Comparison of the Gene Expression Profiles of Human Hematopoietic Stem Cells between Humans and a Humanized Xenograft Model

Hideyuki MATSUZAWA[*1, 2], Hiromichi MATSUSHITA[*2, 3], Takashi YAHATA[*2, 4], Masayuki TANAKA[*1] and Kiyoshi ANDO[*2, 5]

[*1]*Support Center for Medical Research and Education, Tokai University*
[*2]*Division of Hematopoiesis, Research Center for Regenerative Medicine, Tokai University School of Medicine*
[*3]*Department of Laboratory Medicine, Tokai University School of Medicine*
[*4]*Department of Cell Transplantation and Regenerative Medicine, Tokai University School of Medicine*
[*5]*Department of Hematology and Oncology, Tokai University School of Medicine*

(Received November 2, 2016; Accepted February 7, 2017)

The aim of this study is to evaluate the feasibility of NOD/Shi-scid-IL2Rγ^{null} (NOG) mice transplanted with human CD34$^+$/CD38$^-$/Lin$^{-/low}$ hematopoietic cells from cord blood (CB) as an experimental model of the gene expression in human hematopoiesis. We compared the gene expressions of human CD34$^+$/CD38$^-$/Lin$^{-/low}$ cells from human bone marrow (BM) and in xenograft models. The microarray data revealed that 25 KEGG pathways were extracted from the comparison of human CD34$^+$/CD38$^-$/Lin$^{-/low}$ HSCs between CB and BM, and that 17 of them—which were mostly related to cellular survival, RNA metabolism and lymphoid development—were shared with the xenograft model. When the probes that were commonly altered in CD34$^+$/CD38$^-$/Lin$^{-/low}$ cells from both human and xenograft BM were analyzed, most of them, including the genes related hypoxia, hematopoietic differentiation, epigenetic modification, translation initiation, and RNA degradation, were downregulated. These alterations of gene expression suggest a reduced differentiation capacity and likely include key alterations of gene expression for settlement of CB CD34$^+$/CD38$^-$/Lin$^{-/low}$ cells in BM. Our findings demonstrate that the xenograft model of human CB CD34$^+$/CD38$^-$/Lin$^{-/low}$ cells using NOG mice was useful, at least in part, for the evaluation of the gene expression profile of human hematopoietic stem cells.

Key words: human hematopoietic stem cells, cord blood, bone marrow, gene expression profile, NOG mice

INTRODUCTION

The hematopoietic system is important for the maintenance of the systemic oxygen supply and immunological surveillance. In humans, it initially appears in the second week of fetal life in the yolk sac as primitive hematopoiesis. It then moves to the aorta-gonad-mesonephros (AGM) region, where definitive hematopoiesis develops at four to six months of fetal life. The fetal liver takes over the hematopoietic function after that, and at birth, hematopoietic stem cells (HSCs) move to the bone marrow through the blood stream and settle there for lifetime hematopoiesis [1–5].

Human HSCs maintain the definitive hematopoietic system. They are characterized by multipotency and self-renewal and provide all of the hematopoietic lineage cells, including lymphoid and non-lymphoid cells, for the body's lifetime. Their cellular immunophenotype is characterized as CD34$^+$/CD38$^{-/low}$/CD59$^+$/CD90 (THY1)$^+$/CD117 (KIT)$^+$/CD133$^+$/CD135 (FLT3)$^+$/CD164$^+$/CD338$^+$/GATA2$^+$/TdT$^+$/Lin$^-$ [6–12]. They are located in the niche region of the bone marrow, which provides an adequate environment for maintaining their cellular stemness through direct interaction and the function of various kinds of secreted cytokines [13, 14].

The characterization of HSCs and studies on hematopoietic system require *in vivo* evaluations. The development of NOD/Shi-scid-IL2Rγ^{null} (NOG) mice, which lack any functions of NK dendritic cells or T or B cell immunity, by crossing NOD-scid mice with an IL2Rγ-knockout background has facilitated such evaluations, due to the high efficiency of human HSC engraftment [15, 16]. Cord blood (CB) contains young human HSCs that move to the bone marrow through the blood stream at birth and is the most primitive of the available HSC sources. Therefore, NOG mice transplanted with CB-HSCs are believed to be a suitable experimental model for evaluating the development of human hematopoiesis [17–22].

In the present study, to evaluate the feasibility of this xenograft model with particular focus on gene expression alterations, we compared the microarray data of human CD34$^+$/CD38$^-$/Lin$^{-/low}$ cells from bone marrow (BM) and xenograft serial transplantation models with CB CD34$^+$/CD38$^-$/Lin$^{-/low}$ cells.

MATERIALS AND METHODS

1. Materials

The use of CB obtained from full-term deliveries was approved by the Tokai University Committee on Clinical Investigation (Permit number: #12I-46).

Kiyoshi ANDO, Division of Hematopoiesis, Research Center for Regenerative Medicine, and Department of Hematology and Oncology, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan Tel: +81-463-93-1121 Fax: +81-463-92-4750 E-mail: andok@keyaki.cc.u-tokai.ac.jp

These CB samples were used for the xenograft experiments described below (Permit numbers: #10I-24 and #111026). The BM samples were utilized, in accordance with the approval of the Tokai University Committee on Clinical Investigation (Permit number: #14I-15).

2. Preparation of $CD34^+/CD38^-/Lin^{-/low}$ cells

Human $CD34^+/CD38^-/Lin^{-/low}$ cells were prepared from pooled CB and BM in accordance with the method of our previous report, with minor modifications [18, 19]. Briefly, they were initially prepared using the CD34 Progenitor Cell Isolation Kit (Miltenyi Biotec, Sunnyvale, CA, USA). These cells were then stained with allophycocyanin (APC)-conjugated anti-CD45 mAb (Coulter/Immunotech, Marseille, France), and fluorescein isothiocyanate (FITC)-conjugated anti-lineage-specific antigens; CD2, CD3 (UCHT1), CD41 (P2), glycophorin A (11E4B-7-6), CD14 (MfP9), CD19 (SJ25C1), and CD56 (NCAM16.2) (all from BD Biosciences, San Jose, CA, USA), phycoerythrin (PE)-conjugated anti-CD38 (HB7; BD Biosciences), and phycoerythrin-Texas Red (ECD)-conjugated anti-CD34 (581; Coulter/Immunotech) mAbs. The $CD34^+/CD38^-/Lin^{-/low}/CD45^+$ cells were fractionated using the FACSVantage™ flow cytometer (BD Biosciences).

3. Transplantation of $CD34^+/CD38^-/Lin^{-/low}$ cells in a xenograft mouse model

NOG mice were obtained from the Central Institute for Experimental Animals (Kawasaki, Japan) and maintained in the animal facility of the Tokai University School of Medicine in microisolator cages. Nine- to 20-week-old NOG mice were irradiated with 220 cGy of X-rays. A total of 10,000 sorted human $CD34^+/CD38^-/Lin^{-/low}$ cells were injected into the retro-orbital plexus of the NOG mice the day after irradiation [18, 19]. The mice were sacrificed at 18 weeks after transplantation. BM cells were harvested, and human hematopoietic cells were distinguished from mouse cells by the expression of human CD45. $CD34^+/CD38^-/Lin^{-/low}$ cells isolated from the pooled BM cells of three recipient mice were used for the microarray analysis. For serial transplantation, human $CD34^+$ cells from the primary transplantation (1×10^5 cells per recipient) were injected intravenously into irradiated secondary NOG recipients.

4. RNA extraction and microarray analysis

After FACS sorting, cells were pelleted by centrifugation at $300 \times g$ in RNase-free, 1.5-ml microcentrifuge tubes. The pellets were disrupted by vigorous pipetting in 350 µl of Lysis Buffer RLT (Qiagen, Valencia, CA, USA). Total RNA was extracted using the RNeasy Mini-Kit (Qiagen) in accordance with the manufacturer's RNA Purification protocol. The cDNA was synthesized and labeled from 50 ng of total RNA from each sample using a Two-Cycle cDNA Synthesis kit and the IVT Labbering kit (Affymetrix, Santa Clara, CA, USA) to produce targets for hybridization to Affymetrix Human Genome U133 Plus 2.0 Array GeneChip microarrays (Affymetrix) in accordance with the manufacturer's instructions.

Raw signal data were processed and analyzed using the GeneSpring GX software program (version 13.0, Agilent Technologies, Santa Clara, CA, USA). The background subtraction, normalization, and log base 2 transformation of gene signals were carried out using the robust multi-array analysis (RMA) summarization algorithm [23].

The probes demonstrating an expression level of > 80% expression after normalization in each set of the gene expression profiles were selected to detect the differential gene expression. The lists of genes whose expression significantly changed in the two compared groups were analyzed by DAVID bioinformatics resources 6.7 (https://david.ncifcrf.gov) to determine whether or not they included a specific pathway in the KEGG database (http://www.genome.jp/kegg/pathway.html)

RESULTS

1. Study strategy

The strategy of this study is shown in Fig. 1. The human $CD34^+/CD38^-/Lin^{-/low}$ cells from CB were transplanted twice and collected from the xenograft BM at the steady state at each transplantation (18 weeks after transplantation). The $CD34^+/CD38^-/Lin^{-/low}$ cells from the human BM were also separated. The gene expression profiles (GEPs) in these cells were evaluated and compared.

2. Selection of human $CD34^+/CD38^-/Lin^{-/low}$ cells from the xenograft model and primary samples

The frequency of $CD34^+/CD38^-/Lin^{-/low}$ cells in $CD34^+$ cells from the original CB was 23.08% ± 4.49% (n = 3). A total of 10,000 human $CD34^+/CD38^-/Lin^{-/low}$ cells were infused into NOG mice at the first transplantation, and the frequency of $CD34^+/CD38^-/Lin^{-/low}$ cells in the xenograft BM was 1.53% ± 0.31% (n = 3) at 18 weeks after transplantation. In the second transplantation, the number of infused human $CD34^+$ cells was 1×10^5, and their frequency in the xenograft BM was sustained at a comparable level, 1.20% ± 0.84% (n = 3) at 18 weeks after the second transplantation (Fig. 2).

The cells from human BM without myeloid diseases were also sorted using the same strategy. The frequencies of the $CD34^+/CD38^-/Lin^{-/low}$ fraction in human BM $CD34^+$ hematopoietic progenitors from 3 cases were 0.92%, 2.94%, and 15.49%, respectively.

3. Preparation for analyzing the GEPs

The microarray analyses were performed using these human $CD34^+/CD38^-/Lin^{-/low}$ cells, and their GEPs were obtained in the original CB (A), at 18 weeks after the first transplantation in the xenograft model (B), at 18 weeks after the second transplantation in the xenograft model (C), and in the human BM (D). The cell numbers applied for the analysis were 1.0×10^3 per sample from CB and the xenograft models, and 2.7×10^3, 6.9×10^3, and 1.3×10^4, respectively, from the 3 cases.

The probes for analyzing the GEPs were initially filtered by their expression, and the 34,106 probes whose expression was above 80% in all 4 backgrounds were selected for the subsequent comparison of GEPs to identify genes in which the expression was clearly altered by excluding low-expressing genes. The pri-

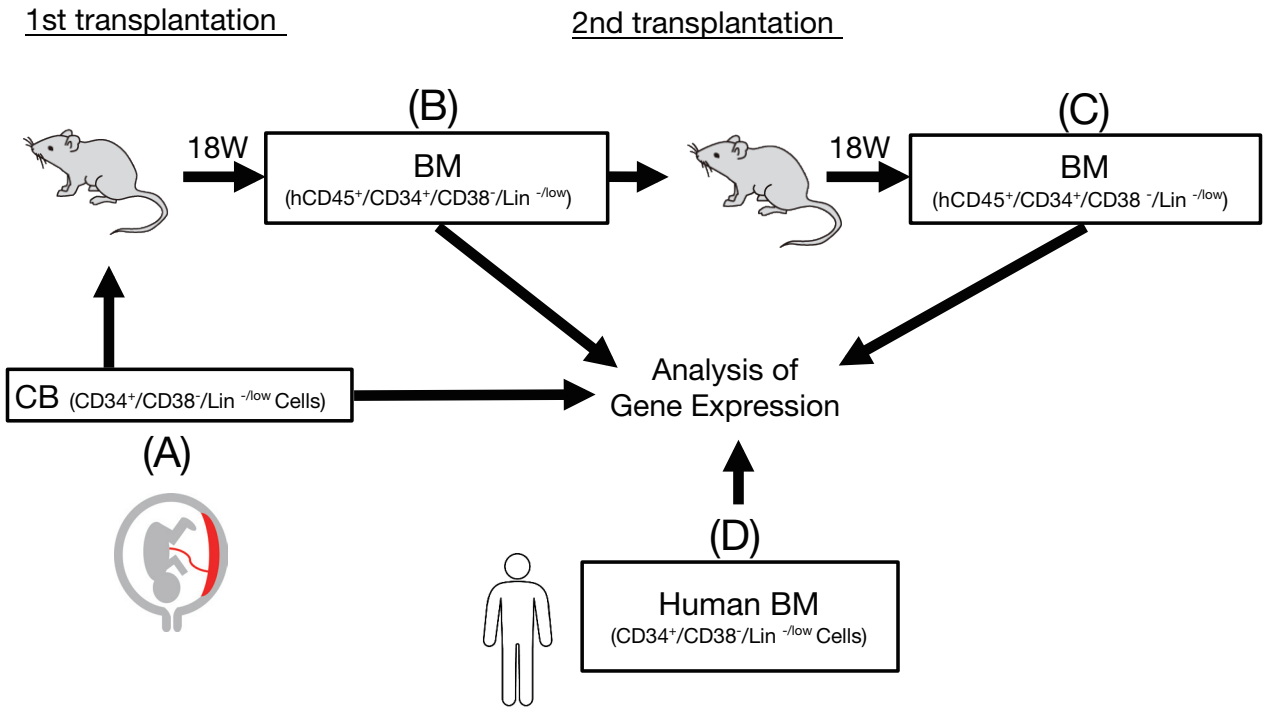


Fig. 1 Experimental design of this study. Cell samples were obtained from cord blood (A), and the bone marrow was obtained from xenogranft models (B and C) and human samples (D). Human CD34$^+$/CD38$^-$/Lin$^-$ cells were fractioned from these sources, and gene expression analyses were performed.

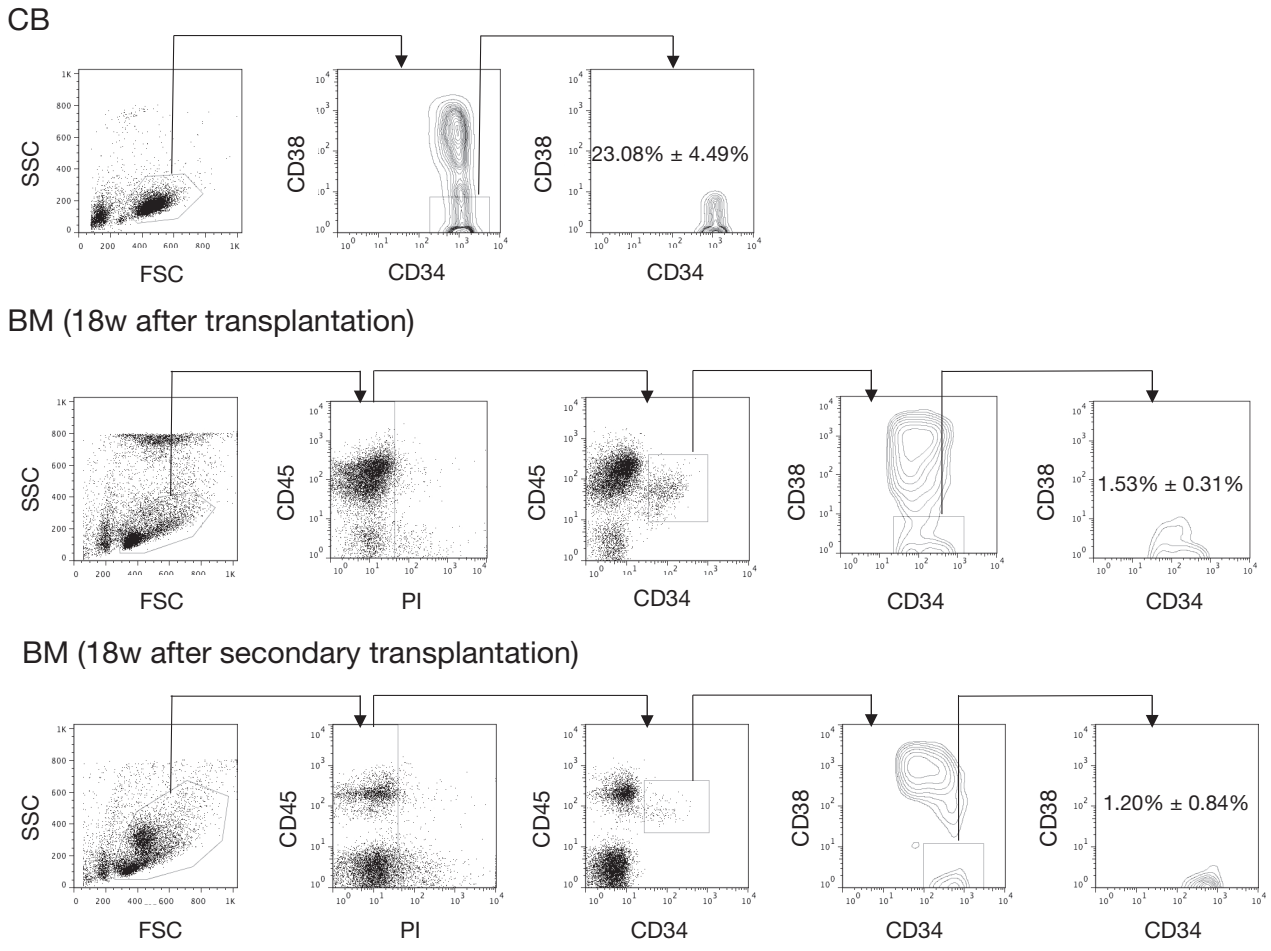


Fig. 2 Collection of human CD34$^+$/CD38$^-$/Lin$^{-/low}$ CB and BM cells from the xenograft model. The target fraction was sorted for microarray analyses using a FACSVantage™ flow cytometer.

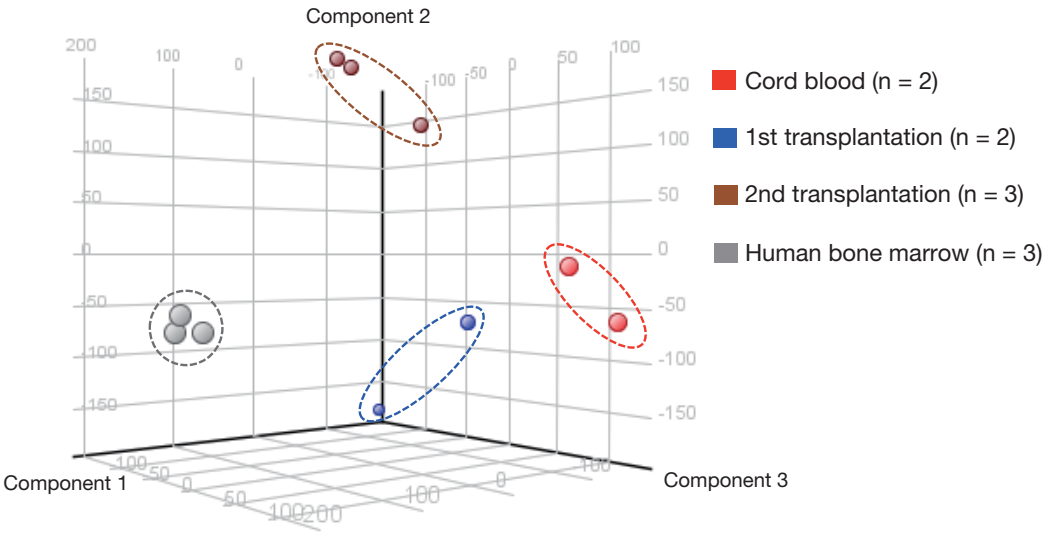


Fig. 3 Primary component analyses of the microarray data. The microarray data from (A) to (D) were separated by their backgrounds.

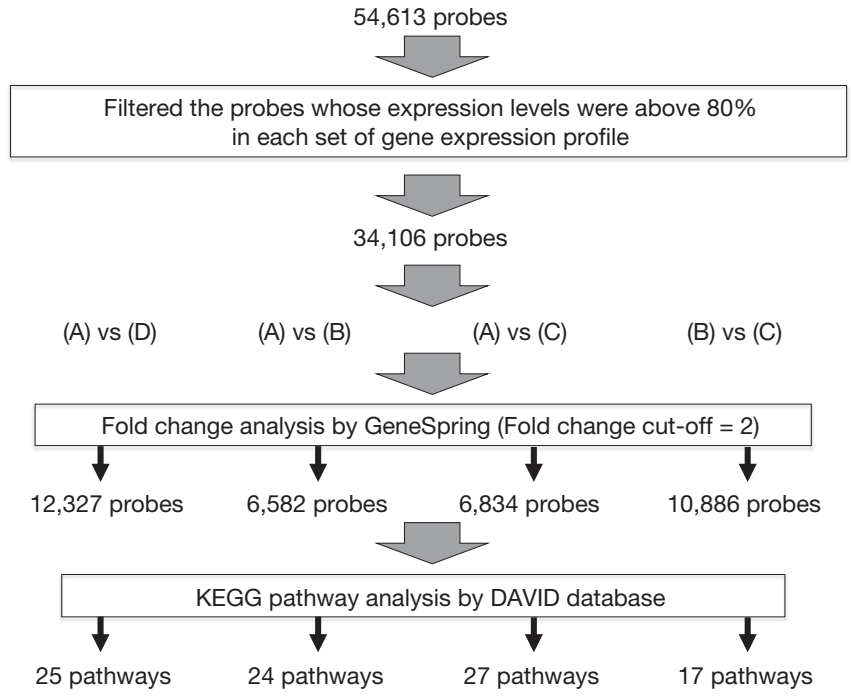


Fig. 4 Identification of KEGG pathways in the comparison between each pair of microarray data from (A) to (D).

mary component analysis (PCA) revealed that the selected probes clustered the GEP according to their backgrounds (Fig. 3).

4. Comparison of GEP

The comparison between the GEP data sets of human HSCs in the current study was performed as follows: (A) vs. (B) and (A) vs. (C) for the evaluation of the alteration of CB-HSCs in xenograft BM; (B) vs. (C) for the development and/or aging of CB-HSCs in xenograft BM; and (A) vs. (D) for their difference in HSCs between CB and BM. The numbers of probes representing the genes that were differently expressed between the compared GEP pairs (more than 2-fold changes) were as follows: 6,582 between (A) vs. (B), 6,834 between (A) vs. (C), 10,886 between (B) vs. (C), and 12,327 between (A) vs. (D). These gene lists were then applied to the KEGG pathway analysis using DAVID bioinformatics resources 6.7. The numbers of identified KEGG pathways identified from (A) vs. (B), (A) vs. (C), (B) vs. (C) and (A) vs. (D) were 24, 27, 17 and 25, respectively (Fig. 4).

The categories of identified KEGG pathways in each gene list are shown Fig. 5A and B. There were three common pathways among these comparisons. In addition, the 14 pathways extracted by the comparison be-

tween CB and human BM, (A) vs. (D), were also found in the comparison between CB and xenograft BM, (A) vs. (B), (A) vs. (C), and (B) vs. (C). In total, 17 of the 25 pathways (68%) extracted from (A) vs. (D) were identified based on comparisons with the xenograft model. In particular, 16 of these 17 pathways were shared with (A) vs. (B) and (A) vs. (D), in contrast to (A) vs. (C) (10 pathways) and (B) vs. (C) (7 pathways) . These 16 pathways accounted for 67% of the pathways extracted from (A) vs. (B).

The 17 pathways included 4 cellular growth-related pathways —"Apoptosis", "Cell cycle", "MAPK signaling pathway", and "p53 signaling pathway"—and 2 RNA metabolism-related pathways —"RNA degradation" and "Spliceosome". There were also 3 tumor-related pathways: "Chronic myeloid leukemia", "Prostate cancer", and "Colorectal cancer". They commonly included the pathways on general cellular biology, such as "Apoptosis", "Cell cycle", "MAPK signaling pathway", and "p53 signaling pathway", which were independently identified as described above, and "PI3K-Akt signaling pathway", which was not identified as a single pathway. These specific tumor-related pathways also include their characteristic pathways: "TGF-β signaling pathway" for "Chronic myeloid leukemia", "Cytokine-cytokine receptor interaction", and "Androgen and estrogen metabolism" for "Prostate cancer", and "Wnt signaling pathway" and "TGF-β signaling pathway" for "Colorectal cancer". Additionally, there were three pathways related to neurodegenerative disorders: "Alzheimer's disease", "Huntington's disease", and "Parkinson's disease". They shared "Apoptosis" and "Oxidative phosphorylation". "Alzheimer's disease" and "Huntington's disease" also share "Calcium signaling pathway", and "Huntington's disease" and "Parkinson's disease" share "Proteasome". Furthermore, "T cell receptor signaling pathway" and "B cell receptor signaling pathway", which include major cellular signaling pathways, were also identified. These pathways, which are related to cellular survival, RNA metabolism, and lymphoid development, might be related to changes in the properties of human $CD34^+/CD38^-/Lin^{-/low}$ cells.

There were eight pathways identified only in the data comparison from xenografts, and all of them were shared with (B) vs. (C), suggesting that these pathways were uniquely related to the development and maintenance of human $CD34^+/CD38^-/Lin^{-/low}$ cells in the xenograft model. They included the pathways related to the electron transport chain, protein degradation, and DNA repair, such as "Oxidative phosphorylation", "Proteasome", "Ubiquitin mediated proteolysis", "Ribosome", "DNA replication", "Nucleotide excision repair" and "Pyrimidine metabolism".

There were also proper pathways in each comparison. The numbers of specific pathways in (A) vs. (B), (A) vs. (C), (B) vs. (C) and (A) vs. (D) were 3, 12, 2, and 8, respectively. The specific pathways extracted from the comparison of CB and human BM, (A) vs. (D), were as follows: "Adherens junction", "Fc gamma R-mediated phagocytosis", "Focal adhesion", "Insulin signaling pathway", "Lysosome", "Phosphatidylinositol signaling system", "Regulation of actin cytoskeleton", and "SNARE interactions in vesicular transport". Except for "Insulin signaling pathway", including "Phosphatidylinositol signaling system" and "MAPK signaling pathway", these are single pathways that are related to fundamental cellular functions, such as adhesion, cytoskeleton, and lysosomal function.

5. Genes whose expression was commonly altered in BM $CD34^+/CD38^-/Lin^{-/low}$ cells from both human and xenograft models

The expression of 10 and 321 probes was commonly up- or downregulated over 5-fold in comparison to CB

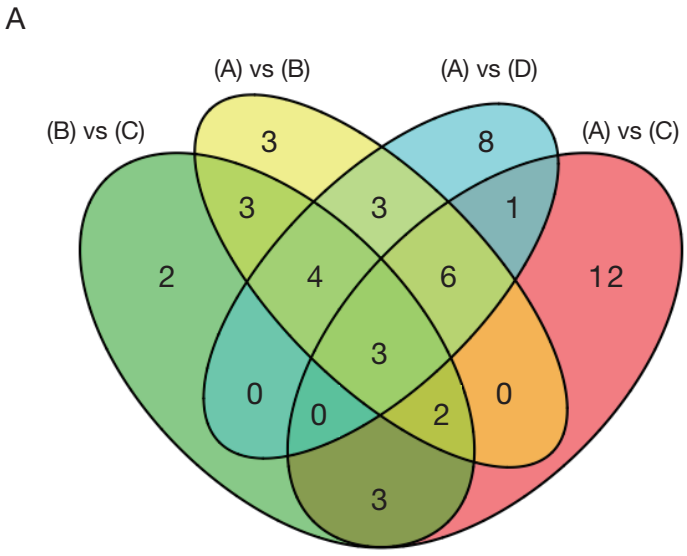
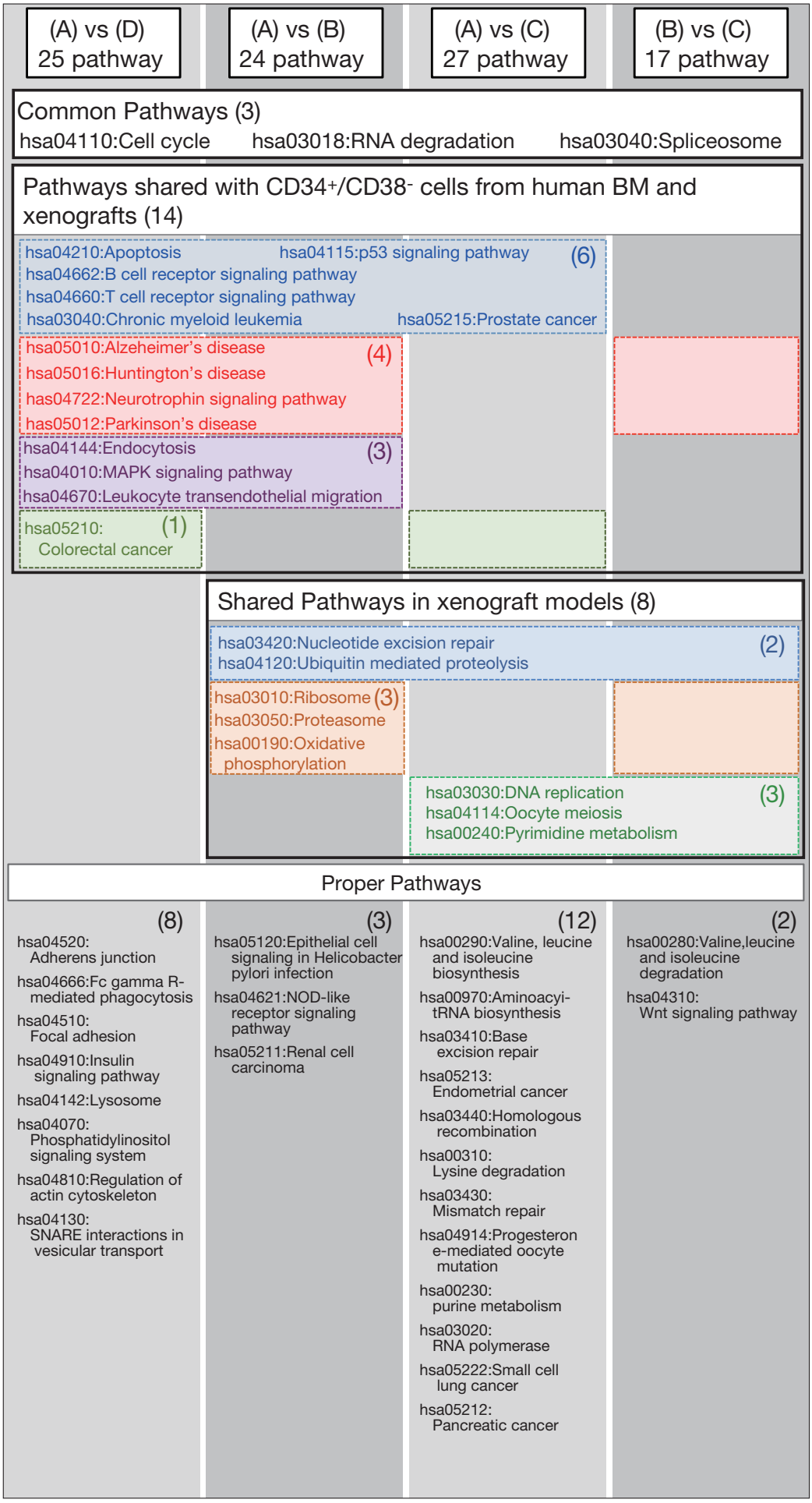


Fig. 5 The common KEGG pathways identified by the comparison between each pair of microarray data from (A) to (D). A Venn diagram (A) and a list of the common pathways (B) are shown.

B


(A) vs (D)
25 pathway
(A) vs (B)
24 pathway
(A) vs (C)
27 pathway
(B) vs (C)
17 pathway
Common Pathways (3)
hsa04110:Cell cycle
hsa03018:RNA degradation
hsa03040:Spliceosome
Pathways shared with CD34+/CD38- cells from human BM and xenografts (14)
hsa04210:Apoptosis
hsa04115:p53 signaling pathway
(6)
hsa04662:B cell receptor signaling pathway
hsa04660:T cell receptor signaling pathway
hsa03040:Chronic myeloid leukemia
hsa05215:Prostate cancer
hsa05010:Alzeheimer's disease
(4)
hsa05016:Huntington's disease
has04722:Neurotrophin signaling pathway
has05012:Parkinson's disease
hsa04144:Endocytosis
(3)
hsa04010:MAPK signaling pathway
hsa04670:Leukocyte transendothelial migration
hsa05210:
Colorectal cancer
(1)
Shared Pathways in xenograft models (8)
hsa03420:Nucleotide excision repair
hsa04120:Ubiquitin mediated proteolysis
(2)
hsa03010:Ribosome
(3)
hsa03050:Proteasome
hsa00190:Oxidative phosphorylation
hsa03030:DNA replication
hsa04114:Oocyte meiosis
hsa00240:Pyrimidine metabolism
(3)
Proper Pathways
(8)
hsa04520:
Adherens junction
hsa04666:Fc gamma R-mediated phagocytosis
hsa04510:
Focal adhesion
hsa04910:Insulin signaling pathway
hsa04142:Lysosome
hsa04070:
Phosphatidylinositol signaling system
hsa04810:Regulation of actin cytoskeleton
hsa04130:
SNARE interactions in vesicular transport
(3)
hsa05120:Epithelial cell signaling in Helicobacter pylori infection
hsa04621:NOD-like receptor signaling pathway
hsa05211:Renal cell carcinoma
(12)
hsa00290:Valine, leucine and isoleucine biosynthesis
hsa00970:Aminoacyi-tRNA biosynthesis
hsa03410:Base excision repair
hsa05213:
Endometrial cancer
hsa03440:Homologous recombination
hsa00310:
Lysine degradation
hsa03430:
Mismatch repair
hsa04914:Progesteron e-mediated oocyte mutation
hsa00230:
purine metabolism
hsa03020:
RNA polymerase
hsa05222:Small cell lung cancer
hsa05212:
Pancreatic cancer
(2)
hsa00280:Valine,leucine and isoleucine degradation
hsa04310:
Wnt signaling pathway

$CD34^+/CD38^-/Lin^{-/low}$ cells (A) in BM $CD34^+/CD38^-/Lin^{-/low}$ cells from human (D) and xenograft models (B), respectively (Fig. 6). We were able to clearly classify (B) and (D) from (A). Most of these probes were downregulated (97% in total 331 probes). The 10 upregulated probes included *MYC* and *MUM1*, transcription factors for differentiation of B and T cells.

The expression of 57 probes was commonly altered (all down-regulated) over 10-fold in (B) and (D) in comparison to (A) (Fig. 7). They included the hypoxia-related gene *HIF1A*; the nucleoporin family gene *NUP98,* which has been found to be rearranged in leukemic cells; the genes related to hematopoietic differentiation such as *BCL6* and *CSF2RB*; the genes related to cellular proliferation such as *AZIN1*, *BTG1*, *BTG3*, *RIT1,* and *RPRD1A*; the epigenetic modifiers such as *KMT2A* (also known as *MLL*), *JMJD1C,* and *CHD1*; the ubiquitination-related genes such as *PELI1*, *RANBP2*, *RNF111,* and *UBA6*; the genes related to translation initiation and RNA degradation such as *DCP1A* and *MEX3C*; and the genes related to the ER and Golgi function such as *CLN8*, *HSPA5, SEC24A,* and *WHAMM*.

The genes extracted from the comparison revealed the differences in the characteristics of human $CD34^+/CD38^-/Lin^{-/low}$ cells between BM and CB at the molecular level and may reflect the differences in their biological properties as well, including cellular proliferation and differentiation.

DISCUSSION

In the current study using NOG mice, we extracted 25 KEGG pathways by the comparison of human $CD34^+/CD38^-/Lin^{-/low}$ cells between CB and BM, and 17 of them were shared with the xenograft model. These extracted pathways included pathways related to cellular survival, RNA metabolism, and lymphoid development. In addition, most of the altered gene expressions that were shared with $CD34^+/CD38^-/Lin^{-/low}$ cells both in human and xenograft BM were downregulated in comparison to CB, including genes related to hypoxia, hematopoietic differentiation, epigenetic modification, translation initiation and RNA degradation. We suspect that these genes had key alterations in the gene expression for settlement of CB $CD34^+/CD38^-/Lin^{-/low}$ cells in BM.

A number of studies have proven the feasibility of the xenograft model using NOG mice for the evaluation of normal and malignant human hematopoiesis [15, 17–21, 24–34]. The advantages of the system include the availability of the phenotypes within a relatively short time period, because of the defective immune systems and forced proliferation by transplantation. However, the resultant phenotypes as well as gene expression may be enhanced or modified simultaneously in the environment of xenograft; although human HSCs exhibit myeloid differentiation, they dominantly develop into B-cells. Our data may have reflected the reproducibility and inconsistency seen in the xenograft model.

Sixteen out of 17 KEGG pathways shared with $CD34^+/CD38^-/Lin^{-/low}$ cells in the human and xenograft models were identified in the first transplantation model. In contrast, only one KEGG pathway was shared with (A) vs. (D) and (A) vs. (C) suggesting that a single transplantation was sufficient to induce changes due to aging, as seen in human BM, because the BM samples were obtained from elderly patients. Repeated transplantation caused additional changes in the gene expressions, resulting in the identification of 8 KEGG pathways from the (B) vs. (C) comparison that were related to fundamental cellular functions, such as cellular metabolism, but these KEGG pathways were not identified in (A) vs. (D). In addition, the 8 unique KEGG pathways in (A) vs. (D) included those related to cell adhesion. It is therefore possible that these alterations in gene expression were exaggerated in the adaptation and survival of human $CD34^+/CD38^-/Lin^{-/low}$ cells to the xenograft environment.

All of the gene expressions in $CD34^+/CD38^-/Lin^{-/low}$ cells both from human and xenograft BM commonly altered over 10-fold compared with that from CB were downregulated. These genes included *HIF1A*, a master transcriptional regulator activated under hypoxic conditions. Previous studies have shown that the oxygen concentration is especially low in CB (20–30 mmHg in umbilical vein, 10–15 mmHg in umbilical artery) [35, 36], although some studies have shown the oxygen concentration to be about 20 mmHg [37]. The downregulation of *HIF1A* might therefore validate our array data. The observed upregulation of *MYC1*, which was found to be over 5-fold in the analysis of the commonly altered expression, also support our data, as MYC expression is inhibited by hypoxia [38]. Interestingly, the global repression of genes was observed in the non-neoplastic $CD34^+/CD38^-/Lin^{-/low}$ cells in spite of the occurrence of *MYC* upregulation, which has been reported to induce either global or specific gene expressions in cancerous cells [39].

There were also several genes related to hematopoiesis and leukemogenesis among the downregulated genes. *KMT2A*, also known as *MLL1*, encodes a transcriptional coactivator with histone H3 lysine 4 (H3K4) methyltransferase activity, which plays an essential role in normal hematopoiesis [40, 41]. *MLL1* is often involved in chromosomal translocation in leukemic cells to generate *MLL1* chimeric genes. *JMJD1C*, a putative histone demethylase that acts as a coactivator but lacks histone demethylase activity, has been reported to maintain leukemic stem cells with *MLL1-AF9* and also exerts similar effects on normal hematopoietic stem cells, albeit to a lesser extent [42, 43]. *CSF2RB* encodes the common β-chain of human cytokine receptors such as GM-CSF, IL-3 and IL-5 [44]. *BCL6*, which is frequently translocated and hypermutated in diffuse large B-cell lymphoma, is a master transcription factor that promotes the development of normal B-cells and follicular helper T-cells [45, 46]. The expression of genes associated with the initiation of transcription, RNA degradation, and the functions of the endoplasmic reticulum and Golgi apparatus, which are required for the generation of proteins for cellular functioning, were also suspected to be repressed. The downregulation of these genes may be related to repression of stem cell function as well as hematopoietic differentiation.

However, the interpretation of the decreased expression of genes related to cellular proliferation and survival is controversial; AZIN1 has been reported to

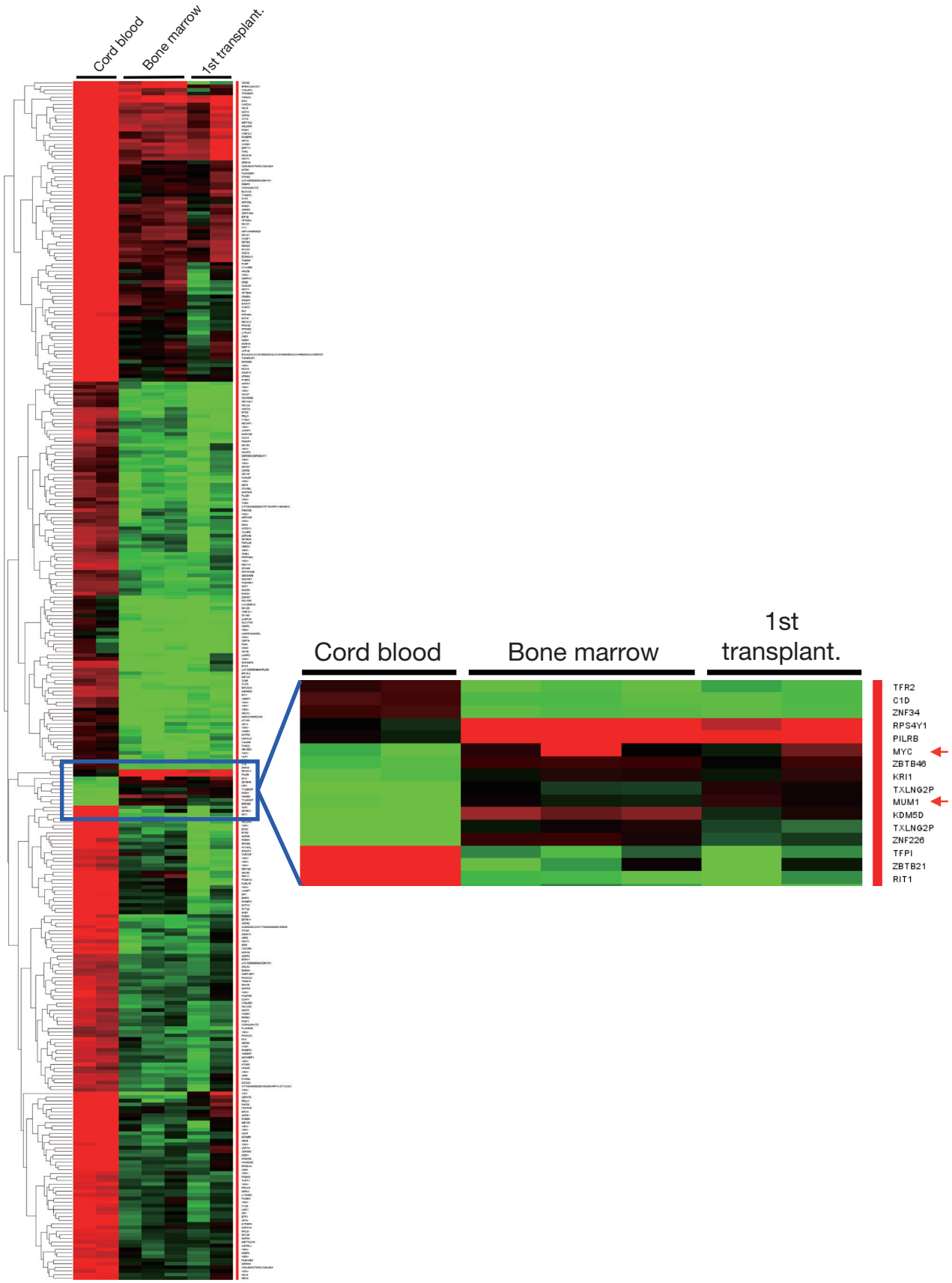


Fig. 6 The expression profile of 331 genes whose expression was commonly altered in $CD34^{+}/CD38^{-}/Lin^{-/low}$ cells from human and xenograft bone marrow in comparison to those from cord blood (more than 5-fold).

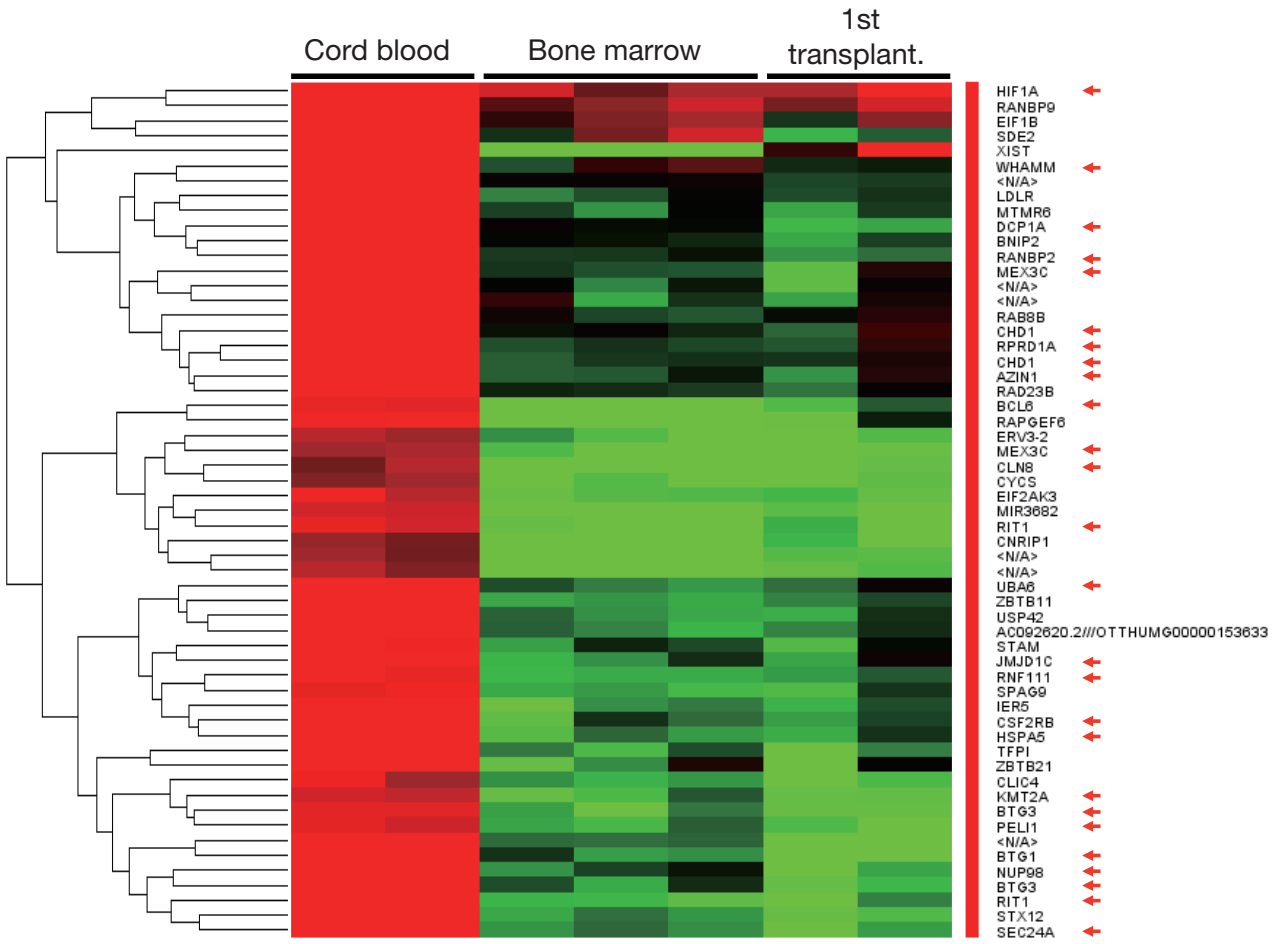


Fig. 7 The expression profile of 57 genes whose expression was commonly altered in CD34^{+}/CD38^{-}/Lin$^{-/low}$ cells from human and xenograft bone marrow in comparison to those from cord blood (more than 10-fold).

be associated with increased proliferation [47]. RIT1, a member of the RAS subfamily of small GTPases, has been found to be important for cellular survival in response to oxidative stress. Mutations in *RIT1* have been reported in myeloid malignancies and lung adenocarcinoma [48, 49]. In contrast, BTG1 and BTG3 are members of the BTG/Tob family, which have anti-proliferative properties [50, 51]. RPRD1A has been reported to be a negative regulator of G1/S phase progression by interacting with INK4B as well as a transcriptional repressor by inhibiting the Wnt/β-catenin signal-mediated gene transcription [52, 53]. *MYC* upregulation was detected, but the MYC function is precisely regulated by ARF and TP53 in non-cancerous cells [54], in sharp contrast to the uncontrolled proliferation in cancerous cells [39].

In summary, our findings demonstrated that human CB CD34^{+}/CD38^{-}/Lin$^{-/low}$ cells adapted to the BM microenvironment in human and xenograft models similarly, with a shared reduced gene expression, and that the xenograft model of human CD34^{+}/CD38^{-}/Lin$^{-/low}$ cells using NOG mice was useful, at least in part, for the evaluation of the gene expression in human hematopoietic stem cells.

ACKNOWLEDGEMENT

This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports and Technology of Japan (KAKENHI Grant Numbers 20790238, 20390276 and 22220007).

REFERENCES

1) Calloni R, Cordero EA, Henriques JA, Bonatto D. Reviewing and updating the major molecular markers for stem cells. Stem Cells Dev. 2013; 22(9): 1455–76. doi: 10.1089/scd.2012.0637. PubMed PMID: 23336433; PubMed Central PMCID: PMCPMC3629778.
2) Medvinsky A, Rybtsov S, Taoudi S. Embryonic origin of the adult hematopoietic system: advances and questions. Development. 2011; 138(6): 1017–31. doi: 10.1242/dev.040998. PubMed PMID: 21343360.
3) O'Donoghue K, Fisk NM. Fetal stem cells. Best Pract Res Clin Obstet Gynaecol. 2004; 18(6): 853–75. doi: 10.1016/j.bpobgyn.2004.06.010. PubMed PMID: 15582543.
4) Bonnet D. Hematopoietic stem cells. Birth Defects Res C Embryo Today. 2003; 69(3): 219–29. doi: 10.1002/bdrc.10018. PubMed PMID: 14671775.
5) Bonnet D. Haematopoietic stem cells. J Pathol. 2002; 197(4): 430–40. doi: 10.1002/path.1153. PubMed PMID: 12115860.
6) Baum CM, Weissman IL, Tsukamoto AS, Buckle AM, Peault B. Isolation of a candidate human hematopoietic stem-cell population. Proceedings of the National Academy of Sciences of the United States of America. 1992; 89(7): 2804–8. Epub 1992/04/01. PubMed PMID: 1372992; PubMed Central PMCID: PMCPMC48751.
7) Hill B, Rozler E, Travis M, Chen S, Zannetino A, Simmons P, *et al.* High-level expression of a novel epitope of CD59 identifies a subset of CD34+ bone marrow cells highly enriched for pluripotent stem cells. Exp Hematol. 1996; 24(8): 936–43. Epub 1996/07/01. PubMed PMID: 8690053.
8) Yin AH, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M,

Leary AG, *et al*. AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood. 1997; 90(12): 5002-12. Epub 1998/01/07. PubMed PMID: 9389720.

9) Watt SM, Buhring HJ, Rappold I, Chan JY, Lee-Prudhoe J, Jones T, *et al*. CD164, a novel sialomucin on CD34(+) and erythroid subsets, is located on human chromosome 6q21. Blood. 1998; 92(3): 849-66. Epub 1998/07/29. PubMed PMID: 9680353.
10) LeBien TW. Fates of human B-cell precursors. Blood. 2000; 96(1): 9-23. Epub 2000/07/13. PubMed PMID: 10891425.
11) Scharenberg CW, Harkey MA, Torok-Storb B. The ABCG2 transporter is an efficient Hoechst 33342 efflux pump and is preferentially expressed by immature human hematopoietic progenitors. Blood. 2002; 99(2): 507-12. Epub 2002/01/10. PubMed PMID: 11781231.
12) Kent D, Copley M, Benz C, Dykstra B, Bowie M, Eaves C. Regulation of hematopoietic stem cells by the steel factor/KIT signaling pathway. Clinical cancer research : an official journal of the American Association for Cancer Research. 2008; 14(7): 1926-30. Epub 2008/04/03. doi: 10.1158/1078-0432.ccr-07-5134. PubMed PMID: 18381929.
13) Schepers K, Campbell TB, Passegue E. Normal and leukemic stem cell niches: insights and therapeutic opportunities. Cell Stem Cell. 2015; 16(3): 254-67. doi: 10.1016/j.stem.2015.02.014. PubMed PMID: 25748932; PubMed Central PMCID: PMCPMC4391962.
14) Mendelson A, Frenette PS. Hematopoietic stem cell niche maintenance during homeostasis and regeneration. Nat Med. 2014;20(8):833-46. doi: 10.1038/nm.3647. PubMed PMID: 25100529; PubMed Central PMCID: PMCPMC4459580.
15) Ito M, Kobayashi K, Nakahata T. NOD/Shi-scid IL2rgamma(null) (NOG) mice more appropriate for humanized mouse models. Curr Top Microbiol Immunol. 2008; 324: 53-76. PubMed PMID: 18481452.
16) Ito M, Hiramatsu H, Kobayashi K, Suzue K, Kawahata M, Hioki K, *et al*. NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. Blood. 2002; 100(9): 3175-82. doi: 10.1182/blood-2001-12-0207. PubMed PMID: 12384415.
17) Wang C, Oshima M, Sashida G, Tomioka T, Hasegawa N, Mochizuki-Kashio M, *et al*. Non-Lethal Ionizing Radiation Promotes Aging-Like Phenotypic Changes of Human Hematopoietic Stem and Progenitor Cells in Humanized Mice. PLoS One. 2015; 10(7): e0132041. doi: 10.1371/journal.pone.0132041. PubMed PMID: 26161905; PubMed Central PMCID: PMCPMC4498777.
18) Yahata T, Takanashi T, Muguruma Y, Ibrahim AA, Matsuzawa H, Uno T, *et al*. Accumulation of oxidative DNA damage restricts the self-renewal capacity of human hematopoietic stem cells. Blood. 2011; 118(11): 2941-50. doi: 10.1182/blood-2011-01-330050. PubMed PMID: 21734240.
19) Yahata T, Muguruma Y, Yumino S, Sheng Y, Uno T, Matsuzawa H, *et al*. Quiescent human hematopoietic stem cells in the bone marrow niches organize the hierarchical structure of hematopoiesis. Stem Cells. 2008; 26(12): 3228-36. doi: 10.1634/stemcells.2008-0552. PubMed PMID: 18787204.
20) Yahata T, Yumino S, Seng Y, Miyatake H, Uno T, Muguruma Y, *et al*. Clonal analysis of thymus-repopulating cells presents direct evidence for self-renewal division of human hematopoietic stem cells. Blood. 2006; 108(7): 2446-54. doi: 10.1182/blood-2006-02-002204. PubMed PMID: 16757689.
21) Nakamura T, Miyakawa Y, Miyamura A, Yamane A, Suzuki H, Ito M, *et al*. A novel nonpeptidyl human c-Mpl activator stimulates human megakaryopoiesis and thrombopoiesis. Blood. 2006; 107(11): 4300-7. doi: 10.1182/blood-2005-11-4433. PubMed PMID: 16484588.
22) Yahata T, Ando K, Miyatake H, Uno T, Sato T, Ito M, *et al*. Competitive repopulation assay of two gene-marked cord blood units in NOD/SCID/gammac(null) mice. Mol Ther. 2004; 10(5): 882-91. doi: 10.1016/j.ymthe.2004.07.029. PubMed PMID: 15509506.
23) Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP. Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res. 2003; 31(4): e15. PubMed PMID: 12582260; PubMed Central PMCID: PMCPMC150247.
24) Wang TY, Chang SJ, Chang MD, Wang HW. Unique biological properties and application potentials of CD34+ CD38- stem cells from various sources. Taiwan J Obstet Gynecol. 2009; 48(4): 356-69. doi: 10.1016/S1028-4559(09)60324-7. PubMed PMID: 20045756.
25) Feraud O, Valogne Y, Melkus MW, Zhang Y, Oudrhiri N, Haddad R, *et al*. Donor Dependent Variations in Hematopoietic Differentiation among Embryonic and Induced Pluripotent Stem Cell Lines. PLoS One. 2016;11(3): e0149291. doi: 10.1371/journal.pone.0149291. PubMed PMID: 26938212; PubMed Central PMCID: PMCPMC4777368.
26) Haji Y, Suzuki M, Moriya K, So T, Hozumi K, Mizuma M, *et al*. Activation of Notch1 promotes development of human CD8(+) single positive T cells in humanized mice. Biochem Biophys Res Commun. 2014; 447(2): 346-51. doi: 10.1016/j.bbrc.2014.04.003. PubMed PMID: 24726647.
27) Levescot A, Flamant S, Basbous S, Jacomet F, Feraud O, Anne Bourgeois E, *et al*. BCR-ABL-induced deregulation of the IL-33/ST2 pathway in CD34+ progenitors from chronic myeloid leukemia patients. Cancer Res. 2014; 74(10): 2669-76. doi: 10.1158/0008-5472.CAN-13-2797. PubMed PMID: 24675360.
28) Matsushita H, Yahata T, Sheng Y, Nakamura Y, Muguruma Y, Matsuzawa H, *et al*. Establishment of a humanized APL model via the transplantation of PML-RARA-transduced human common myeloid progenitors into immunodeficient mice. PLoS One. 2014; 9(11): e111082. doi: 10.1371/journal.pone.0111082. PubMed PMID: 25369030; PubMed Central PMCID: PMCPMC4219701.
29) Negishi N, Suzuki D, Ito R, Irie N, Matsuo K, Yahata T, *et al*. Effective expansion of engrafted human hematopoietic stem cells in bone marrow of mice expressing human Jagged1. Exp Hematol. 2014; 42(6):487-94 e1. doi: 10.1016/j.exphem.2014.02.001. PubMed PMID: 24530466.
30) Moriya K, Suzuki M, Watanabe Y, Takahashi T, Aoki Y, Uchiyama T, *et al*. Development of a multi-step leukemogenesis model of MLL-rearranged leukemia using humanized mice. PLoS One. 2012; 7(6): e37892. doi: 10.1371/journal.pone.0037892. PubMed PMID: 22745659; PubMed Central PMCID: PMCPMC3380045.
31) Ito R, Negishi N, Irie N, Matsuo K, Suzuki D, Katano I, *et al*. Osteosclerosis and inhibition of human hematopoiesis in NOG mice expressing human Delta-like 1 in osteoblasts. Exp Hematol. 2012; 40(11): 953-63 e3. doi: 10.1016/j.exphem.2012.07.002. PubMed PMID: 22771497.
32) Bruns I, Cadeddu RP, Brueckmann I, Frobel J, Geyh S, Bust S, *et al*. Multiple myeloma-related deregulation of bone marrow-derived CD34(+) hematopoietic stem and progenitor cells. Blood. 2012; 120(13): 2620-30. doi: 10.1182/blood-2011-04-347484. PubMed PMID: 22517906; PubMed Central PMCID: PMCPMC3460684.
33) Muguruma Y, Matsushita H, Yahata T, Yumino S, Tanaka Y, Miyachi H, *et al*. Establishment of a xenograft model of human myelodysplastic syndromes. Haematologica. 2011; 96(4): 543-51. doi: 10.3324/haematol.2010.027557. PubMed PMID: 21193418; PubMed Central PMCID: PMCPMC3069231.
34) Yamane A, Nakamura T, Suzuki H, Ito M, Ohnishi Y, Ikeda Y, *et al*. Interferon-alpha 2b-induced thrombocytopenia is caused by inhibition of platelet production but not proliferation and endomitosis in human megakaryocytes. Blood. 2008;112(3): 542-50. doi: 10.1182/blood-2007-12-125906. PubMed PMID: 18523149.
35) Carreau A, El Hafny-Rahbi B, Matejuk A, Grillon C, Kieda C. Why is the partial oxygen pressure of human tissues a crucial parameter? Small molecules and hypoxia. J Cell Mol Med. 2011; 15(6): 1239-53. doi: 10.1111/j.1582-4934.2011.01258.x. PubMed PMID: 21251211; PubMed Central PMCID: PMCPMC4373326.
36) Arikan GM, Scholz HS, Petru E, Haeusler MC, Haas J, Weiss PA. Cord blood oxygen saturation in vigorous infants at birth: what is normal? BJOG. 2000; 107(8): 987-94. PubMed PMID: 10955430.
37) Spencer JA, Ferraro F, Roussakis E, Klein A, Wu J, Runnels JM, *et al*. Direct measurement of local oxygen concentration in the bone marrow of live animals. Nature. 2014; 508(7495):

269–73. doi: 10.1038/nature13034. PubMed PMID: 24590072; PubMed Central PMCID: PMCPMC3984353.
38) Stine ZE, Walton ZE, Altman BJ, Hsieh AL, Dang CV. MYC, Metabolism, and Cancer. Cancer Discov. 2015; 5(10): 1024–39. doi: 10.1158/2159-8290.CD-15-0507. PubMed PMID: 26382145; PubMed Central PMCID: PMCPMC4592441.
39) Dang CV. Gene regulation: fine-tuned amplification in cells. Nature. 2014; 511(7510): 417–8. doi: 10.1038/nature13518. PubMed PMID: 25043013.
40) Haladyna JN, Yamauchi T, Neff T, Bernt KM. Epigenetic modifiers in normal and malignant hematopoiesis. Epigenomics. 2015; 7(2): 301–20. doi: 10.2217/epi.14.88. PubMed PMID: 25942537.
41) Yip BH, So CW. Mixed lineage leukemia protein in normal and leukemic stem cells. Exp Biol Med (Maywood). 2013; 238(3): 315–23. doi: 10.1177/1535370213480717. PubMed PMID: 23598978.
42) Zhu N, Chen M, Eng R, DeJong J, Sinha AU, Rahnamay NF, *et al*. MLL-AF9- and HOXA9-mediated acute myeloid leukemia stem cell self-renewal requires JMJD1C. J Clin Invest. 2016; 126(3): 997–1011. doi: 10.1172/JCI82978. PubMed PMID: 26878175; PubMed Central PMCID: PMCPMC4767347.
43) Sroczynska P, Cruickshank VA, Bukowski JP, Miyagi S, Bagger FO, Walfridsson J, *et al*. shRNA screening identifies JMJD1C as being required for leukemia maintenance. Blood. 2014; 123(12): 1870–82. doi: 10.1182/blood-2013-08-522094. PubMed PMID: 24501218.
44) Broughton SE, Nero TL, Dhagat U, Kan WL, Hercus TR, Tvorogov D, *et al*. The betac receptor family - Structural insights and their functional implications. Cytokine. 2015; 74(2): 247–58. doi: 10.1016/j.cyto.2015.02.005. PubMed PMID: 25982846.
45) Bunting KL, Melnick AM. New effector functions and regulatory mechanisms of BCL6 in normal and malignant lymphocytes. Curr Opin Immunol. 2013; 25(3): 339–46. doi: 10.1016/j.coi.2013.05.003. PubMed PMID: 23725655; PubMed Central PMCID: PMCPMC4075446.
46) Choi YS, Yang JA, Crotty S. Dynamic regulation of Bcl6 in follicular helper CD4 T (Tfh) cells. Curr Opin Immunol. 2013; 25(3): 366–72. doi: 10.1016/j.coi.2013.04.003. PubMed PMID: 23688737; PubMed Central PMCID: PMCPMC3763739.
47) Silva TM, Cirenajwis H, Wallace HM, Oredsson S, Persson L. A role for antizyme inhibitor in cell proliferation. Amino Acids. 2015; 47(7): 1341–52. doi: 10.1007/s00726-015-1957-6. PubMed PMID: 25813938; PubMed Central PMCID: PMCPMC4458265.
48) Berger AH, Imielinski M, Duke F, Wala J, Kaplan N, Shi GX, *et al*. Oncogenic RIT1 mutations in lung adenocarcinoma. Oncogene. 2014; 33(35): 4418–23. doi: 10.1038/onc.2013.581. PubMed PMID: 24469055; PubMed Central PMCID: PMCPMC4150988.
49) Gomez-Segui I, Makishima H, Jerez A, Yoshida K, Przychodzen B, Miyano S, *et al*. Novel recurrent mutations in the RAS-like GTP-binding gene RIT1 in myeloid malignancies. Leukemia. 2013; 27(9): 1943–6. doi: 10.1038/leu.2013.179. PubMed PMID: 23765226.
50) Winkler GS. The mammalian anti-proliferative BTG/Tob protein family. J Cell Physiol. 2010; 222(1): 66–72. doi: 10.1002/jcp.21919. PubMed PMID: 19746446.
51) Mauxion F, Chen CY, Seraphin B, Shyu AB. BTG/TOB factors impact deadenylases. Trends Biochem Sci. 2009; 34(12): 640–7. doi: 10.1016/j.tibs.2009.07.008. PubMed PMID: 19828319; PubMed Central PMCID: PMCPMC2787745.
52) Liu C, Zhang Y, Li J, Wang Y, Ren F, Zhou Y, *et al*. p15RS/RPRD1A (p15INK4b-related sequence/regulation of nuclear pre-mRNA domain-containing protein 1A) interacts with HDAC2 in inhibition of the Wnt/beta-catenin signaling pathway. J Biol Chem. 2015; 290(15): 9701–13. doi: 10.1074/jbc.M114.620872. PubMed PMID: 25697359; PubMed Central PMCID: PMCPMC4392270.
53) Liu J, Liu H, Zhang X, Gao P, Wang J, Hu Z. Identification and characterization of P15RS, a novel P15(INK4b) related gene on G1/S progression. Biochem Biophys Res Commun. 2002; 299(5): 880–5. PubMed PMID: 12470661.
54) Gustafson WC, Weiss WA. Myc proteins as therapeutic targets. Oncogene. 2010; 29(9): 1249–59. doi: 10.1038/onc.2009.512. PubMed PMID: 20101214; PubMed Central PMCID: PMCPMC2904682.