Genetic Network Analysis of Human CD34+ Hematopoietic Stem/Precursor Cells

Shing-Jyh Chang[1,2], Tse-Sung Huang[3], Kung-Liahng Wang[4,5,6], Tao-Yeuan Wang[7], Yuh-Cheng Yang[4], Margaret Dah-Tsyr Chang[2], Yu-Hsuan Wu[3], Hsei-Wei Wang[3,8,9,10]*

[1]Department of Obstetrics and Gynecology, Mackay Memorial Hospital, [2]National Tsing Hua University, HsinChu, [3]Institute of Microbiology and Immunology, National Yang-Ming University, [4]Department of Obstetrics and Gynecology, Mackay Memorial Hospital, [5]National Taipei College of Nursing, [6]Mackay Medicine, Nursing and Management College, [7]Department of Pathology, Mackay Memorial Hospital, [8]Institute of Clinical Medicine and [9]VGH-YM Genome Center, National Yang-Ming University, and [10]Department of Education and Research, Taipei City Hospital, Taipei, Taiwan.

SUMMARY

Objective: Somatic CD34+ hematopoietic stem/precursor cells (HSPCs) give rise to hematopoietic cells and endothelial cells and have been used in clinical applications. Understanding the genes responsible for stemness and how they interact with each other will help us to manipulate these cells more efficiently in the future.

Materials and Methods: We performed microarray analysis on human CD34+ HSPCs and on two different progeny cell types, i.e. microvascular endothelial cells and peripheral blood mononuclear cells. Systems biology and advanced bioinformatics tools were used to help clarify the genetic networks associated with these stem cell genes.

Results: We identified CD34+ HSPC genes and found that they were involved in critical biologic processes such as cell cycle regulation, chromosome organization, and DNA repair. We also identified a novel precursor gene cluster on chromosome 19p13.3. Analysis of HSPC-enriched genes using systems biology tools revealed a complex genetic network functioning in CD34+ cells, in which several genes acted as hubs to maintain the stability (such as GATA1) or connectivity (such as hepatic growth factor) of the whole network.

Conclusion: This study provides the foundation for a more detailed understanding of CD34+ HSPCs. [*Taiwan J Obstet Gynecol* 2008;47(4):422–430]

Key Words: CD34 antigen, GATA2, genetic network, hematopoietic stem cells

Introduction

Regenerative medicine can accelerate the healing of tissues that are unable to repair themselves. Embryonic and somatic stem cells have the potential for self-renewal and differentiation, and show great promise for clinical usage. Hematopoietic stem cells (HSCs) are currently used in clinical stem cell transplantation [1]. However, it is difficult to expand CD34+ HSCs and to maintain them undifferentiated. A better understanding of the mechanisms regulating "stemness" is needed in order to improve our ability to utilize HSCs more effectively.

HSC isolation is performed by making use of the CD34 antigen [2,3]. Bone marrow, peripheral blood and cord blood are all excellent sources of CD34+ HSCs. However, the CD34+ population also contains few lineage-committed progenitors that are directly responsible for the generation of mature blood cells or endothelial cells. For example, the CD133+/VEGFR3+ subpopulation was defined as precursors of lymphatic endothelial cells [4], and CD133+/VEGFR2+ cells were also identified as functional endothelial precursors in circulating human CD34+ cells [5]. Thus, CD34 is usually combined with other selection markers for the isolation of HSCs [6]; long-term and short-term functional HSC are identified by the surface markers CD34+ CD33−CD38−Rho^{low} and CD34+CD33−CD38−Rho^{high},

*Correspondence to: Dr Hsei-Wei Wang, Institute of Microbiology and Immunology, National Yang-Ming University, 155, Section 2, Li-Nong Street, Taipei, Taiwan.
E-mail: hwwang@ym.edu.tw
Accepted: March 7, 2008

respectively. The CD133 antigen has also recently been used for HSC isolation [7,8].

The gene expression patterns in human and mouse CD34+ hematopoietic stem/precursor cells (HSPCs) have been widely characterized by serial analysis of gene expression or microarray analysis in several studies, which have identified genes involved in self-renewal, differentiation and lineage choice [2,3,6,9–13]. However, assigning biologic significance to these genes remains difficult. In this post-genomics era, modern bioinformatics and systems biology tools can help to predict the biologic behaviors of gene products. Deductions about *de novo* gene relationships can be made using exploratory computational tools and systems biology algorithms, and can provide a systematic approach for discovering novel molecular events and relationships [14,15]. The combination of microarray and computational results will enable biologists to speed up their research.

This study aimed to identify the genes responsible for the stem cell properties of CD34+ HSPCs, and to provide a global genetic network for them. We compared the gene expression profile of CD34+ HSPCs to those of their matured progenies, including endothelial cells and hematopoietic cells. This is a novel strategy that has not been used before.

Materials and Methods

CD34+ cells and primary microvascular endothelial cells (MVECs)

Human CD34+ cells from healthy individuals were isolated from bone marrow, peripheral blood or cord blood (Poietics; Cambrex, USA). In order to induce endothelial differentiation, isolated CD34+ cells were plated onto fibronectin-coated wells (Sigma-Aldrich, St Louis, MO, USA) and maintained in endothelial basal medium-2 (EBM2) supplemented with EGM2-MV SingleQuots (Clonetics Cambrex, Emerainville, France). Microarray analysis was performed using CD34+ cells from bone marrow and peripheral blood, while those from cord blood were processed for quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) analysis. Human primary MVECs (Clonetics Cambrex, Emerainville, France) and the human endothelial cell line HMEC1 were cultured in EGM-2 MV BulletKit medium (Cambrex, Walkersville, MD, USA).

Real-time RT-PCR and cyber green quantitative polymerase chain reaction (qPCR)

For real-time RT-PCR, primers and probes for the human glyceraldehyde 3-phosphate dehydrogenase gene (endogenous control) were designed using the Primer Express software program (Applied Biosystems, Foster City, CA, USA). Specific products were detected and analyzed using a Perkin-Elmer 7900 sequence detector (Applied Biosystems, Foster City, CA, USA).

Array probe preparation, data analysis, and functional network analysis

Total RNA collection, complementary RNA probe preparation, array hybridization and data analysis were performed as previously described [16]. Expression profiles of CD34+ HSPCs, MVECs and peripheral blood mononuclear cells were identified using the Affymetrix HG-U133 Plus 2.0 (Affymetrix, Santa Clara, CA, USA). Gene annotation enrichment analysis was performed using the DAVID 2007 tool (http://david.abcc.ncifcrf.gov/) [17]. EASE score, a Fisher's exact test-based scoring system, was used to calculate *p* values, determining the probability that the number of genes was specifically enriched in a given gene ontology (GO) or occurred by random chance. The Ingenuity Pathway Analysis (IPA) web tool developed by Ingenuity Co. (www.ingenuity.com) was used to construct functional regulatory networks of gene profiles. IPA uses the Ingenuity Pathways Knowledge Base to identify known interactions between focus genes and other genes that are not in the gene list. IPA then determines a statistical score for each network according to the fit of the network to the set of focus genes. The score is the negative log of *p* and denotes the likelihood of the focus genes in the network being found together by chance.

Results

Molecular signatures of CD34+ HSPCs

To access molecular signature genes for CD34+ cells, we obtained CD34+ HSPCs from human bone marrow and granulocyte-macrophage colony-stimulating factor-mobilized peripheral blood from healthy individuals. When freshly isolated CD34+ cells were subjected to endothelial differentiation conditions for 14 days [18,19], endothelial-like cells formed characteristic capillary tube structures, suggesting the presence of endothelial stem/precursor cells in the CD34+ cell preparations. We also obtained human dermal MVECs that were positive for the endothelial cell markers, CD31 and von Willebrand factor.

The gene expression profiles of HSPCs and MVECs were determined and compared with each other. CD34+ cells can also give rise to hematopoietic cells, and peripheral blood mononuclear cells were, therefore, also included in the comparison. The gene expression profiles of these three cell types were identified at least

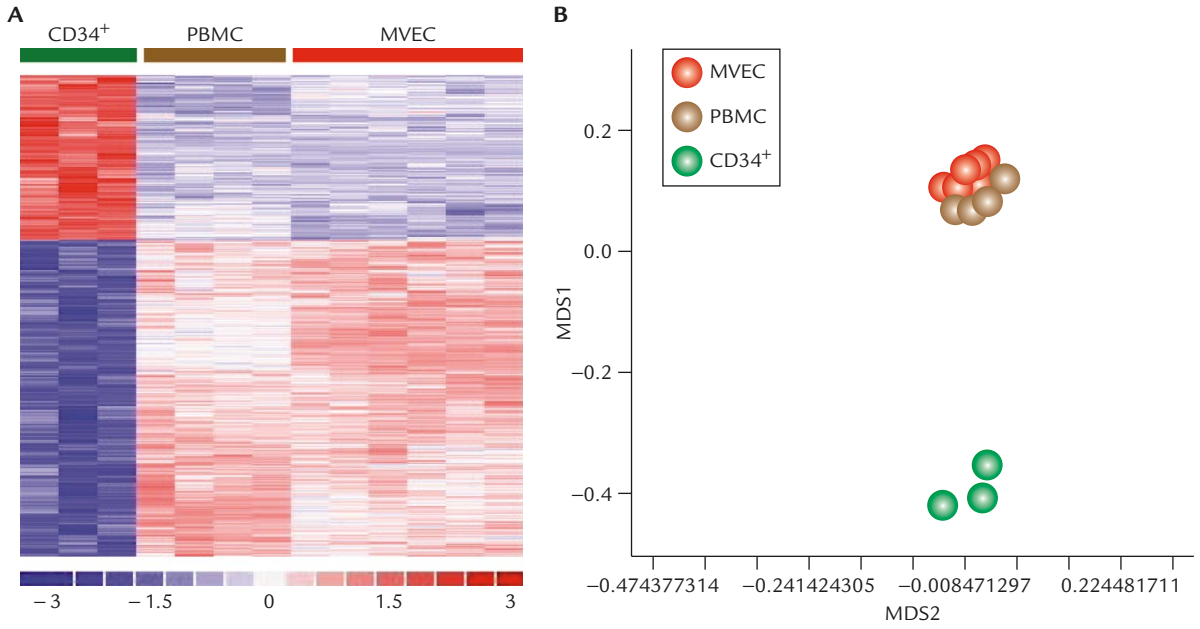

Figure 1. Characterization of CD34+ hematopoietic stem-progenitor cells (HSPCs) and primary microvascular endothelial cells (MVECs). (A) A heatmap shows genes enriched in CD34+ HSPCs. Red indicates increased gene expression; blue indicates decreased gene expression. (B) A multidimensional scaling (MDS) plot shows the discriminant ability of the obtained molecular signatures for cell groups. Each spot represents a single array sample.

in triplicate, using the Affymetrix™ HG-U133 Plus 2.0 whole-genome chip. According to the statistical methods used previously [16], genes differentially expressed between each cell type (the molecular signature) were identified. A total of 1,282 probe sets (corresponding to 1,042 genes) were uniquely expressed in CD34+ HSPCs (with a false-positive discovery rate threshold $q < 0.001$). All but five of these signature genes showed at least a 1.5-fold expression difference when compared with the other cell types. A gene expression heatmap for this signature indicated its unique expression pattern in CD34+ HSPCs (Figure 1A). Its discriminant ability was also assessed using multidimensional scaling (Figure 1B).

GO mapping of differentially expressed HSPC genes

There were 439 probe sets (345 genes) specifically enriched in CD34+ cells (Figure 1A), and these were most likely to be involved in self-renewal and cell proliferation. To gain more insights into their functional roles in CD34+ cells, these 439 probe sets were subjected to a GO database search [20] to identify statistically over-represented functional groups within this gene list. The DAVID 2007 web-based tool, a graph theory evidence-based method to agglomerate gene/protein identifiers [17], was used for this task. Because the chip we used represented the whole human transcriptome, this analysis was not biased toward the coverage of the microarray.

The GO tree of biologic processes that were statistically over-represented ($p < 0.05$) is shown in Table 1. The predominant processes included those pertaining to the cell cycle (especially M phase), DNA and RNA metabolism (replication and transcription), chromosome organization and biogenesis, cell proliferation, and DNA repair. Genes belonging to broad biologic process categories are summarized in Table 2. Many of these CD34+ cell-enriched genes have previously been identified as HSC-specific gene products, using a global subtractive hybridization screen [21] or by other microarray analyses [2,3,6,9–13]. GATA2, KIT and MYB were shown to be enriched in pluripotent HSCs [22–24]. PROM1 (CD133) is also known as a hemangioblast marker [8]. GATA1 has been identified as an erythroid-specific transcription factor essential during the relatively early stages of erythrocytic differentiation [25]. GATA1 and RUNX1 are coexpressed and cooperate in megakaryocytic differentiation [26]. Defective vascular remodeling in RUNX1 homozygous mutant mice could be rescued by the addition of HSCs or angiopoietin-1, supporting the role of angiopoietin-1 secreted by HSCs in angiogenesis [27]. Many other known HSC markers, such as FLT3, KLF1, LMO2 and PBX1, were also present. These consistent findings demonstrated the reliability of our gene list (Table 2, italics).

Chromosomal locations of CD34+ signature genes

A significant number of CD34+ cell-enriched genes were located at cytoband 19p13.3 (11 genes; $p = 0.00002$; Figure 2A). Among them, the genes for azurocidin (AZU1), neutrophil elastase (ELA2) and proteinase-3 (PRTN3) encoded primary components of neutrophil

Table 1. Biologic processes enriched in CD34+ hematopoietic stem-progenitor cells (HSPCs). Selected biologic processes over-represented by genes enriched in CD34+ cells. A total of 439 probe sets enriched in CD34+ cells were subjected to DAVID 2007 analysis. These categories were selected from the biologic process organizing principle in the Gene Ontology project. The number of genes, their percentage in those 439 probe sets, and *p* values for each category that was significantly ($p < 0.05$) over-represented are listed

Biological process	Gene no.	%	*p*
Metabolism			
Nucleo-base, -side, -tide and nucleic acid metabolism	96	24.2	6.54×10^{-8}
DNA replication	19	4.8	5.41×10^{-9}
DNA-dependent DNA replication	13	3.3	2.92×10^{-8}
DNA replication initiation	8	2	2.23×10^{-7}
Transcription	57	14.4	3.18×10^{-3}
Transcription, DNA-dependent	45	13.6	2.30×10^{-3}
DNA metabolism	32	8.1	6.54×10^{-8}
Nucleotide metabolism	10	2.5	1.20×10^{-3}
Cellular metabolism			
Regulation of cellular metabolism			
Positive regulation of cellular metabolism	8	2	1.43×10^{-2}
Cellular biosynthesis	34	8.6	9.66×10^{-3}
Biopolymer metabolism	61	15.4	2.03×10^{-3}
Regulation of metabolism	61	15.4	1.39×10^{-3}
Biosynthesis	35	8.8	2.46×10^{-2}
Cellular physiologic process			
Cell cycle			
Regulation of cell cycle	15	3.9	2.06×10^{-2}
M phase	9	2.3	1.43×10^{-2}
Cell organization and biogenesis			
Organelle organization and biogenesis			
Chromosome organization and biogenesis			
Chromosome organization and biogenesis (sensu Eukaryota)	10	2.5	4.58×10^{-2}
Response to endogenous stimulus	11	2.8	1.43×10^{-2}
Response to DNA damage stimulus	10	2.5	2.45×10^{-2}
DNA repair	10	2.5	1.37×10^{-2}
Regulation of physiologic process	73	18.4	1.05×10^{-2}

azurophilic granules [28] and were closely located in a region of approximately 50 kb (Figure 2B). These genes have been shown to be expressed in a coordinated fashion, and changes in chromatin organization at this locus are associated with myeloid cell differentiation [28]. We additionally found that an adjacent gene, that for bone marrow proteoglycan 2 (PRG2), was also upregulated in CD34+ cells (Figure 2B).

Coordinated changes in functional networks of CD34+ signature genes

In order to understand how genes enriched in CD34+ cells are related, we performed functional network analysis using the IPA web tool and the Ingenuity knowledge database (see Materials and Methods). Two major networks and three interacting pairs were identified (Figure 2A). One of the major networks (33 genes) included most of the known stemness-related or pro-proliferation genes. Among these CTSG (cathepsin G), a member of the peptidase S1 protein family encoded on chromosome 14q11.2, is another azurophil granular protein. GATA1 and KLF1 (EKLF) are precursors and definitive differentiation-associated genes; HGF (hepatic growth factor) and KIT are HSC-associated genes; GATA2, LMO2, MPO and MYB support self-renewal and keep HSCs in an undifferentiated state; FOXO3A and PLAGL1 are involved in inhibition of the cell cycle [29]. CDK6 is a factor promoting the G1 phase in HSCs [30].

In addition to confirming what was already known about CD34+ HSPCs, novel genes that may play crucial roles in self-renewal and differentiation were also revealed. Genes not previously implicated in stemness,

Table 2. Genes enriched in CD34$^+$ HPCs

Cell cycle/cell proliferation	CDCA7, *CDK6*, CDT1, CHAF1A, DDX11, DKC1, DNTT, DUT, KNTC1, *MCM2*, *MCM3*, *MCM4*, *MCM5*, *MCM6*, *MCM7*, MSH6, ORC1L, PLAGL1, POLD1, PPP3R1, RCC2, RFC3, SET, SMC2L1, TFDP2, TOP1MT, TSGA2, WEE1
Cytokines & cognate receptors	*ANGPT1, FLT3,* HGF, *KIT*, NENF, TFR2, THRB, TMPO
Response to DNA damage stimulus	APEX1, BRIP1, FANCL, PMS2L1, RAD51AP1, TRH, UNG
Cell adhesion	CDH1, CLDN10, COL14A1, COL24A1, DSG2, EPDR1, GMDS, ICAM4, ITGA9, PRG2
Transcription	ADNP, BRCA1, CHD4, ELF2, ETV6, FOXO3A, *GATA2*, JUND, KCNH2, L3MBTL4, LOC148203, LYL1, MLF1IP, MXD3, *MYB*, NR1I2, NRIP1, NSBP1, PHB2, PRDM16, RAI17, SSBP2, TGIF2, YEASTS2, ZNF6, ZNF44, ZNF135, ZNF334, ZNF667
Protein metabolism	APOC1, CPXM, CTSG, DARS, DPEP3, ELA2, FBXO11, HS6ST1, NEDD4L, PRSS21, PRTN3, PTPMT1, PTPRD, RHAG, RPL7A, RPL15, RPL18A, RPS10, RPS15A, RPS17, STT3B, TPSAB1, ZNRF1
Development	APOE, AZU1, CHRM3, CLGN, DACH1, DPPA4, DNMT3B, EREG, ETS2, FZD7, *GATA1*, HESX1, HLF, *KLF1*, *LMO2*, MAML3, MAP7, MPPED2, NDN, NPR3, *PBX1*, PHGDH, *PROM1 (AC133)*, REC8L1, RTN4R, *RUNX1*, SCML2, SLC22A16, SPATA11, SPTA1, ST6GAL2, TMPRSS6, TRO, WHSC1
Signal transduction	ASB13, CRHBP, DEPDC1B, DEPDC6, GNB1, GPSM2, GUCY1A3, HTR1F, KCNK17, LGR7, NET1, NPW, PDZK3, PDZK8, PGDS, RAB7B, SUCNR1, TGFBRAP1
Transport	EMID1, HBD, RYR3, SFXN2, SFXN4, SHMT1, SLC16A14, SLC18A2, SLC1A6, SLC22A16, SLC25A15, SLC25A27, SLC27A2, SLC27A5, SLC2A5, SLC39A3, SLC39A4, SLC43A1, SLC7A5, SLC8A3, SLC9A3, SNX26, SV2A
Others	B4GALT6, C5orf13, CALN1, Cep70, CRISPLD1, DNAJA5, ENO1, EXOSC4, HNRPA0, HNRPA1, HPRT1, KLHL23, MGC21644, MNS1, MPO, MSI2, MUC1, PPP1R13B, RNF24, SEPP1, SESN3, SNRPD1, SNRPE, SS18, TCBA1, YPEL2, ZBED3

Genes are represented by the HGNC official symbols (www.gene.ucl.ac.uk/nomenclature/). Genes discussed in the text are in italics.

but crucial in carcinogenesis, were identified, including BRCA1, CDH1, ELF2, JUND, TACSTD1 and MSH6. Similarly, genes without previously known roles in hematopoiesis or endothelial differentiation but with known roles in the development of other organs, including NCL, PIK3R1, THRB1 and TRH, were identified [31,32]. NR1I2 (PXR), UQCRH (ubiquinol-cytochrome-c reductase hinge protein) and WEE1 (an anti-apoptotic protein involved in the DNA damage checkpoint), whose functions have not previously been linked to stem cell activities, were also shown. The network also revealed five genes whose roles may be more crucial than others in CD34$^+$ cells; GATA1, HGF, MYB, THRB and RUNX1 were the "hub" genes (genes involved in most interactions with other components) that either preserved the stability of the whole network or linked sub-modules in the same network to each other.

Another major network contained genes mainly involved in minichromosome maintenance (MCM) and DNA replication, including CDT1, MCM2, MCM3, MCM4, MCM5, MCM6, MCM7 and ORC1L (eight genes; Figure 3A). The upregulation of these S-phase inducers [33] suggests that a portion of CD34$^+$ cells may enter the cell cycle. Consistent with this, the M-phase potential of CD34$^+$ cells was indicated by high expression levels of genes crucial in M phase mitosis ($p = 1.43 \times 10^{-2}$; Table 1).

Abundant expression of some network genes (including GATA1, JUND and MCM5) in CD34$^+$ cells was verified by qPCR (Figure 3B). The qPCR results showed a high degree of correlation with microarray results ($r \geq 0.95$).

Discussion

Stem cells are currently attracting much attention because of their unique biologic behavior and potential clinical usefulness. In this study, we performed extensive comparative transcriptomic and gene network analyses of CD34$^+$ HSPCs. The results will help to unravel the riddle of HSPCs and contribute to cell-based therapy.

In addition to identifying specific genes, we also used refined computational methods to highlight key functional networks. There is increasing recognition that a systematic approach is necessary to achieve an overview

A

Term	Count	%	*p*
19p13.3	11	2.76	0.00002
21q22.3	6	1.51	0.00334
1p13.3	4	1.01	0.00495
9q34	3	0.75	0.01053
12p13	3	0.75	0.01151
14q11.2–q12	2	0.50	0.02177
2p16	2	0.50	0.02177
4q28	2	0.50	0.02416
2q21	2	0.50	0.03129
17q22	3	0.75	0.03731
2q33–q35	2	0.50	0.03837
12q22	3	0.75	0.04349
3q23	3	0.75	0.04701

B

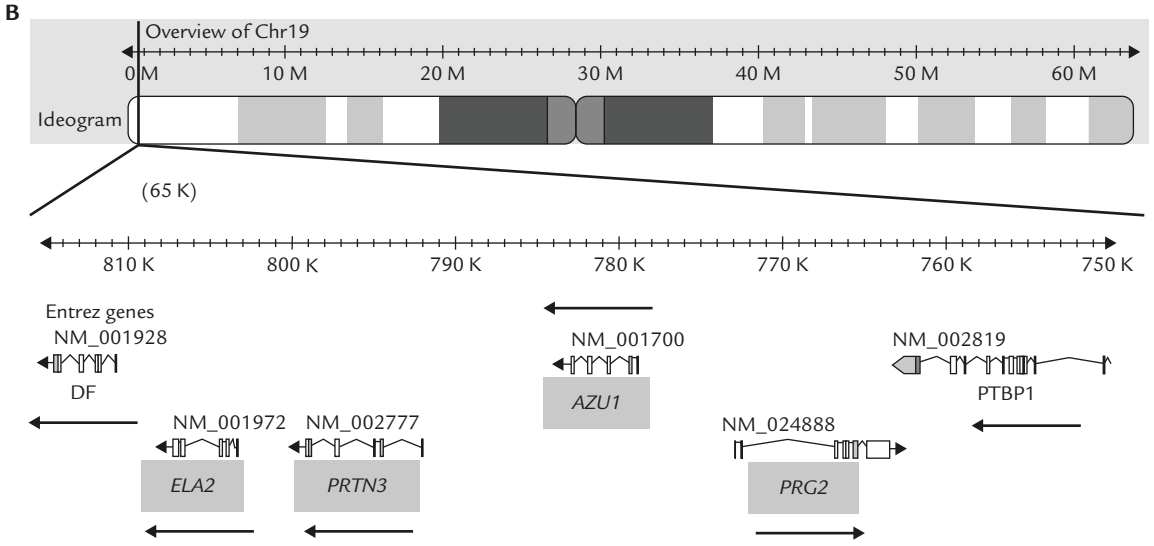

Figure 2. A novel gene cluster identified in CD34+ hematopoietic stem-progenitor cells (HSPCs). (A) Distribution of CD34+ HSPC-enriched genes on human chromosomes. A total of 439 probe sets enriched in CD34+ cells were subjected to DAVID 2006 analysis. The number of genes, their percentage in those 439 probe sets, and *p* values for each cytoband that was significantly ($p < 0.05$) over-represented are all listed. (B) Chromosomal location of a gene cluster in CD34+ HSPCs. The cytoband containing the most stemness genes was 19p13.3. Of those 11 genes, four, including AZU1, ELA2, PRG2 and PRTN3 (in gray), were closely linked in a region of approximately 50 kb (810–760 kb).

of the molecular events responsible for a given biologic process [34,35]. In this study, we used systems biology tools to reveal the interactions between CD34+ signature genes. Two functional networks and three interacting pairs of CD34+ HSPCs-enriched genes were identified, though only 9.6% of CD34+ genes were involved (Figure 3A). The genetic networks, which were identified by *in silico* approaches based on knowledge-based databases, provided further information (such as key hub genes) that could not be readily extracted using one-dimensional gene list analysis. For example, GATA1 plays an essential role in the promotion of hematopoietic cell differentiation [25], but GATA2 blocks cells differentiation and stimulates the proliferation of immature cells [24]. Differential expression of the GATA2 and MYB genes also correlates with the potential of α4-integrin+ embryonic stem cells to differentiate into hematopoietic cells [23,36].

Our analyses have limitations: the functional networks were mapped based on pre-existing knowledge databases (GO and IPA), in which molecular interactions were established in various physiologic or pathologic conditions. It is known that molecular functions vary depending on cellular and tissue contexts, and the stemness genes may, therefore, not interact in a similar way in human CD34+ cells. Functional analyses will be needed for the rigorous evaluation of individual gene interactions deduced by an *in silico* modeling approach.

The functions of many of the genes found to be enriched in CD34+ cells remain unknown. This study provides a novel strategy that can be used in further genetic network studies on CD34+ HSCs. The methods used can also be applied to the analysis of differentiation and self-renewal paradigms in other stem cells, and for the analysis of many other complex biologic processes, such as tumorigenesis.

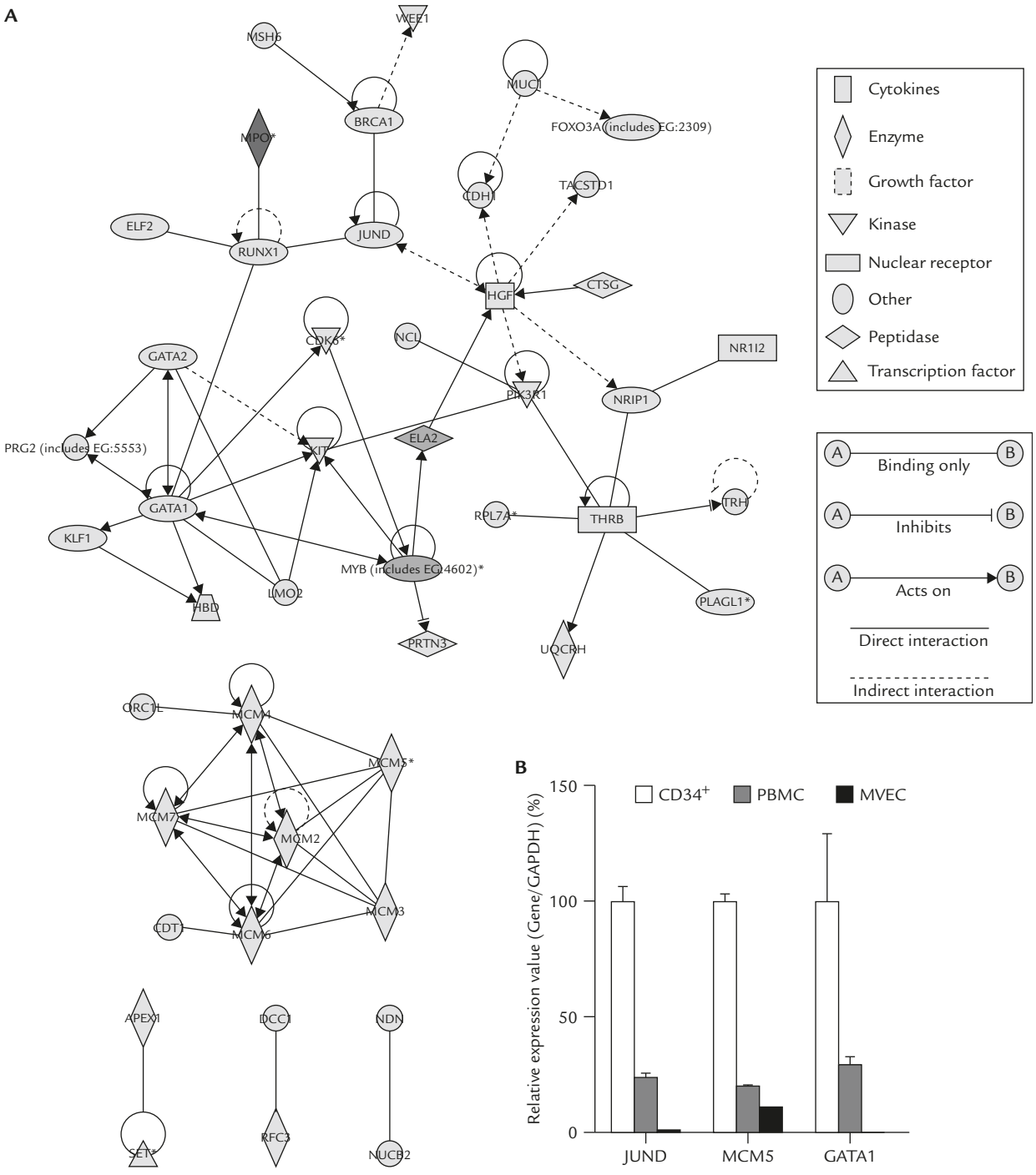

Figure 3. Protein–protein interaction networks as a framework for the interpretation of CD34$^+$ stem cell biology. (A) Functional networks composed of multiple genes, some of which (such as GATA2, HGF and KIT) have previously been implicated in CD34$^+$ stemness, while most of them have never been described. These networks are displayed graphically as nodes (gene products) and edges (biologic relationships between nodes) mapped using the Ingenuity Pathway Analysis tool. The intensity of the node color indicates the degree of upregulation. (B) Validation of network genes by real-time reverse transcription polymerase chain reaction. GATA1, JUND and MCM5 were deemed to be of particular interest because of their differential expression in CD34$^+$ cells and/or their interaction in the knowledge-based network analysis. Mean expression levels of target genes were compared with that of glyceraldehydes phosphate dehydrogenase control.

Acknowledgments

The authors acknowledge the technical services provided by the Microarray and Gene Expression Analysis Core Facility of the National Yang-Ming University VGH Genome Research Center. The Gene Expression Analysis Core Facility is supported by National Research Program for Genomic Medicine, National Science Council. This

work was supported by grants from the National Science Council (NSC 96-3112-B-010-009 and NSC 96-2320-B-010-026), the Yen Tjing Lin Medical Foundation (CI-96-11), VGHUST Joint Research Program, Tsou's Foundation (V97ER2-002), the Taipei City Hospital (95002-62-086), and the Ministry of Education's Aim for the Top University Plan.

References

1. Brunstein CG, Baker KS, Wagner JE. Umbilical cord blood transplantation for myeloid malignancies. *Curr Opin Hematol* 2007;14:162–9.
2. Ng YY, van Kessel B, Lokhorst HM, Baert MR, van den Burg CM, Bloem AC, Staal FJ. Gene-expression profiling of CD34+ cells from various hematopoietic stem-cell sources reveals functional differences in stem-cell activity. *J Leukoc Biol* 2004;75:314–23.
3. Oswald J, Steudel C, Salchert K, et al. Gene-expression profiling of CD34+ hematopoietic cells expanded in a collagen I matrix. *Stem Cells* 2006;24:494–500.
4. Salven P, Mustjoki S, Alitalo R, Alitalo K, Rafii S. VEGFR-3 and CD133 identify a population of CD34+ lymphatic/vascular endothelial precursor cells. *Blood* 2003;101:168–72.
5. Peichev M, Naiyer AJ, Pereira D, et al. Expression of VEGFR-2 and AC133 by circulating human CD34+ cells identifies a population of functional endothelial precursors. *Blood* 2000;95:952–8.
6. Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, Lemischka IR. A stem cell molecular signature. *Science* 2002;298:601–4.
7. Loges S, Fehse B, Brockmann MA, et al. Identification of the adult human hemangioblast. *Stem Cells Dev* 2004;13:229–42.
8. Jaatinen T, Hemmoranta H, Hautaniemi S, et al. Global gene expression profile of human cord blood-derived CD133+ cells. *Stem Cells* 2006;24:631–41.
9. Phillips RL, Ernst RE, Brunk B, et al. The genetic program of hematopoietic stem cells. *Science* 2000;288:1635–40.
10. Park IK, He Y, Lin F, et al. Differential gene expression profiling of adult murine hematopoietic stem cells. *Blood* 2002;99:488–98.
11. Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA. "Stemness": transcriptional profiling of embryonic and adult stem cells. *Science* 2002;298:597–600.
12. Bruno L, Hoffmann R, McBlane F, et al. Molecular signatures of self-renewal, differentiation, and lineage choice in multipotential hemopoietic progenitor cells in vitro. *Mol Cell Biol* 2004;24:741–56.
13. Georgantas RW 3rd, Tanadve V, Malehorn M, et al. Microarray and serial analysis of gene expression analyses identify known and novel transcripts overexpressed in hematopoietic stem cells. *Cancer Res* 2004;64:4434–41.
14. Featherstone DE, Broadie K. Wrestling with pleiotropy: genomic and topological analysis of the yeast gene expression network. *Bioessays* 2002;24:267–74.
15. Jen CH, Manfield IW, Michalopoulos I, et al. The Arabidopsis co-expression tool (ACT): a WWW-based tool and database for microarray-based gene expression analysis. *Plant J* 2006;46:336–48.
16. Wang HW, Trotter MW, Lagos D, et al. Kaposi sarcoma herpesvirus-induced cellular reprogramming contributes to the lymphatic endothelial gene expression in Kaposi sarcoma. *Nat Genet* 2004;36:687–93.
17. Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol* 2003;4:P3.
18. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;275:964–7.
19. Bompais H, Chagraoui J, Canron X, et al. Human endothelial cells derived from circulating progenitors display specific functional properties compared with mature vessel wall endothelial cells. *Blood* 2004;103:2577–84.
20. Harris MA, Clark J, Ireland A, et al. The Gene Ontology (GO) database and informatics resource. *Nucleic Acids Res* 2004;32:D258–61.
21. Shojaei F, Gallacher L, Bhatia M. Differential gene expression of human stem progenitor cells derived from early stages of in utero human hematopoiesis. *Blood* 2004;103:2530–40.
22. Orlic D, Anderson S, Biesecker LG, Sorrentino BP, Bodine DM. Pluripotent hematopoietic stem cells contain high levels of mRNA for *c-kit*, GATA-2, p45 NF-E2, and *c-myb* and low levels or no mRNA for *c-fms* and the receptors for granulocyte colony-stimulating factor and interleukins 5 and 7. *Proc Natl Acad Sci USA* 1995;92:4601–5.
23. Ogawa M, Kizumoto M, Nishikawa S, Fujimoto T, Kodama H, Nishikawa SI. Expression of α4-integrin defines the earliest precursor of hematopoietic cell lineage diverged from endothelial cells. *Blood* 1999;93:1168–77.
24. Minegishi N, Suzuki N, Yokomizo T, et al. Expression and domain-specific function of GATA-2 during differentiation of the hematopoietic precursor cells in midgestation mouse embryos. *Blood* 2003;102:896–905.
25. Kawabata H, Germain RS, Ikezoe T, Tong X, Green EM, Gombart AF, Koeffler HP. Regulation of expression of murine transferrin receptor 2. *Blood* 2001;98:1949–54.
26. Elagib KE, Racke FK, Mogass M, Khetawat R, Delehanty LL, Goldfarb AN. RUNX1 and GATA-1 coexpression and cooperation in megakaryocytic differentiation. *Blood* 2003;101:4333–41.
27. Takakura N, Watanabe T, Suenobu S, et al. A role for hematopoietic stem cells in promoting angiogenesis. *Cell* 2000;102:199–209.
28. Wong ET, Jenne DE, Zimmer M, Porter SD, Gilks CB. Changes in chromatin organization at the neutrophil elastase locus associated with myeloid cell differentiation. *Blood* 1999;94:3730–6.
29. Schmidt M, Fernandez de Mattos S, van der Horst A, et al. Cell cycle inhibition by FoxO forkhead transcription factors involves downregulation of cyclin D. *Mol Cell Biol* 2002;22:7842–52.
30. Leibundgut K, Schmitz NM, Hirt A. Catalytic activities of G1 cyclin-dependent kinases and phosphorylation of retinoblastoma protein in mobilized peripheral blood CD34+ hematopoietic progenitor cells. *Stem Cells* 2005;23:1002–11.

31. Forrest D, Erway LC, Ng L, Altschuler R, Curran T. Thyroid hormone receptor beta is essential for development of auditory function. *Nat Genet* 1996;13:354–7.
32. White R, Leonardsson G, Rosewell I, Ann Jacobs M, Milligan S, Parker M. The nuclear receptor co-repressor nrip1 (RIP140) is essential for female fertility. *Nat Med* 2000;6:1368–74.
33. Blow JJ, Dutta A. Preventing re-replication of chromosomal DNA. *Nat Rev Mol Cell Biol* 2005;6:476–86.
34. Barabasi AL, Oltvai ZN. Network biology: understanding the cell's functional organization. *Nat Rev Genet* 2004;5: 101–13.
35. Janes KA, Yaffe MB. Data-driven modelling of signal-transduction networks. *Nat Rev Mol Cell Biol* 2006;7:820–8.
36. Mucenski ML, McLain K, Kier AB, et al. A functional c-*myb* gene is required for normal murine fetal hepatic hematopoiesis. *Cell* 1991;65:677–89.