SHORT REPORT

Genetic and epigenetic alterations of bone marrow stromal cells in myelodysplastic syndrome and acute myeloid leukemia patients

Yonggoo Kim\textsuperscript{a,b}, Dong Wook Jekar\textsuperscript{a,b}, Jiyeon Kim\textsuperscript{b}, AhIm Kwon\textsuperscript{b}, Hayoung Choi\textsuperscript{b}, Seungok Lee\textsuperscript{a,b}, Yoo-Jin Kim\textsuperscript{c}, Hee-Je Kim\textsuperscript{c}, Yonghwan Kim\textsuperscript{d}, Il-Hoan Oh\textsuperscript{e}, Myungshin Kim\textsuperscript{a,b,*}

\textsuperscript{a} Department of Laboratory Medicine, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
\textsuperscript{b} Catholic Genetic Laboratory Center, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
\textsuperscript{c} Department of Internal Medicine, Catholic Blood and Marrow Transplantation Center, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
\textsuperscript{d} Department of Life Systems, Sookmyung Women's University, Seoul, Republic of Korea
\textsuperscript{e} Catholic High-Performance Cell Therapy Center, Research Center for Stem Cell Therapeutics Evaluation, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

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Abstract We evaluated the characteristics of bone marrow stromal cells (BMSCs) and hematopoietic cells (HCS) from patients of myelodysplastic syndrome (MDS, n = 21) and acute myeloid leukemia (AML, n = 58), and compared the results with control BMSCs derived from healthy donors (n = 8). The patient BMSCs had lower proliferative activity than that of the controls due to increased senescence. This retarded proliferation induced failure to obtain enough metaphase cells for karyotyping in patient BMSCs (10%). Patient BMSCs were genetically altered which was demonstrated by chromosome abnormalities in 5% of the patients (one MDS and three AML), whereas no clonal abnormalities were detected in the controls. The most common abnormality of the BMSCs was an extra chromosome 5, followed by an extra chromosome 7 and balanced translocations. The proportion of the abnormal metaphase cells was low (17.8%). We also analyzed the epigenetic changes of long interspersed nucleotide element 1 (LINE-1) repetitive element and \textit{CDKN2B} using pyrosequencing. The quantitative measurement of global LINE-1 methylation demonstrated that patient BMSCs revealed global hypomethylation (68.2 ± 3.8) compared with controls (72.9 ± 3.4, \(P < 0.001\)) and that the global hypomethylation of BMSCs were more significant in AML than in MDS patients (67.9 ± 3.8, 69.4 ± 4.2, respectively). These findings seem worthy of further evaluation of their association with ineffective hematopoiesis and leukemogenesis.

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* Corresponding author at: Department of Laboratory Medicine, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 137-701, Republic of Korea. Fax: +82 2 2258 1719.
E-mail address: microkim@catholic.ac.kr (M. Kim).

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Introduction

Bone marrow (BM) is the site of hematopoiesis and is composed of hematopoietic cells (HCs) and their niche, which includes BM stromal cells (BMSCs), osteoblastic cells, osteoclasts, skeletal stem cells, endothelial cells, endosteal monocytes/macrophages, and sympathetic nervous system neurons (Sacchetti et al., 2007; Smith and Calvi, 2013). The hematopoietic niche is the physical locale of the microenvironment that regulates self-renewal, proliferation and differentiation of HCs and protects HCs from oncogenic, physical and chemical damage (Oh and Humphries, 2012). BMSCs have been reported to control hematopoiesis through the production of cytokines that are active in effective hematopoiesis and to support T cell and B cell survival by preventing apoptosis (English, 2013; Majumdar et al., 2000).

Myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) are HC neoplasias, and their leukemogenic process is initiated by the accumulation of inherited and randomly acquired genetic aberrations. Cytogetic abnormalities and gene mutations in MDS and AML are well-known and have been recognized in the World Health Organization classification (Swedlow et al., 2008) and revised international prognostic scoring system for MDS (Greenberg et al., 2012). In addition, many hematologic neoplasias also acquire epigenetic changes, which may have a profound effect at the level of gene expression (Bonifer and Bowen, 2010; Jelinek et al., 2011). For example, the inactivation of tumor suppressor genes by promoter hypermethylation contributes to the initiation and progression of MDS and AML (Bies et al., 2010; Cechova et al., 2012).

A few studies have characterized abnormalities of the BM microenvironment in hematologic malignancies. Some reported that BMSCs in MDS are cytogenetically and functionally normal and maintain their original function of supporting normal hematopoiesis (Flores-Figueroa et al., 2008; Klaus et al., 2010; Soenen-Cornu et al., 2005), whereas others have reported that BMSCs in MDS and AML showed numerical and structural chromosomal abnormalities, especially the loss of chromosomes (Blau et al., 2007; Flores-Figueroa et al., 2005). Recent data demonstrated that BMSCs of MDS and AML patients have distinct genetic abnormalities compared with leukemic blasts and that these genetic alterations in BMSCs may constitute a particular mechanism of leukemogenesis and affect patient prognosis (Blau et al., 2011). In addition, Geyh et al. defined epigenetic changes, including a specific methylation pattern of MDS-derived BMSCs, and their association with the functional properties of BMSCs (Geyh et al., 2013).

In this study, we evaluated genetic and epigenetic abnormalities of HCs and BMSCs in MDS and AML patients. Genetic changes were analyzed using conventional cytogenetics, fluorescence in situ hybridization (FISH) and array comparative genomic hybridization (aCGH) techniques. Epigenetic changes were analyzed by measurement of global and focal methylation using pyrosequencing.

Patients and methods

Patients

This study included a total of 79 patients (21 with MDS and 58 with AML) who were diagnosed and treated in the Catholic Blood and Marrow Transplantation Center. The diagnoses were based on the World Health Organization criteria (Swedlow et al., 2008). Multicolor flow cytometry was performed using myeloid lineage antigens, including CD33, CD13, CD117 (CD34), CD14, CD64, and CD41 (BD Biosciences/Pharmingen, CA, USA), to establish the blast lineage. Multiplex RT-PCR was performed to detect the presence of common genetic rearrangements using a HemaVision kit (Bio-Rad Laboratories, CA, USA). Signed informed consent was obtained from all patients, and this study was performed in accordance with the Declaration of Helsinki and approved by our local institutional review board (SCMC07BR131). BMSCs from eight healthy donors, four of which were isolated and cultured in our laboratory and four of which were obtained from the Texas A&M Health Science Center College of Medicine Institute for Regenerative Medicine at Scott & White (Temple, TX, USA), served as the controls for this study. The characteristics of the control and patient cells are summarized in Table 1.

Isolation, culture, and characterization of BMSCs

BMSCs were isolated from fresh BM aspirates using the RosetteSep human mesenchymal stem cell enrichment cocktail (StemCell Technologies, Vancouver, BC, Canada) at the time of initial diagnosis. In brief, 50 μL RosetteSep solution and 2 mL BM aspirate were mixed and incubated at room temperature for 20 min. After addition of Ficoll-paque Plus (Amersham Bioscience, Freiburg, Germany) and centrifugation, the unwanted cells were precipitated, and the BMSC-enriched fraction was collected and seeded at an initial concentration of approximately 1 × 10^6 cells/mL. The cells were cultured in α-MEM (Gibco BRL®, NY, USA) supplemented with 10% fetal bovine serum (Gibco BRL®) and 1% penicillin–streptomycin (Gibco BRL®) at 37 °C and 5% CO2.

For adherence and characterization of BMSCs, non-adherent cells were removed and the media were changed every three to four days until the BMSCs reached 70% confluence. To define the characteristics of BMSCs, immunophenotypic features were analyzed with antibodies against CD73, CD90, CD105, HLA-DR, CD11b, CD79a, CD34, and CD45 (BD Biosciences/Pharmingen). The samples were analyzed on a FACS Aria, and the resulting data were processed using CellQuest software (BD Biosciences). BMSCs at passages 3 to 4 were used for the experiments. The proliferation activity of BMSCs was analyzed to compare growth kinetics between the control and patient BMSCs using representative specimens (4 normal samples and 3 patients; 2 MDS and 1 AML). Population doubling time (PDT) was used as an indicator of the proliferative activity of the BMSCs.

Senescence-associated β-galactosidase staining assay

Beta-galactosidase, which is specifically expressed in senescent cells and corresponds to an accumulation of lysosomal endogenous β-galactosidase, is active at pH 6.0 (Debacq-Chainiaux et al., 2009). We evaluated the expression of β-galactosidase using the senescence-associated β-galactosidase (SA-β-gal) staining kit (Cell Signaling Technology, Boston, MA, USA), according to the manufacturer’s instructions. The percentage of blue-stained senescent cells was obtained by
observing all cells in at least 10 random fields under a microscope.

In vitro differentiation

Each sample was differentiated as follows: the cells were harvested by trypsinization and seeded in 6-well culture dishes (BD Biosciences) at a concentration of $1 \times 10^5$/well. After expanding the BMSCs in growth medium to 70% confluency, the growth medium was replaced with differentiation induction medium. The cells were incubated at 37 °C for three weeks, and the fresh medium was replaced every three days. To evaluate adipogenic differentiation of the BMSC samples, the cells were cultured in adipogenesis differentiation basal medium (Gibco) containing an adipogenesis supplement, such as 1 μM dexamethasone, 0.5 mM isobutylmethylxanthine, and 200 μM indomethacin. The cytoplasmic inclusions of neutral lipids were then stained with oil red O (Sigma-Aldrich, St. Louis, MO, USA). The cells were fixed with 4% paraformaldehyde (PFA) solution and incubated in 60% isopropanol for 5 min at room temperature; then, the isopropanol was aspirated. The cells were stained with oil red O (Sigma) solution for 30–60 min.

For chondrogenic differentiation, $4 \times 10^5$ cells of the BMSC samples were added to 15-mL conical tubes (BD Biosciences), which were centrifuged at 150 g for 5 min. Then, the cells were cultivated in chondrogenic induction medium (Gibco) for four weeks. Control cells were maintained in standard culture medium with 10% FBS during the same time period. To assess chondrogenic differentiation, pellets were fixed in 4% formaldehyde and embedded in paraffin. The sections were stained with Alcian Blue (Sigma-Aldrich).

Osteogenic differentiation was assessed by incubating the cells in osteogenesis differentiation medium (Gibco) containing an osteogenesis supplement, such as 100 nM dexamethasone, 50 μM ascorbic acid-2-phosphate and 10 ng/mL recombinant human bone morphogenetic protein. Osteoblasts were identified by visualizing calcium deposits using the von Kossa method and a calcium kit (Polysciences, Warrington, PA, USA). The cells were fixed with 4% PFA solution and incubated in 3% silver nitrate solution under UV light for 1 h. After rinsing, the cells were incubated in 5% sodium thiosulfate for 2 min.

Cytogenetic analysis of HCs and BMSCs

Karyotyping of HCs and BMSCs was performed by a standard method of G-banding and described by the International System for Human Cytogenetic Nomenclature (ISCN), 2009 (Willatt and Morgan, 2009). To confirm cytogenetic abnormalities identified by G-banding, FISH was performed with appropriate probes, according to the manufacturer’s recommendations. The following probes were used: LSI EGR2/D5S23, D5S721, D7S486/CEP7, CEP8, CEPX/CEPY (Abbott Laboratories, IL, USA), MYBL2, and D20S150/D20S108 (Cytocell, UK).

Array comparative genomic hybridization

Briefly, genomic DNA was extracted from HCs and BMSCs using a QiAamp DNA Blood Mini Kit (Qiagen, CA, USA). Sex-matched DNA from Promega (Napeen, Canada) was used as the reference DNA. The patient and reference DNAs were labeled with Cy3 and Cy5 dyes, respectively, by random primer labeling. Labeled genomic DNA was co-hybridized to the NimbleGen CGX 12 × 175 K array (Roche NimbleGen Inc., Madison, WI, USA). The arrays were washed, and fluorescent signals were acquired with a NimbleGen MS200 scanner (Roche NimbleGen Inc.). The data were analyzed using DEVa Project Manager 1.0.2 software (Roche NimbleGen Inc.).

Pyrosequencing for quantitative analysis of long interspersed nucleotide element 1 (LINE-1) repetitive element and CDKN2B methylation

Bisulfite conversion was performed with 2 μg DNA using the EpitTect Bisulfite Kit (Qiagen) according to the manufacturer’s instructions. Denaturation was performed at 95 °C for 5 min. PCR and pyrosequencing primers were designed using PSQ Assay Design software (Biotage, Uppsala, Sweden) (Supplementary Table S1). The PCR mixture contained 100 ng bisulfate-converted DNA, 10 × PCR buffer, 10 mM dNTP, DNA Taq polymerase, and 10 pmol of the forward and 5′ biotinylated reverse primers in a 25 μL reaction. The PCR conditions were as follows: pre-denaturation at 95 °C for 15 min, followed by 45 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min.

### Table 1 Demographics of enrolled individuals.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MDS</th>
<th>AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
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<td>21</td>
<td>58</td>
</tr>
<tr>
<td>Age, years</td>
<td>32.3 ± 15.1</td>
<td>47.9 ± 14.1</td>
<td>38.7 ± 19.2</td>
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<td>Sex (M/F)</td>
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<td>11/10</td>
<td>29/29</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RCMD (n = 7)</td>
<td>RAEB-1 (n = 4)</td>
<td>AML with t(8;21) (n = 8)</td>
</tr>
<tr>
<td></td>
<td>RAEB-2 (n = 41)</td>
<td></td>
<td>APL with t(15;17) (n = 8)</td>
</tr>
<tr>
<td></td>
<td>Very low (n = 1)</td>
<td></td>
<td>AML with inv(16) (n = 5)</td>
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<tr>
<td></td>
<td>Low (n = 3)</td>
<td></td>
<td>AML NOS (n = 37)</td>
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<tr>
<td></td>
<td>Intermediate (n = 10)</td>
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<tr>
<td></td>
<td>High (n = 5)</td>
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<td></td>
<td>Very high (n = 2)</td>
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Abbreviations: MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; RCMD, refractory cytopenia with multilineage dysplasia; RAEB, refractory anemia with excess blasts; APL, acute promyelocytic leukemia; NOS, not otherwise specified.
Pyrosequencing was performed with the PyroGold Q96 Reagent kit (Qiagen) and PyroMark ID system™ (Biotage, Uppsala, Sweden) according to the manufacturer’s recommendations. CpG site quantification was performed with the new methylation software PyroQ-CpG™ (Biotage) (Supplementary Fig. 2). The criteria for Pyrogram selection were as follows: sufficient peak height of >15 units (calculated by the software), symmetric peaks without any irregularities or side peaks, wide reading length with high reliability, and absence of significant signals at the position of the bisulfate treatment control.

**Statistical analysis**

The LINE-1 and CDKN2B methylation percentages in the normal and patient BMSCs were compared by the Mann Whitney U test. The methylation percentages in BMSCs were compared with those of the corresponding HCs by the Wilcoxon signed rank test. The correlations of the LINE-1 and CDKN2B methylation percentages between the patient BMSCs and their corresponding HCs were determined using Spearman’s rank method. Significance was defined as P < 0.05. All calculations were performed using Medcalc software 9.0 (Medcalc, Mariakerke, Belgium).

**Results**

**BMSCs from patients demonstrated impaired growth kinetics**

The isolated BMSCs from both healthy donors and patients were adherent cells with typical fibroblast-like morphology. They were positive for CD73, CD90 and CD105 (>95% of cells), negative for HLA-DR, CD11b, CD79a, CD34 and CD45 (<2% of cells) and possessed the potential to differentiate into adipocytes, chondroblasts and osteoblasts (Supplementary Fig. S1).

PDT was increased with increasing passages. The patient BMSCs had retarded proliferation rates when compared with the control BMSCs indicated by a longer PDT after passage 3 (patient 51.84 ± 6.56 vs. control 42.53 ± 6.12, P = 0.040), and this difference was increased with increasing passages (patient PDT: 259.80 ± 56.85 vs. control PDT: 111.67 ± 53.45 at passage 7, P = 0.001). SA-β-gal staining revealed that a greater number of patient BMSCs entered senescence than control BMSCs (30.3 ± 0.5% vs. 8.9 ± 2.1%) at passage 3. Cells were considered to be senescent if they were stained blue which were usually enlarged and had a flat shape. Retarded growth rates were also explained by these SA-β-gal staining results (Fig. 1).

**Genetic abnormalities of BMSCs from patients**

Genetic abnormality enhancement has been well-documented in HCs isolated from patients presenting with MDS and AML. Accumulated data has shown that BMSCs play a critical role in many aspects of HC growth, and this raises the question of whether BMSC and HC genetic alterations are correlated. We investigated patient BMSCs to detect increased genomic instability. In addition, we compared the findings of patient BMSCs with their paired HCs to identify correlations in genomic changes. The genetic findings were evaluated by cytogenetic analysis, FISH, and aCGH. The cytogenetic analysis was successfully performed using a conventional G-banding technique in all patient HCs. We obtained enough metaphase cells (at least 15) in all control BMSCs derived from healthy donors, and all of the control BMSCs were found to have normal karyotypes. On the other hand, we failed to obtain enough metaphase cells in the BMSC samples of eight patients (10.1%; one MDS and seven AML), mainly because of their low proliferative activities.

A cytogenetic abnormality is usually considered to be clonal when more than two metaphase cells exhibit it. We found clonal cytogenetic abnormalities in four patient BMSC samples, and the percentage of abnormal metaphase cells

![Figure 1](image-url) Proliferation activity of control and patient bone marrow stromal cells (BMSC). (a) Population doubling time is prolonged in patient BMSCs after 3rd passage (*P = 0.040, **P = 0.001). (b) Representative pictures of BMSCs during culture. Cell density is lower in BMSC myelodysplastic syndrome (MDS) patient (lower) than in control (upper) at 4th and 7th passages. Wide and granular cells are also observed in patient BMSCs in later passage. (c) Senescence associated β galactosidase staining in MDS patient BMSCs (lower) revealed higher positive cells than control.
was low (average 17.8%) (Supplementary Table S2). Among MDS patients, clonal cytogenetic abnormalities were identified in 61.9% (n = 13) of HCs and 5.0% (n = 1) of BMSCs. An MDS patient (R86) with a normal HC karyotype had an abnormal BMSC karyotype, including t(4;15) (q12;q24), tetrasomy 5, and trisomy 7, 8, and 9, in 10 out of 50 metaphase cells (Fig. 2a). We used FISH and aCGH to evaluate whether this aberration was found in both HCs and BMSCs and confirmed that only BMSCs had the aberration. Among AML patients, cytogenetic abnormalities were identified in 58.6% (n = 34) of HCs and 5.9% (n = 3) of BMSCs. An AML (A62) patient with a normal HC karyotype displayed trisomy 5 in three of 15 analyzed BMSCs. This karyotype abnormality was only detected in BMSCs; this was confirmed by FISH but was not confirmed by aCGH because of the low mosaicism (Fig. 2b). For an AML patient (A106) with HCs displaying monosomy 7 and inv(3) (p25q29), the corresponding BMSCs displayed trisomy 5 and 1q gain (Fig. 2c). The numerical aberrations observed in HCs and BMSCs were verified by FISH.

aCGH results were available for seven healthy controls and 18 MDS and 51 AML patients. In HCs, four MDS (22.2%) and 14 AML (27.5%) patients revealed abnormal aCGH findings. In BMSCs, only 1 MDS patient (R86) had abnormal aCGH findings, which included an extra chromosome 5 and the loss of the short arm of chromosome X (Fig. 2a). In the HCs of one AML patient (A106), aCGH detected the loss of chromosome 7 and additional genetic abnormalities that were not observed by karyotyping, but it did not detect numerical aberrations in the corresponding BMSCs (Fig. 2c). This may have occurred because aCGH cannot sufficiently detect a low proportion of cells with abnormalities (low mosaicism) and balanced translocations. However, aCGH detected additional copy number aberrations that were not

![Figure 2](https://example.com/figure2.png)

**Figure 2** Representative karyotype, fluorescence in situ hybridization (FISH) and array comparative genomic hybridization (aCGH) results. (a) Normal karyotype of hematopoietic cells (HC) (upper) and abnormal karyotype of bone marrow stromal cells (BMSC) (medium) from a patient with refractory anemia with excess blasts-2 (R86). Patient’s aCGH of chromosome 5 (lower). HC with normal pattern and BMSCs showing gain of whole chromosome 5. (b) Normal karyotype of HC (upper) and trisomy 5 of BMSC (medium) from a patient with acute myeloid leukemia (AML) with maturation (A62). FISH of chromosome 5 revealed normal pattern of 2 green signals (G) and 2 red signals (R) in HC (①, ②) (upper) and coexistence of abnormal (3G3R in ③, 3G2R in ④) and normal (⑤) in BMSC. (c) Abnormal karyotype including monosomy 7 in HC (upper) and mosaicism of trisomy 5 and dicentric (1;19) in BMSC (medium) from a patient with AML without maturation (A106). aCGH of chromosome 7 (lower). HC showing loss of whole chromosome 7 and BMSC with normal pattern.
Epigenetic abnormalities in BMSCs from patients with MDS and AML

Global methylation was quantitatively determined by LINE-1 pyrosequencing. The LINE-1 methylation percentage in the patient BMSCs was significantly lower than that in the control BMSCs (68.2 ± 3.8 vs. 72.9 ± 3.4, P < 0.001) (Fig. 3a). The LINE-1 methylation percentage in BMSCs was typically lower than that in the corresponding HCs (68.2 ± 3.8 vs. 71.5 ± 6.0, P = 0.001), whose proportion significantly correlated with each other (r = 0.722, P < 0.001) (Fig. 3a). For the BMSCs of the AML patients, significant LINE-1 hypomethylation was observed compared with the control BMSCs (67.9 ± 3.8, P < 0.001). However, in MDS patients, this difference was not statistically significant (69.4 ± 4.2, P = 0.152) (Fig. 3b).

We chose CDKN2B as a marker for local methylation because CDKN2B is a commonly methylated gene in HCs of MDS and AML. The CDKN2B methylation percentages in patient BMSCs were not different from those in the normal controls (4.2 ± 4.7 vs. 4.2 ± 1.5). The CDKN2B methylation percentages were higher in HCs (11.2 ± 10.2) than in BMSCs (P < 0.001), and the CDKN2B methylation percentages in HCs and BMSCs were significantly correlated with each other (r = 0.517, P = 0.003) (Supplementary Fig. S2).

Discussion

In this study, we evaluated the genetic and epigenetic changes in BMSCs from patients diagnosed with MDS and AML, and we compared these results with those obtained from control BMSCs derived from healthy donors. Patient BMSCs had lower proliferative activity than controls, which was confirmed by growth kinetics. The proliferative activity of patient BMSCs was dramatically decreased after the 3rd passage. This retarded proliferation was explained by SA-β-gal staining, which indicated that senescence had occurred in the greater part of patient BMSCs at an earlier passage than in the controls. This is one of the reasons that we did not obtain enough metaphase cells for karyotyping from approximately 10% of the patient samples. Results from this study have indicated that cytogenetic abnormalities are more frequent in patient BMSCs than in controls. Approximately 5% of patients were found to have clonal cytogenetic abnormalities, the most common of which was an extra chromosome 5. None of the observed cytogenetic abnormalities in BMSCs were the same as those found in HCs. Additionally, there were more genetic changes in the BMSCs of AML patients than in the BMSCs of MDS patients.

A previous study reported karyotype abnormalities in four out of 13 MDS patients (trisomy 5 and 7) compared with one out of five healthy individuals (trisomy 5) (Klaus et al., 2010). Another study found similar abnormalities in chromosome 5 in cultured BMSCs produced in cell therapy facilities. Those authors commented that the occurrence of aneuploidy in cultured BMSCs was not related to the culture process but could be donor dependent (Tarte et al., 2010). These changes in chromosome number and structure were major contributors to the genomic instability of BMSC, which is induced by cellular aging rather than oncogenic transformation (Estrada et al., 2013; Tarte et al., 2010). The proportion of metaphase cells with chromosomal aberrations was low; thus, genetic abnormalities were more easily detected by cytogenetics and FISH methods than by aCGH. On the other hand, aCGH has higher resolution; thus, more genetic changes can be recognized by aCGH than by other methods.

Another potential source of genomic instability is epigenetic-induced reductions in the expression of DNA repair genes. Commonly observed changes in DNA methylation are global hypomethylation, including repetitive element hypomethylation, and focal hypermethylation of promoter CpG islands. In this study, we analyzed methylation of the LINE-1 repetitive elements and CDKN2B as surrogate markers of global genomic and focal methylation, respectively (Yang et al., 2004) and identified global hypomethylation in patient BMSCs. LINE-1 is a repetitive element that constitutes 17–25% of the human genome (Deininger et al., 2003; Lander et al., 2001), is variably hypomethylated in cancers, and is associated with microsatellite instability (Estécio et al., 2007). In this study, the LINE-1 methylation level in patient HCs was comparable with that in

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**Figure 3** Comparison of methylation status between hematopoietic cells (HCs) and bone marrow stromal cells (BMSCs). (a) Correlation of LINE-1 methylation between HCs and BMSCs (P < 0.001). (b) The percentage of LINE-1 methylation in control, myelodysplastic syndrome and acute myeloid leukemia patient HCs and BMSCs.
healthy controls, and this finding was consistent with results from a previous study (Kroeger et al., 2008). Global LINE-1 hypomethylation can repress genome-wide gene expression (Aporntewan et al., 2011); therefore, we postulated that LINE-1 hypomethylation in patient BMSCs may be one of the causative factors of impaired stromal support and the resultant ineffective hematopoiesis in those patients (Geyh et al., 2013). It is notable that LINE-1 was hypomethylated in AML patient BMSCs compared with their corresponding HCs and control BMSCs. In MDS patient BMSCs, a similar phenomenon was observed but it was not statistically significant. This may have occurred because MDS is a heterogeneous spectrum of disorders. Some of them progress into AML which are considered preleukemic. The quantitative measurement using pyrosequencing technique can easily discriminate this difference and may indicate that the level of global LINE-1 methylation is associated with the initiation and progression of MDS and AML. In addition, these results were partially supported by the hypotheses that leukemic cells disrupt niche signaling and that niche changes promote leukemogenesis (Raaijmakers, 2011). In vitro studies using co-culture methods revealed that stromal cells could alter their behavior, resulting in increased proliferation and altered cytokine profiles of leukemic cells (Bruserud et al., 2004; Hatfield et al., 2006).

In summary, our data demonstrated that BMSCs from patients with MDS and AML are genetically and epigenetically altered. Cytogenetic abnormalities developed more frequently in patient BMSCs than in healthy control BMSCs; they developed due to genomic instability, and they induced cellular senescence. Epigenetic alterations in patient BMSCs were characterized by global hypomethylation represented by LINE-1 gene hypomethylation. The epigenetic changes were more severe in the BMSCs of AML patients than in the BMSCs of MDS patients. Further studies including functional investigations will enhance our understanding of the effects of genomic instability in BMSCs and their potential as a therapeutic target.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2015.01.004.

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