works have, to a certain degree, helped to narrow down the candidate genes predominantly expressed in HSCs, revealed a list of stemness genes, and indirectly demonstrated the wide-open chromatin state of stem cells and, with it, the molecular basis of the multipotentiality of stem cells.

INTRODUCTION

Many different methods have been developed for determining differential gene expression. These include subtractive hybridization (1,2), differential display PCR (DD-PCR) (3), representational difference analysis (RDA) (4), expressed sequence tag (EST) analysis (5), serial analysis of gene expression (SAGE) (6), and the nucleic acid array technology (7–10). These technologies have been used for the identification of molecules that are either specifically expressed in hematopoietic stem cells (HSCs) or potentially involved in the regulation of HSC development. For example, a novel DNA sequence was identified from sorted CD34⁺ CD38^{lo} hematopoietic progenitor cells using subtractive hybridization (11); both DD-PCR and RDA were used for the isolation of cDNAs from HSC populations (3,4); and a great number of cDNAs have been identified from both human and murine HSC populations using random sequence analysis of ESTs (12,13) and SAGE (4,14).

Each of these technologies has its advantages. Among these technologies, the combination of large-scale cDNA array, microarray, and oligonucleotide arrays affords unprecedented opportunities to systematically analyze gene expression in a given complex biological system and to identify molecules that are involved in the regulation of the development of this system. These tools enable the analysis of not just one gene at a time but thousands or even tens of thousands and allow the comparison of gene sets expressed

at various differentiation stages in HSCs. They also assist in the identification of molecules and the underlying genetic pathways that potentially control the developmental switches in HSCs.

The widely used AtlasTM Array (BD Biosciences Clontech, Palo Alto, CA, USA) is an example of a cDNA array in which cDNAs are deposited at a high density onto nylon filters using a robot. These can be used for either analyzing differential expression genes or defining housekeeping and commonly expressed genes for removal from further analyses. Microarray is another type of robotic system for spotting cDNAs or PCR-amplified cDNA fragments onto glass slides (8). The advantage of microarray is that it allows small-volume hybridization. This is essential for the analysis of material isolated from an extremely limited numbers of HSCs. The microarray system also allows utilization of dual-fluorescent dyes and probes derived from different subpopulations of cells, labeled with different fluorescence dyes. This allows for robust comparisons between expression profiles. A variation of this approach is the oligonucleotide array system (GeneChip[®] 640; Affymetrix, Santa Clara, CA, USA) based on direct synthesis of oligonucleotides onto a silicon surface that are hybridized with the fluorescently labeled target cRNAs derived from tissues or cells (9,15). Given that both the sequence information of human and mouse genomes have been defined and the cost for performing oligonucleotide array hybridization has been reduced, the GeneChip oligonucleotide array has recently gained in popularity.

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USING MICROARRAY TO IDENTIFY HEMATOPOIETIC STEM CELL DIFFERENTIALLY EXPRESSED GENES

The unique property of HSCs is their ability to possess both self-renewal capacity and differentiation potential. Although some signal molecules have been revealed to be involved in the control of self-renewal of HSCs, such as Notch, Wnt, HoxB4, *Bmi*, and *Shh* (16–23), the molecular mechanisms that direct stem cells to choose self-renewal rather than differentiation remain largely unknown. At different stages of HSC development, different subpopulations of cells express different sets of genes. This has led to a hypothesis that different sets of expressed genes, in turn, determine the fate of HSCs and the downstream lineage subpopulation (24). It has also been argued that key aspects of stem cell regulation result as a combination of molecular components and/or genetic programs in stem cells (25). Therefore, several different approaches have been used to uncover the molecular components that are specific to HSCs (24–26).

Phillips et al. (25) constructed a PCR-based cDNA library derived from murine fetal liver HSCs (FLHSCs; identified by markers Lin[−]Sca-1⁺AA4.1⁺c-Kit⁺). The FLHSC cDNA library was then subtracted by a secondary cDNA library derived from AA4.1[−] cells to remove housekeeping genes (25). The subtracted cDNA library, enriched with stem cell-specific genes, was subjected to large-scale sequence analysis, high-density array hybridization, and subsequent bioinformatics annotation. A large number of known genes and ESTs with unknown functions were revealed by this approach (see <http://stemcell.princeton.edu>). Tersikh et al. (26) took a similar approach but focused on adult bone marrow (BM) cells. Two-hundred twenty-three nonredundant contigs out of 1500 genes/ESTs representing sequences specifically expressed in highly purified HSCs (Lin[−]Sca-1⁺c-Kit⁺Thy-1^{lo}) were identified following a subtractive procedure using cDNAs from BM cells (PCR-SelectTM cDNA Subtraction kit; BD Biosciences Clontech). Interestingly, some of the genes that were differentially expressed in the HSC population were also expressed in neurospheres, which are enriched with neural stem cells (NSCs), suggesting that HSCs and NSCs share overlapping genetic programs. For example, *Ku80*, which encodes a DNA binding protein, is required for both lymphogenesis and neurogenesis (27).

In another study, a combined cDNA high-density array and microarray approach was taken to interrogate gene expression profiles in adult HSCs (24). Two cDNA libraries were generated; one derived from rigorously purified HSCs (Lin[−]Sca-1⁺c-Kit⁺Thy-1^{lo}) and the other derived from cell populations (Lin[−]Sca-1⁺), including both HSCs and early hematopoietic progenitors. These two cDNA libraries were spotted onto nylon filters (cDNA arrays) and hybridized with cDNA probes derived from BM cells to eliminate housekeeping and mature cell-expressed gene products from subsequent analyses. Approximately 5000 genes/ESTs were identified through this subtraction process, and they were then spotted onto glass slides for microarray analysis. Two PCR-amplified cDNA probe sets, one from rhodamine 123 (Rh)^{lo}Lin[−]Sca-1⁺c-Kit⁺ and the other from Rh^{hi}Lin[−]Sca-1⁺c-Kit⁺ populations of cells, were hybridized to the microarray of the resultant 5000 genes/ESTs. While the

Rh^{lo}Lin[−]Sca-1⁺c-Kit⁺ population of cells is enriched with arrested long-term (LT)-HSCs, the Rh^{hi}Lin[−]Sca-1⁺c-Kit⁺ population is enriched with cycling short-term (ST)-HSCs and multipotent progenitor (MPP) cells (24,28–30). Thus, differentially expressed genes between LT-HSCs and ST-HSCs/MPP cells were identified (see http://db.systemsbio.net/projects/local/stem_cell/).

Table 1 shows a comparison of differentially expressed HSC genes compiled from these three different approaches (24–26). The components of evolutionally conserved and developmentally regulatory pathways were prominent, including those from the Wnt pathway (*Lef1*, *Tcf4*, and *Dsh*); the TGF- β superfamily (*BMP4*, *activin C*, serine and threonine kinases *NIK* and *Ski*); the sonic hedgehog (*Shh*) pathway [*smoothed (SMO)*]; the Notch family (*Notch1* and *Manic Fringe*); members of the homeobox regulatory cascade (*Hoxa9*, *Meis-1*, *TGIF*, and *Enx-1*); and *Bmi*. Accumulated evidence indicates that these molecules and the underlying genetic pathways are involved in the regulation of stem cell self-renewal or maintenance. Abnormal activation of the Wnt signal has been associated with intestinal epithelial and other types of tumors (31,32). Activation of the Wnt pathway enhances the self-renewal of HSCs (19). Similarly, the *Shh* signal pathway is associated with skin basal cell carcinoma and with self-renewal of stem cells (23,33,34). *Notch1* was identified initially as a gene responsible for a form of T-lymphoma (*Tan-1*) (35), and its function in maintaining the state of progenitor cells is well-documented (36). Multiple studies have demonstrated the important role of Notch in the maintenance and enhanced repopulation of HSCs (16–18). Cooperation between *Hoxa9* and *Meis-1* leads to leukemia and is implicated in the regulation of self-renewal of stem cells (37–39). *Bmi-1*, the suppressor of the *Ink4* (*itl*) locus (encoding p16 and p19 cell growth inhibitors), is essential for the determination of the proliferation potential of HSCs (21,22).

Several groups have reported gene expression profiles of human HSC and progenitor cells. Human CD34⁺ HSCs are clinically used in autologous and allogeneic transplantation. Gomes et al. (40) identified 285 genes that are expressed in CD34⁺ HSC and progenitor cells (40). Among these genes, 106 are potential transcriptional factors. A portion of these transcriptional factors has corresponding homologs in murine HSCs, including *Tcf4*, *Runx1*, *Nfix*, *TGIF*, *SKI*, *Myb*, and *Bmi* (40). It has been shown that transplanting CD34⁺ HSCs from peripheral blood (PB) after mobilization has a higher success rate than using CD34⁺ HSCs isolated from BM (41). A comparison of the molecular components and the related genetic-program changes in CD34⁺ HSCs under different physiological conditions may explain these clinical findings. Two groups have carried out such a comparison (41,42). They found that the BM CD34⁺ HSC and progenitor cells express more cell cycle progression genes than the mobilized PB CD34⁺ cells, which suggests that the circulating PB CD34⁺ cells are more quiescent than those in the BM.

GENE EXPRESSION PROFILING OF STEM CELLS AND STEMNESS GENES

Stem cells are defined by their common features of self-renewal and pluripotentiality or multipotentiality. This leads to a hypothesis that all stem cells express a common set of genes

Table 1. Comparison of Representative Genes Expressed in Hematopoietic Stem Cells (HSCs) That Have Been Identified by Three Groups

Name	Description	Phillips et al. (25)	Tersikh et al. (26)	Park et al. (21)
Transcription factors			Yes	
<i>Bmi-1</i>	Polycomb group; involved in cell senescence.			Yes
<i>Enx-1</i>	Controls the expression of several homeobox genes.	Yes		Yes
<i>Hoxa9</i>	Homeobox transcription factor.			Yes
<i>Meis-1</i>	Ecotropic viral integration site 1; a PBX1-related homeobox gene.	Yes	Yes	Yes
<i>Nfe2</i>	AP-1 related DNA binding protein; leucine zipper protein.			Yes
<i>TBX2</i>	Immortalizing gene with tumorigenic potential.			Yes
<i>Ash1</i>	A SET domain, a PHD finger, four AT hooks, and a bromodomain.			Yes
<i>Scmh1</i>	Polycomb group protein; interacts with RAE28/mph1.			Yes
<i>Tcf4</i>	Wnt downstream transcription factor.			Yes
<i>LEF-1S</i>	Contains an HMG domain; Wnt downstream transcription factor.			Yes
<i>Evi-1</i>	Two sets of ZF; causes abnormalities in megakaryopoiesis.	Yes	Yes	Yes
<i>Evi-9</i>	A ZF protein; interacts with BCL6.			Yes
<i>AML-1</i>	Essential for hematopoiesis.	Yes		
Limo only 2	Oncogene and a partner of SCL/TAL.			Yes
<i>Testin</i>	Triple Lim domain protein.	Yes		
<i>Ku80</i>	A DNA binding subunit of DNA-PK.		Yes	
Membrane proteins				
<i>Notch1</i>	Controls cell fate choices by inhibiting differentiation.	Yes		Yes
<i>Notch4</i>	Regulating cell differentiation and proliferation.			Yes
<i>Manic fringe</i>	Notch pathway, regulating Notch and ligand affinity.	Yes		
<i>CD34</i>	HSC antigen.	Yes	Yes	Yes
<i>CD27</i>	Short-term HSC marker, receptor for CD70.	Yes	Yes	
<i>CD69</i>	Surface antigen.	Yes		
<i>c-Kit</i>	Stem cell survival.		Yes	Yes
<i>Cyt28</i>	Seven TM domains.	Yes	Yes	
<i>mETL1</i>	EGF-like domain and TM domain.		Yes	
<i>TM7XN1/GPR56</i>	EGF-like domain and seven TM domains.	Yes	Yes	
<i>ABCD</i>	MDR family.	Yes	Yes	Yes
<i>Flk-2/flt3</i>	Receptor tyrosine kinase.	Yes		Yes
Secreted molecules				
<i>BMP4</i>	TGF- β family; exhibits mesoderm inducing activity.			Yes
<i>IL-12</i>	Hematopoietic growth factor.	Yes		
<i>IL-16</i>	Ligand for CD4.	Yes		
<i>Mip2/Mip1a</i>	Chemokine.	Yes		Yes
<i>SDF</i>	A signal peptide.			Yes
<i>Slit</i>	Ligand for Robot.	Yes		
<i>DOKL</i>	Modulates signal Abl.	Yes		
<i>Angiopoietin 1</i>	Tie2/TEK ligand.	Yes	Yes	
<i>Activin C</i>	TGF- β family.			Yes
<i>TAX</i>	Malignancy-related C140.	Yes		Yes
<i>CDC42-like</i>	Hematopoietic-specific.			Yes
Apoptosis				
<i>Mcl-1</i>	Myeloid leukemia sequence 1; a bcl-2 related gene.			
<i>HP-1</i>	Heterochromatin protein 1.			
Signaling molecules				
<i>Disheveled-1</i>	Wnt pathway.	Yes		
<i>SMO</i>	Hedgehog pathway.	Yes		
<i>Nore1</i>	Ras-related.	Yes		
<i>WNK1</i>	Nine TM, three coil-coil; similar to MEKK.			Yes
<i>Braf</i>	S/T kinase; transforming gene.			Yes
<i>STK-1</i>	Expressed in proliferating cells.			Yes
<i>SKI</i>	TGF family; arrests differentiation cells.	Yes		
truncated form of <i>c-kit</i>	Kinase domain only.			Yes
<i>RGS2</i>	RGS box; GAP for trimeric G-protein.	Yes		Yes
Cell cycle				
<i>Cyclin H</i>	Associates with cyclin-dependent kinase 7.			Yes
<i>Cyclin D1</i>			Yes	
<i>Clk4</i>	CDK-like kinase 4.			Yes
<i>PACT</i>	p53 and pRB binding protein.	Yes		
<i>TOK-1</i>	Binds to p21Cip1/Waf1 and inhibits CDK2.			Yes
Miscellaneous				
<i>HMG-1</i>	DNA binding motif; higher-order nucleoprotein complexes.	Yes		Yes
<i>HMG-1</i> related protein	DNA binding components of the V-(D)-J recombinase.		Yes	Yes
<i>H2A.Z</i>	Maintains chromatin structure.	Yes		Yes
<i>H2A1.2</i> variant	Chromatin structure and function.	Yes	Yes	Yes
<i>HAT1</i>	The GCN5-related N-acetyltransferase.			Yes
<i>Dnmt3</i>	DNA methylase.	Yes	Yes	
PBX1, pre-B cell leukemia transcription factor 1; SET, su(var)3-9, enhancer of zeste, trithorax; PHD, plant homeodomain; HMG, high-mobility group; ZF, zinc finger; SCL/TAL, stem cell leukemia/T cell acute leukemia; DNA-PK, DNA-dependent protein kinase; EGF, epidermal growth factor; TM, transmembrane; MDR, multiple drug resistant; TGF, transforming growth factor; TEK, endothelial-specific receptor tyrosine kinase; MEKK, MAPK/ERK kinase kinase; RGS, regulator of G-protein signaling; GAP, GTPase-activating protein.				

Table 2. Essential Attributes of Stemness

1	Active JAK/STAT, transforming growth factor- β (TGF- β), Yes kinase, and Notch signaling.
2	The capacity to sense growth hormone and thrombin.
3	Interaction with extracellular matrix molecules integrin $\alpha 6/\beta 1$, Adam9, and bystin.
4	Cell cycle regulatory engagement.
5	Stress-resistance molecules including DNA repair, protein folding, ubiquitin, and deoxyfier systems.
6	Chromatin remodeling molecules.
7	Translational regulation factors.

Adapted from Ramalho-Santos et al. (44).

and, with their products, determine the special properties of stem cells. Identification of these common genes will provide important insight into understanding the molecular components and the underlying genetic pathways that may be involved in the maintenance and the regulation of self-renewal of stem cells (43,44). However, there are different types of stem cells. Embryonic stem cells (ESCs), generated from the inner cell mass of blastocysts (45), have the totipotent capacity to give rise to all types of tissues in the body and are located at the top of the hierarchical developmental tree (46). Somatic stem cells have a multipotent ability to produce tissue-specific types of cells. Adult HSCs, derived from BM (47), and NSCs, derived from the subventricular zone (SVZ) of the nervous system (48), are two well-characterized somatic stem cells. Identification of the set of commonly expressed genes in these three stem cell populations will unravel the molecular components of stem cells. Two groups have taken the identical approach of using oligonucleotide array technology to compare the gene expression profiles of ESCs, HSCs, and NSCs and have identified lists of genes representing stemness or a stem cell molecular signature (43,44).

Figure 1. Illustration of changes in accessibility for hematopoietic and nonhematopoietic gene transcriptions during early hematopoiesis. Adapted from Akashi et al. (59). HSC, hematopoietic stem cell; MPP, multipotent progenitor; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; T, T lymphocyte; B, B lymphocyte; NK, natural killer cell.

Ivanova and co-workers (43) have identified 283 genes that are commonly expressed in all ESCs, HSCs, and NSCs (<http://www.sciencemag.org/cgi/content/full/1072530/DC1>). These genes fall into different functional categories, such as signal transduction, transcriptional factors, (such as Edr1, EfnB2 Hes1, and Tcf3), translational regulation, cell cycle control, extracellular matrix, chromatin regulation, RNA binding, and apoptosis (43). The expression of *SMO*, encoding a receptor for the *Shh* signal pathway, is consistent with the role of *Shh* in enhancing the self-renewal ability of stem cells (23). Several Wnt receptors, Fzd6, 7, and 8, are detected in stem cells, which further supports the role of Wnt in the control of stem cell self-renewal and fate determination (31,32).

Similarly, Ramalho-Santos et al. (44) have identified 216 stemness genes that are expressed in all three stem cell populations and belong to a variety of functional groups (Table 2).

These are important studies for unraveling the molecular components of stem cells. Burns and Zon (49) have written a comprehensive review comparing these two studies and provide further insight. As discussed in their review, approximately 70% of the transcripts between fetal and adult HSCs overlap, and 40% of enriched genes are found in both human and mouse HSCs, further narrowing down the selective gene list. Both groups revealed a list of genes that could potentially play a role in the determination of stem cell behavior. A comparison of the list of stemness genes identified independently by these two groups yielded only 15 common genes (<10%); this might be due to a significant difference in methodology, including cell resources and purification, transcript amplification, and data analyses. Recently, using identical statistical methods, Ivanova et al. (50) have reanalyzed the previous data and found 605 overlap genes common to the two sets of their gene list (65.7% overlap). Nevertheless, for the first time, the two papers provide a snapshot of genes and the underlying

regulatory pathways to define stemness. As with other hypotheses, the concept of stemness genes requires further testing to generate more biological evidence. For example, the finding that Nanog is critical for the self-renewal and maintenance of ESCs (51,52) and expressed only in ESCs, but not in NSCs and HSCs, appears to challenge this concept. However, the stemness concept may refer to a broader definition of a specific combination of genes rather than individual genes that endow stem cells with their unique properties.

THE GENERALLY OPEN CHROMATIN STRUCTURE IN HEMATOPOIETIC STEM CELLS MAY DETERMINE THEIR MULTIPOTENTIALITY

HSCs develop first into MPPs, which then give rise to common lymphoid progenitor (CLP) and common myeloid progenitor (CMP) and finally differentiate into mature blood/immune cells of various types, depending on the differentiation programs that orchestrate transcription of lineage-specific genes. For specific genetic

programs to be activated, local chromatin must be accessible to transcription machinery (53,54), and it has been shown that activation of chromatin remodeling can occur prior to significant expression of genes in the region of interest (55,56). These results led to the hypothesis that open chromatin structure is maintained in early HSC/MPP cells, enabling multilineage differentiation programs to be readily accessible (57). This priming of genes affiliated with multiple lineages affords flexibility in cell fate decisions and allows multipotent precursors to rapidly respond to environmental cues (58). To examine this process, a global assessment of gene expression during early hematopoiesis (see <http://stowers-institute.org/labs/LiLab/hscdb.asp>) reveals that HSCs coexpress both hematopoietic as well as nonhematopoietic genes; MPP cells coexpress a more limited gene set that displays a more hematopoietic-specific character. As cells differentiate into the various hematopoietic progenitors (CMP or CLP), the gene sets available for expression are more and more restricted, reflecting their myeloid or lymphoid lineage commitment (Figure 1). This progressive decrease in the transcription accessibility during differentiation correlates with the stepwise restricted lineage potential of the cells (59). Thus, the generally open chromatin state maintained by HSCs may determine their multipotentiality.

If many genes are in fact primed at MPP stages, then these progenitors should then promiscuously express genes of multiple lineages at the single-cell level. The coexpression of genes related to multiple lineages was confirmed by single-cell reverse transcription PCR (RT-PCR) analyses

(60). Approximately 60% of single CMPs coexpressed all of these granulocyte/monocyte (GM)- and megakaryocyte/erythrocyte (MegE)-affiliated genes (Figure 2). In marked contrast, all single GM progenitors (GMPs) expressed GM-related genes such as myeloperoxidase (*MPO*) and granulocyte colony-stimulating factor receptor (*G-CSFR*) but did not express MegE-related genes such as β -globin or erythropoietin receptor (*EpoR*) (Figure 2). Conversely, all single MegE progenitors (MEPs) expressed β -globin and/or *EpoR* but not *MPO* or *G-CSFR* (Figure 2). Myelo-erythroid transcription factors were also coexpressed in single CMPs (60). In the lymphoid pathway, approximately 20% of single CLPs coexpressed B-lymphoid, Pax-5 and λ 5, and T-lymphoid CD3 δ and GATA-3. These data demonstrate directly that the priming of genes of multiple lineages occurs in hematopoietic MPPs such as CMPs and CLPs and suggest that lineage-promiscuous priming might be a common transcriptional feature in uncommitted stem and progenitor cells. Primed genes may represent the full and immediate differentiation potential of each subset assayed (60).

HSCs possess transcriptional accessibility for nonhematopoietic genes associated with multiple organ systems: CD45⁺ HSCs express a great number of nonhematopoietic genes, including genes characteristic of neuronal, endothelial, pancreatic, kidney, liver, heart, hair, epithelial, and muscle cell types (59). These nonhematopoietic genes were expressed in CD45⁺ HSCs at the single-cell to 10-cell level, whereas this broad transcriptional usage is lost as HSCs generate CMPs and CLPs. Thus, in the hematopoietic system, HSCs may

Figure 2. Transcriptional priming stages in single hematopoietic progenitors. Single-cell multiplex reverse transcription PCR (RT-PCR) assays for myeloid-related [β -globin and erythropoietin receptor (*EpoR*) for erythroid and granulocyte colony-stimulating factor receptor (*G-CSFR*) and myeloperoxidase (*MPO*) for myelomonocytic] and lymphoid-related genes (λ 5 and Pax-5 for B lymphoid and GATA-3 and CD3 δ for T lymphoid). Red boxes indicate lineage promiscuous expression, and the white boxes indicate no expression. More than 50% of single common myeloid progenitors (CMPs) coexpress both erythroid and myelomonocytic genes, and approximately 20% of single common lymphoid progenitors (CLPs) coexpress both B and T lymphoid-related genes. These data strongly suggest that (i) the expression of lineage-related genes precedes commitment; (ii) relevant genes are up-regulated upon commitment; and (iii) down-regulation of irrelevant genes is likewise important in fate decisions from oligopotent progenitors. This figure was adapted from Miyamoto et al (60).

maintain accessibility for nonhematopoietic tissues, which may represent their stemness or multipotentiality.

Does the accessibility for nonhematopoietic tissue genes represent HSC ability to transdifferentiate into nonhematopoietic tissue cells? Murine BM reportedly contains cells capable of differentiation into cells homing to multiple organs—including endothelial cells, skeletal and cardiac muscle (61), neurons and glia (62), parenchymal liver cells (63), and/or epithelial cells (64)—in addition to the expected hematopoietic lineages. While these reports suggest that the BM may be special for harboring precursors of nonhematopoietic fates, the notion of HSC plasticity was challenged by a study in which mice reconstituted with single green fluorescent protein (GFP)⁺ HSCs were extensively analyzed for GFP expression in nonhematopoietic tissues (65). These clonal experiments demonstrated that HSCs very rarely contributed to nonhematopoietic fates, suggesting that transdifferentiation of HSCs is unlikely (65). Furthermore, recent reports show that BM cells adopt the phenotype of other tissue cells by spontaneous cell fusion *in vitro* (66) and suggest that the observed regeneration of hepatocytes following BM transplantation occurs through the cell fusion of damaged hepatocytes and BM-derived cells (67,68). The primed nonhematopoietic gene expression in HSCs may contribute to the reprogramming of gene expression in the genome of the other partner after cell fusion. Nonetheless, transdifferentiation in the settings of increased tissue renewal or tissue damage remains to be precisely addressed using bona fide isolated HSCs. However, because HSCs normally express many nonhematopoietic genes, it will be interesting to determine whether any of these are candidate molecules that instruct nonhematopoietic fate outcomes in definitive HSCs.

CONCLUSION

Microarray-based gene expression profiling of stem cells provides a fundamental platform for unraveling the molecular components of stem cells. Here we have summarized the research of various groups using microarray technology and other approaches to determine gene expression profiles in stem cells, focusing primarily on HSCs. These works have identified a list of candidate genes that are differentially expressed in HSCs, proposed a new concept of stemness and the underlying stem cell signature genes, and revealed a potential molecular basis of multipotentiality of stem cells as a wide-open chromatin state in stem cells. The integration of this enormous fund of information in conjunction with other approaches, including molecular, cellular, genetic, and developmental biology, to eventually uncover the mystery of stem cells will be the challenge for all stem cell biologists.

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