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Altered Gene Expression in the Bone Marrow Microenvironment of Myelodysplastic Syndromes

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Altered Gene Expression in the Bone Marrow Microenvironment of Myelodysplastic Syndromes

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이 논문을 의학박사 학위논문으로 제출함

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Abstract

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Introduction: Myelodysplastic syndromes (MDS) are a group of heterogenous diseases characterized by ineffective hematopoiesis and variable degree of increase in blasts with multiple evolutionary stages. Molecular pathogenesis of MDS has been extensively studied, while the molecular change of the MDS bone marrow (BM) microenvironment is rarely known. In this study, we performed cDNA microarray with BM mesenchymal cells of non-hematopoietic origin, and analyzed the differential gene expression in MDS BM microenvironment.

Materials and Methods: Primary culture of adherent cell layers (considered as mesenchymal cells) was performed using BM hematopoietic cells obtained from 7 adult normal controls and 7 MDS patients (3 RCMD, 3 RAEB-1 and 1 RAEB-2). cDNA microarray analysis was performed using humanHT-12 expression v.4 bead array (Illumina, San Diego, CA, USA). Data were analyzed using Illumina GenomeStudio v2009.2 (Illumina), and genes with fold change > 1.5 or < – 1.5, $p < 0.05$ and FDR < 0.05 were considered as differentially expressed genes. Using Ingenuity Pathway Analysis (Ingenuity Systems, www.ingenuity.com) and Cytoscape (www.cytoscape.org), we

figured out the gene networks with clinical significance.

Results: A total of 669 genes were differentially expressed in MDS (RCMD + RAEB) BM mesenchymal cells – 246 overexpressed and 423 underexpressed. Interferon stimulated genes (ISGs) were consistently overexpressed. Disturbances in genes related with ECM and cytoskeleton composition, transcriptional regulation, *ERK*-GTPase pathway, *PI3K* pathway and *NFkB* pathway were notable. A total of 683 genes and 740 genes were differentially expressed in RCMD BM mesenchymal cells and RAEB BM mesenchymal cells compared with normal. Among them, only 48 genes were in common. Genes related with ECM and cytoskeleton composition and transcriptional regulation were altered in RCMD BM mesenchymal cells, while ISGs and apoptosis related genes were altered in RAEB BM mesenchymal cells. In direct comparison between RCMD and RAEB BM mesenchymal cells, 368 genes were differentially expressed – 86 overexpressed and 282 underexpressed. The dysregulation of cell cycle genes was notable. The differentially expressed genes generated hierarchical clustering which does not clearly separate MDS and normal BM mesenchymal cells, or RCMD and RAEB BM mesenchymal cells.

Conclusion and Discussion: Our study provided an overview of altered gene expression of BM microenvironment in adult MDS. The overexpression of ISGs is considered to be the result of the increased level of inflammatory cytokines including interferon-γ in MDS. The alterations in the other pathways probably cause disturbance in normal supportive activity of BM microenvironment, and promote the apoptosis of MDS hematopoietic cells.

Only a small portion of genes were commonly observed as differentially expressed genes in RCMD and RAEB BM microenvironment, suggesting that different genetic pathways are involved in the development of RCMD and RAEB, and the alteration in gene expression in BM microenvironment is important in the progression of MDS. Nevertheless, the gene expression profile itself does not clearly distinguish the BM microenvironment of MDS from that of normal, or RAEB from RCMD, probably because BM microenvironment reflect the characteristics of MDS that it is not overt leukemic status or it is a disease of heterogeneity.

Keywords: myelodysplastic syndromes, microenvironment, mesenchymal cell, gene expression

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1. Introduction

Myelodysplastic syndromes (MDS) are a group of heterogenous clonal stem cell disorders characterized by peripheral cytopenia and paradoxical hypercellular bone marrow (BM) [1-7]. The features of MDS are explained by intramedullary apoptosis due to ineffective hematopoiesis resulting in peripheral deficiency maturing blood cells and dysplastic features of hematopoietic cells, and dysregulation of cell cycle resulting in uncontrolled proliferation [1-7]. Development of MDS is believed to occur through multiple evolutionary stages and the clinical course is divided into several distinct phases depending on the number of lineages involved in dysplasia, the percentage of blasts in BM and peripheral blood and the types of chromosomal abnormalities [1-8]. Refractory cytopenia with unilineage dysplasia (RCUD) is considered as the earliest stage, and cytopenia is the only manifestation in this stage. The clonal cell population with a failure in differentiation proliferates with accelerated cell-cycle transition and increased

apoptosis [9,10]. The clinical course is indolent with low progression rate to acute myeloid leukemia (AML) (at 5 years, 2%). Refractory cytopenia with multilineage dysplasia (RCMD) shows dysplasia in multiple lineages with intermediate prognosis (evolution to AML at 2 years, 10%). Refractory anemia with excess blast-1 (RAEB-1) and refractory anemia with excess blast-2 (RAEB-2) are more advanced types with increased blasts (5-19%) and high rate of progression to AML (about 30%) renders them as preleukemic conditions. Sustained elevation in proliferative fraction of clonal blasts compared with relatively decreased apoptotic fraction is similar to the features observed in AML.

Although the pathobiology of MDS has not been well defined, the genetic alterations of hematopoietic stem cells (HSC) are implicated to promote the initiation and the progression of MDS [2,8,12-19]. Clonal karyotypic abnormalities are observed in approximately 40–50% of de novo MDS and considered as key components in predicting prognosis [15,16,20]. The chromosomal abnormalities are interpreted as the prime molecular markers or

inducers of somatic DNA injury, defective DNA repair, impaired immune surveillance and dysregulated signal transduction [8,12,14-16]. Partial or complete chromosome loss involving chromosome 5, 7, 20, 11 and Y is common, probably resulting in haploinsufficiency of important genes [11,15]. Investigation of MDS with loss of chromosome 5 (or 5q) provides insight into the relationship between haploinsufficiency and chromosomal deletion [15]. *RPS14*, *EGR1*, *CTNNA* and *HSPA9* genes are located on the commonly deleted region (CDR) of chromosome 5, and *NPM* and *APC* are located outside CDR [15]. Haploinsufficiency of *RPS14* is related to upregulation of *TP53*, and contributes to the abnormal erythroid differentiation and apoptosis [21-23]. Reduced expression of *HSPA9* also results in delayed erythroid maturation and increased apoptosis in mice [24]. Haploinsufficiency of *EGR1* enhances self-renewal of stem cell and increases the risk of leukemia in mice [25,26]. Hypermethylation of *CTNNA* is associated with del(5q)-transformed AML in humans [27]. $NPM^{+/-}$ mice develop erythroid macrocytosis and megakaryocytic dysplasia [28]. $APC^{+/-}$ mice also have alterations in stem cell

compartment [29,30]. *EZH2* gene which regulates histone function and maintains the transcriptional repression is located on CDR of chromosome 7 [31,32].

Other genes frequently mutated in MDS include oncogenes (*N-RAS, WT1*), cell cycle regulatory genes (*P15INK4B*, *EVI-1*), apoptotic genes (*BCL2*, *C-MYC*, *TP53*), DNA methylation regulating genes (*DNMT3A*, *TET2*, *IDH1/IDH2*) and histone deacetylation regulating genes (*H3K27*, *EZH2*, *UTX*, *ASXL1*). Overexpression of *N-RAS* by point mutations is associated with disease progression and poor survival in MDS [12,33-35]. Expression of *WT1* gene is higher in advanced MDS and useful for risk assessment [36]. *P15INK4B* regulates cell cycle progression and suppressed expression by promoter hypermethylation is associated with disease progression and leukemic transformation in MDS. [12,14,37]. Overexpression of *EVI1* inhibits the differentiation of hematopoietic progenitor into erythroid and granuloid lineages [12]. Increased expression of *C-MYC* and *BCL2* is common in early and advanced MDS, respectively [12,20]. Genes related to DNA methylation

(*DNMT3A*, *TET2*, *IDH1/IDH2*) and histone deacetylation (*H3K27*, *EZH2*, *UTX*, *ASXL1*) regulates the expression of other genes epigenetically, and their mutations are associated with the initiation and progression in MDS [15]. Mutations in growth factor and angiogenesis genes may be related with elevated levels of TNF- α, TGF-β, IL-1β, IL-6, IFN-γ, VEGF, basic mesenchymal cell growth factor, and hepatocyte growth factor [12,20]. The cDNA microarray facilitated the identification of many other genes with altered expression in MDS and their clinical significance [8,12-16,20].

The pathogenesis of MDS is not solely dependent on the alteration of hematopoietic elements. It is speculated that the reciprocal interaction between aberrant hematopoietic cells and BM microenvironment (stroma) also plays an important role in the pathogenesis of MDS [1,2,7,38-40]. The BM microenvironment contributes to regulation of the self-renewal, commitment, differentiation, and proliferation of stem cells and hematopoietic progenitors [40]. The roles of BM microenvironment in hematopoietic malignancy can be summarized as follows: (i) the BM microenvironment can

be modified by the malignant hematopoietic cells which induce an altered production of adhesion and extracellular matrix molecules and cytokines; (ii) the BM microenvironment can be permanently modified by the microenvironment cells derived from malignant hematopoietic clones, such as BM macrophages, mast cells, or lymphocytes; (iii) the intrinsically modified BM microenvironment generates conditions favorable to the development of the malignant clone [40]. The dynamic reciprcal interaction between the BM microenvironment and the malignant clone continues throughout disease progression. There are four types of representative cells composing the BM microenvironment - mesenchymal cells, adipocytes, endothelial cells, and macrophages [40,41]. Among them, adherently growing mesenchymal cells are considered to reflect the true nature of BM microenvironment and the main target of the researches [40,41]. They are characterized by adherent growth in in-vitro culture, and expression of CD29, CD44, CD90 and CD105, but not CD45 [39].

Previous functional researches on cytokine release or colony formation

provided the insights of the role of BM microenvironment in the pathobiology of MDS. MDS is considered as chronic inflammatory status in which T-cell mediated immune response is suppressed and cytokines inducing apoptosis such as TNF-α, IL-1 or IL-6 are elevated. Apoptosis of F-36P cells on the MDS BM microenvironment was augmented compared with normal BM microenvironment [3]. The media conditioned by MDS BM microenvironment facilitates the apoptosis of CD34+ cells [5]. Derangement of cytokines including TNF-α, leukemia inhibitory factor, IL-32, IL1β, IL-6 and hepatocyte growth factor is observed in MDS BM microenvironment [6,7,38]. TNF-α promotes the apoptosis of MDS clones by increasing the proinflammatory cytokines (IL-6, IL-8, IL-32) and the expression of pro-apoptotic genes such as BH3 interacting domain death agonist (*BID*) gene [6]. However, controversial findings also exist that the MDS BM microenvironment is not the main source of inflammation. Klaus et al. have shown that MDS BM mesenchymal cells were not significantly different from normal BM mesenchymal cells in terms of immunosuppressive function

(inhibition of mitogen-induced T cell proliferation) or production of proinflammatory or growth-promoting cytokines [42].

In contrast to the active investigations on the altered gene expression of MDS hematopoietic cells, the global expression profile of MDS BM microenvronment has rarely been reported [1]. In the study with BM mesenchymal cells of pediatric MDS, Roela et al. has reported that genes related to the biological processes including the regulation of cell cycle, RNA metabolism and vesicle-mediated transport were differentially expressed in pediatric MDS BM mesenchymal cells compared with normal BM mesenchymal cells. In the present study, we analyzed the gene expression profile of the BM microenvironment in adult MDS using cDNA array technology. Gene expression profile of BM derived mesenchymal cells cultured from 3 early MDS and 4 advanced MDS patients were compared with the result from 7 normal controls. Pathway analysis was performed to gain insight into the probable mechanism of gene alteration in MDS BM microenvironment.

2. Materials and methods

2.1. Patients

The study included 7 adults referred for staging of lymphoma and diagnosed as no evidence of BM involvement of lymphoma as normal controls and 7 adults diagnosed as MDS in Seoul National University Hospital from March 2010 to September 2011. The MDS patients consist of 3 RCMD, 3 RAEB-1 and 1 RAEB-2 (6 males and 1 females, age 41-73 years) according to the WHO 2008 classification [11], and the laboratory findings are summarized in Table 1. Bone marrow aspirates obtained at the time of initial diagnosis were used to establish adherent mesenchymal cell layer. The study was approved by the Institutional Research Board of Seoul National University Hospital (IRB No. C-1008-012-326).

2.2. Establishment of adherent cell layers

The heparinized or EDTA-anticoagulated BM aspirate was used after

centrifugation at 2000 rpm for 5 mins. Buffy coat layer was mixed with prewarmed 5 mL of ACK lysis buffer (Lonza, Walkersville, MD, USA) and incubated for 5-10 mins, then centrifuged at 1200 rpm for 5 mins. After suctioning the supernatant, the cells were washed with 10mL of phosphate buffered saline (PBS) and centrifuged the mixture at 1200 rpm for 5 mins. The supernatant was suctined and the cell pellet was mixed with 1.2 mL of prewarmed MLR media composed of RPMI1640 media (JBI, Seoul, South Korea), 10% FBS (Gibco, Grand Island, NY, USA), 100 units/ml of an antibiotic–antimycotic agent (Gibco), 10 mM HEPES (Gibco), 1 mM sodium pyruvate (Gibco), 4.5 g/L glucose (Gibco) and 0.05 mM 2-mercaptoethanol (Amresco, Solon, OH, USA). The cells were plated onto the 20 by 20 mm glass slides on two 6-well plates. The plates were incubated at 37°C, 5% CO_2 incubator until adherent cells were observed. When the confluency of adherent cells reached to 30-40%, we replaced the culture media every 2-3 days. The media was suctioned when the confluency reached to 70-80%, and The cells were washed in each well with 2mL of PBS. The cells were

incubated with 500 uL of EDTA-trypsin until the adhered cells were detached (usually takes 3-5 mins), and mixed with 5 mL of MLR culture media. The mixture was moved onto 50 mL tube and centrifuged at 1200 rpm for 5 mins. After the supernatant was removed, the cell pellet was mixed with 10 mL of MLR media, plated onto culture dishes and incubated at the same condition (37°C, 5% CO_2). Cell pellets of 3^{rd} or 4^{th} passages were mixed with 1 mL of PBS, counted (usually 1×10^5 - 1×10^6) and stored at −70°C or −140°C with DMSO with 20% FBS.

2.3. Immunophenotypic characterization of mesenchymal cells

The expression of surface antigens was monitored by flow cytometry using Navios (Beckman Coulter, Brea, CA, USA) and analyzed using Kaluza software (Beckman Coulter). Following antibodies were used: phycoerythrin-cyanin 5 (PC5) mouse-anti-human CD34 (581, Beckman Coulter), allophycocyanin (APC) mouse anti-human CD45 (J.33, Beckman Coulter), fluorescein isothiocyanate (FITC, Beckman Coulter) mouse anti-human CD29

(4B4LDC9LDH8, Beckman Coulter), FITC mouse-anti-human CD44 (J.173, Beckman Coulter), phycoerythrin (PE) mouse anti-human CD90 (Thy1/310, Beckman Coulter), and PE mouse anti-human CD105 (1G2, Beckman Coulter).

2.4. cDNA microarray

2.4.1. RNA preparation

Total RNA was extracted using Trizol (Invitrogen Life Technologies, Carlsbad, CA, USA), and purified using RNeasy columns (Qiagen, Valencia, CA, USA) according to the manufacturers' protocol. After processing with DNase digestion and clean-up procedures, RNA samples were quantified, aliquoted and stored at −80°C until use. For quality control, RNA purity and integrity were evaluated by using denaturing gel electrophoresis and OD 260/280 ratio analyzed on Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA).

2.4.2. Labeling and purification

Total RNA was amplified and purified using the Ambion Illumina RNA amplification kit (Ambion, Austin, TX, USA) to yield biotinylated cRNA according to the manufacturer's instructions. Briefly, 550 ng of total RNA was reverse-transcribed to cDNA using a T7 oligo(dT) primer. Second-strand cDNA was synthesized, in vitro transcribed, and labeled with biotin-NTP. After purification, the cRNA was quantified using the ND-1000 Spectrophotometer (NanoDrop, Wilmington, DE, USA).

2.4.3. Hybridization and data export

A total of 750 ng labeled cRNA samples were hybridized to each humanHT-12 expression v.4 bead array for 16-18 h at 58°C, according to the manufacturer's instructions (Illumina, San Diego, CA, USA). The array uses 47231 probes and detects the expression of 34694 genes. Detection of array signal was carried out using Amersham fluorolink streptavidin-Cy3 (GE Healthcare Bio-Sciences, Little Chalfont, UK) following the bead array manual. Arrays were scanned with an Illumina bead array Reader confocal scanner according to the manufacturer's instructions. Array data export

processing and analysis was performed using Illumina BeadStudio v3.1.3 (Gene Expression Module v3.3.8).

2.4.4. Raw data preparation and Statistic analysis

The quality of hybridization and overall chip performance were monitored by visual inspection of both internal quality control checks and the raw-scan data. Raw data were extracted using the software provided by the manufacturer (Illumina GenomeStudio v2009.2 (Gene Expression Module v1.5.4)). A data set containing gene (or chemical) identifiers and corresponding expression values was uploaded into in the application. Each identifier was mapped to its corresponding object in the Ingenuity® Knowledge Base using Ingenuity Pathway Analysis 7.5 software (Ingenuity systems, Redwood City, CA, USA) and Cytoscape (www.cytoscape.org). The p-value < 0.05 (similar to signal to noise) and fold change > 1.5 or < -1.5 were set to identify molecules whose expression was significantly differentially regulated. These molecules, called Network Eligible molecules, were overlaid onto a global molecular network developed from information contained in the Ingenuity Knowledge Base.

Networks of Network Eligible Molecules were then algorithmically generated based on their connectivity.

2.5. Quantitative real-time PCR

The expression data for selected genes of interest were validated in controls and MDS patients using quantitative real-time PCR and appropriate primers (Applied Biosystems, Foster City, CA, USA).

2.5.1. cDNA synthesis

cDNA was produced using the Superscript™ II RT-PCR System (Invitrogen, Karlsruhe, Germany) according to the manufacture's recommendations for oligo(dT)20 primed cDNA-synthesis. cDNA synthesis was performed on 500 ng of RNA, at 42°C. Finally, cDNA was diluted 1:2 prior use.

2.5.2. Quantitative TaqMan PCR

PCR was performed in ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) in 384-well microtiter plates using a final volume of 10ul. Optimum reaction conditions were obtained with 5ul of Universal

Master Mix (Applied Biosystems) containing dNUTPs, $MgCl_2$, reaction buffer and Ampli Taq Gold, 90 nM of primer(s) and 250 nM fluorescence-labeled TaqMan probe. Finally, 2 uL template cDNA was added to the reaction mixture. The primer/TaqMan probe combinations were designed on each target sequences. Amplifications were performed starting with a 10min template denaturation step at 95°C, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. All samples were amplified on triplicate and data were analyzed with Sequence Detector software (Applied Biosystems).

2.5.3. Anlaysis of real-time PCR data

The threshold cycle (C_T) value was defined as the fraction cycle number and set at 10 times the standard deviation above the mean baseline fluorescence calculated from cycles 3 to 15. The fold changes in the target genes normalized to *GAPDH* and relative expression of control were calculated for each sample using the $2^{-\Delta\Delta C_T}$ method, where $-\Delta\Delta C_T = (C_{T,\ \text{Target}} - C_{T,\ GAPDH})$ Patient's sample $- (C_{T,\ \text{Target}} - C_{T,\ GAPDH})$ Normal control.

3. Results

3.1. Development of mesenchymal cell layer and immnophenotypic characterization

Adherent cell layers with adequate number of cells for cDNA array were developed in 3 RCMD, 3 RAEB-1, 1 RAEB-2 and 7 normal controls (Fig 1-a). The immunophenotyping analysis at the end of culture demonstrated that the cultured cells were homogenous population expressing CD29, CD44, CD90 and CD105, but not CD34 or CD45 (Fig 1-b), consistent with the immunophenotype of mesenchymal cell.

3.2. Differentially expressed genes in MDS BM microenvironment compared with controls

We identified MDS BM mesenchymal cell genes with significantly different expression level into following comparisons: (i) MDS (RCMD + RAEB) vs. control; (ii) RCMD vs. control; (iii) RAEB vs. control. A total of 669 genes

were differentially expressed in MDS (RCMD + RAEB) BM mesenchymal cells – 246 genes were overexpressed and 423 genes were underexpressed. The differentially expressed genes generated hierarchical clustering which did not clearly separate MDS and normal BM microenvironment (Fig 2). RAEB02 was clustered with controls except for Control06 and Control07 which were clustered together with RCMD and RAEB. Differentially expressed genes in the comparison between MDS (RCMD + RAEB) and controls generated networks (Fig 3) and can be categorized as follows.

3.2.1. Interferon stimulated genes

Many interferon stimulated genes (ISGs) were overexpressed, and most of them were related with interferon-α (IFN- α): *OAS2*, *IFITM1, GBP2, MX2, IFITM2, IFITM3, IRF7, IFI6, ISG15, MX1, UBA7, IFIT1*. *IFIT1* is also related with interferon-β (IFN- β), along with *XAF1*. *IL18BP*, an interferon-γ (IFN-γ) induced gene, was overexpressed. *NFkB* pathway could be affected by IFN- γ, while *PI3K* pathway could be affected by IFN- β.

3.2.2. *NFkB* pathway related genes

Disturbance in the expression of *NFkB* pathway related genes were observed: *IL18BP, UNC93B1* and *TNFSF14* were overexpressed, while *PPM1D, SGK1* and *CARD10* were underexpressed.

3.2.3. *PI3K* pathway related genes

Disturbance in the expression of *PI3K* pathway related genes were observed: *MDK* and *CXCL16* were overexpressed, while *PODXL* were underexpressed. *PODXL* also affects the expression of *VIM*, connecting the network to vimentin-actin pathway.

3.2.4. Extracellular matrix and cytoskeleton related genes

The expression of *VIM*, *SPARC* and *LAMA3* were decreased, while *ACTN4* was increased. *PARP*, a *VIM* related gene, and *TIPARP* were underexpressed. Through *UCHL1*, PARP regulating gene connects the pathway to the transcriptional regulating genes. *VIM* expression is also related with GTPase genes.

3.2.5. Transcriptional regulation related genes

The expression of genes related to histone acetylation was altered: *ADA,*

KAT8 and *MSL3* were overexpressed, while *MORF4L1* was underexpressed. A DNA methylation related gene, DMAP1, was overexpressed. CBX1 and UCHL1, transcriptional modification genes were underexpressed.

3.2.6. *ERK* pathway - GTPase related genes

CNN2, WWOX and *PTPRA* were overexpressed genes related to *ERK* pathway. *PCDCD10, MOB4, PAQR3, BINP2, MYC* and *RGS4* were underexpressed genes related to *ERK* pathway. Including *RGS4*, GTPase related genes were underexpressed such as *RP2* and *RAP1GDS*.

3.3. Differentially expressed genes in RCMD and RAEB BM microenvironment

In the comparison between BM mesenchymal cells from RCMD and controls, 683 genes were differentially expressed – 252 genes were overexpressed and 441 genes were underexpressed. Hierarchical clustering showed the gene expression profile of BM mesenchymal cells of RCMD02 were similar to those of controls (Fig 4). BM mesenchymal cells from RAEB showed 740

differentially expressed genes compared with those from normal controls – 185 genes were overexpressed and 555 genes were underexpressed. The gene expression profile of BM mesenchymal cells of Control06 and Control07 were similar to those of RAEB (Fig 5). In the direct comparison between RCMD and RAEB BM mesenchymal cells, 368 genes were differentially expressed – 86 were overexpressed and 282 were underexpressed in RAEB BM mesenchymal cells compared with RCMD BM mesenchymal cells. BM mesenchymal cells from RAEB04 showed similar gene expression pattern to BM mesenchymal cells from RCMD patients (Fig 6).

Only 48 genes were commonly observed as differentially expressed genes in both comparisons (RCMD vs. controls and RAEB vs. controls). Among them, 45 showed the same pattern of differential expression (either underexpression or overexpression) and similar fold change: *ADA*, *ATAD1*, *ATP11C*, *ATP2C1*, *BMP6*, *CCDC137*, *CFH*, *COMMD10*, *DCAF6*, *DKK3*, *H1F0*, *HIGD1A*, *IFI35*, *IFITM3*, *ISCA1*, *KIAA1671*, *KRT19*, *LCLAT1*, *MAN2A1*, *ME2*, *MN1*, *MORF4L1*, *MYC*, *NAP1L1*, *NFIC*, *NPTN*, *NTN4*, *OSTC*, *PHLDB2*, *PKD2*,

PLEKHA4, *PPP2R3C*, *PSG5*, *PSG6*, *PSG9*, *RECK*, *SLC39A10*, *SLK*, *SPCS3*, *SUMF2*, *TMEM106B*, *TSPAN13*, *UBASH3B*, *WRB*, and *ZNF654*. Three genes showed different pattern of expression: *KRT81* and *LOC100131866* were overexpressed in RCMD, but underexpressed in RAEB BM mesenchymal cells; *MACF1* was underexpressed in RCMD, but overexpressed in RAEB BM mesenchymal cells. These genes did not suggest any certain network in pathway analysis.

We performed pathway analysis with the genes with differential expression either in RCMD or in RAEB BM mesenchymal cells. The alteration of genes related with ECM and cytoskeleton, and transcriptional regulation were notable in RCMD BM mesenchymal cells (Fig 7). Among ECM and cytoskeleton related genes, *APOE, COL6A1, COL6A2, PLTP* and *VCAM1* were overexpressed in RCMD BM mesenchymal cells compared with control BM mesenchymal cells. *CAV2, CDH6, CDH13, CTGF, CTNNA, CYR61, FAT1, ITGB5, PRNP, SPARC* and *VEGFA* were underexpressed in RCMD BM mesenchymal cells compared with control BM mesenchymal cells. Among

transcriptional regulation related genes, *MED25, HIST2H2AC, RALY, RBM39* and *RDBP* were overexpressed, *ARID1A, BCLAF, CREBBP, HIST2H2BE, KAT2B, PLAGL1, SAP18, SMAD4* and *SON* were underexpressed in RCMD BM mesenchymal cells. In RAEB BM mesenchymal cells, the alteration of interferon stimulated genes and apoptosis related genes were notable (Fig 8). *IFI6, IL18BP, ISG15, MX1, OAS2, TNFRSF14, TNFRSF19* and *XAF1* were overexpressed, while *AVEN, FADD* and *FAS* were underexpressed in RAEB BM mesenchymal cells compared with control BM mesenchymal cells.

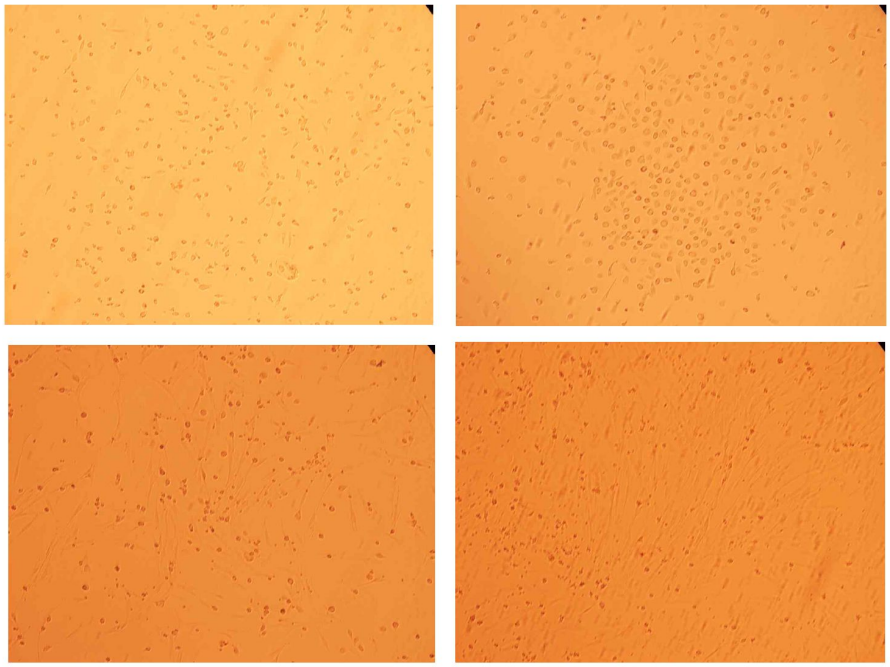
3.4. Validation of cDNA microarray data

Seven genes were chosen to validate cDNA microarray data by RT-PCR considering the fold change in each subgroup analysis: (i) MDS (RCMD + RAEB) vs. control - *IFI6* and *IFITM1*; (ii) RCMD vs. control - *CDC20* and *RGS4*; (iii) RAEB vs. control - *CCND2*, *HSPB6* and *PRICKLE1*. Except for *RGS4* (undexpressed more than –3 fold), the other 6 genes were overexpressed (more than +3 fold) in cDNA array analysis. RT-PCR results

were consistent with those determined by cDNA microarray showing a similar tendency of gene expression (Fig 9).

Figure 1. Culture and characterization of bone marrow mesenchymal cells. (a) Appearance under the inverted microscope (×100) of representative adherent cell layers derived from bone marrow hematopoietic cells. A few adherent cells of spindle shape considered as mesenchymal cells are observed together with round cells which are considered as adipocytes (upper left). The number of spindle shaped adherent cells and round cells are increased (upper right). Round cells are decreased and spindle shaped adherent cells are increased, reaching the confluency of about 30-40% (lower left). Spindle shaped adherent cells are increased, reaching the confluency of 70-80% (lower right). ; (b) Immunophenotypic characterization of bone marrow derived adherent cells. Cultured cells were negative for CD45 and showed low side scatter (Gate S). The histograms show the gated cells are positive for CD29, CD44, CD90 and CD105 and negative for CD34, consistent with the known immunophenotype of mesenchymal cells.

(a)



(b)

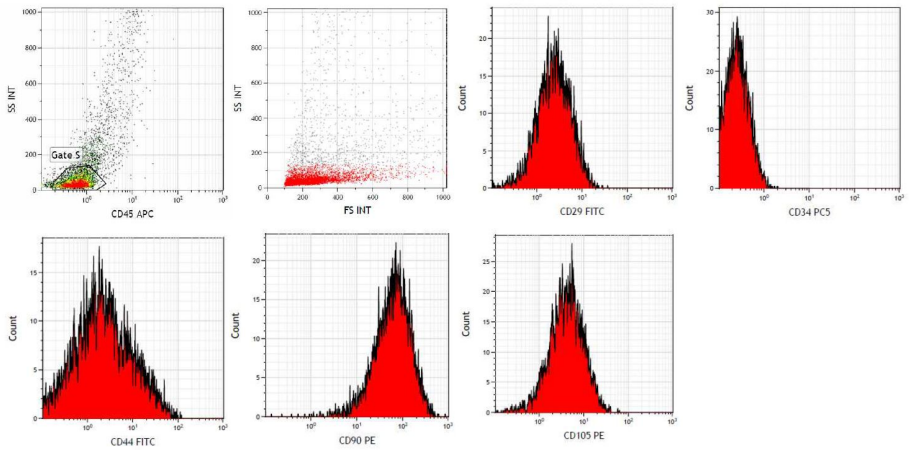

SS INT
Gate S
CD45 APC
SS INT
FS INT
Count
CD29 FITC
Count
CD34 PC5
Count
CD44 FITC
Count
CD90 PE
Count
CD105 PE

Figure 2. Hierarchical clustering was generated for genes differentially expressed in the comparison between MDS (RCMD + RAEB) and normal (control) bone marrow microenvironment. The gene expression profile does not clearly distinguish one group from the other. Color intensity is scaled within each row so that the highest expression value corresponds to bright red and the lowest to bright green.

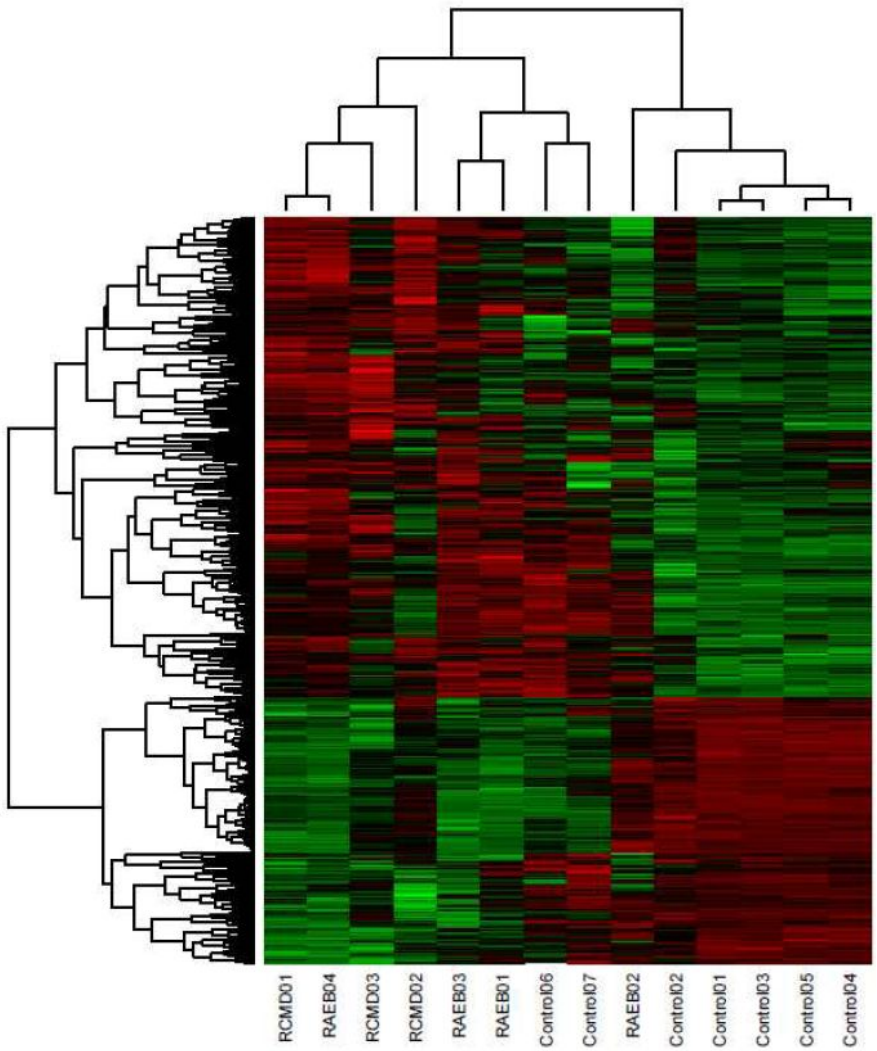


Figure 3. Significantly differentially expressed genes between MDS (RCMD + RAEB) and normal controls are mapped to the pathway. Overexpressed genes were written in red and underexpressed genes were written in green. The figure is modified from networks generated by Ingenuity Pathway Analysis program and Cytoscape.

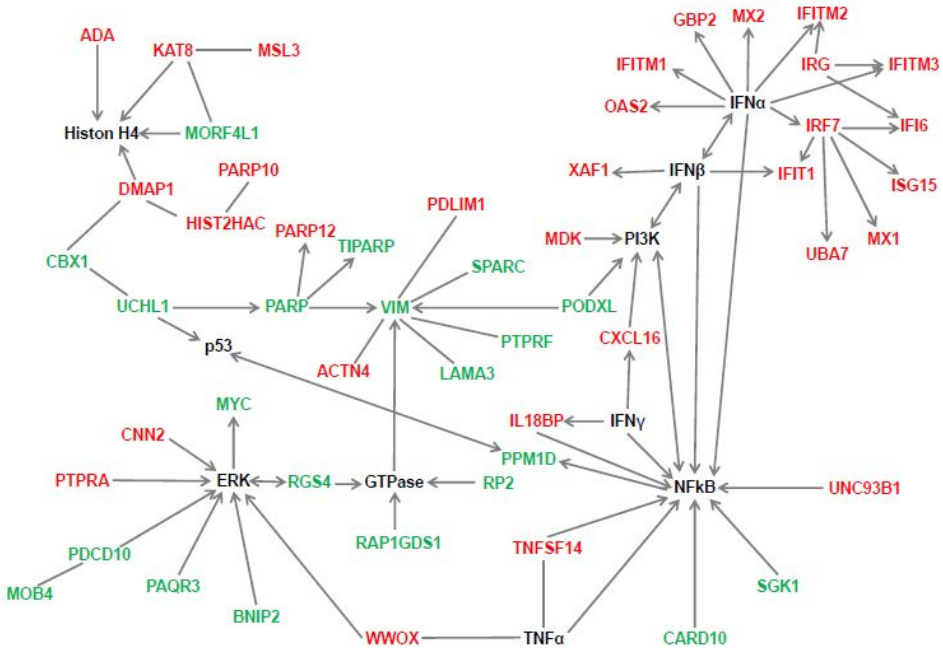


Figure 4. Hierarchical clustering was generated for genes differentially expressed in the comparison between RCMD and normal (control) bone marrow microenvironment. The gene expression profile does not clearly distinguish one group from the other. Color intensity is scaled within each row so that the highest expression value corresponds to bright red and the lowest to bright green.

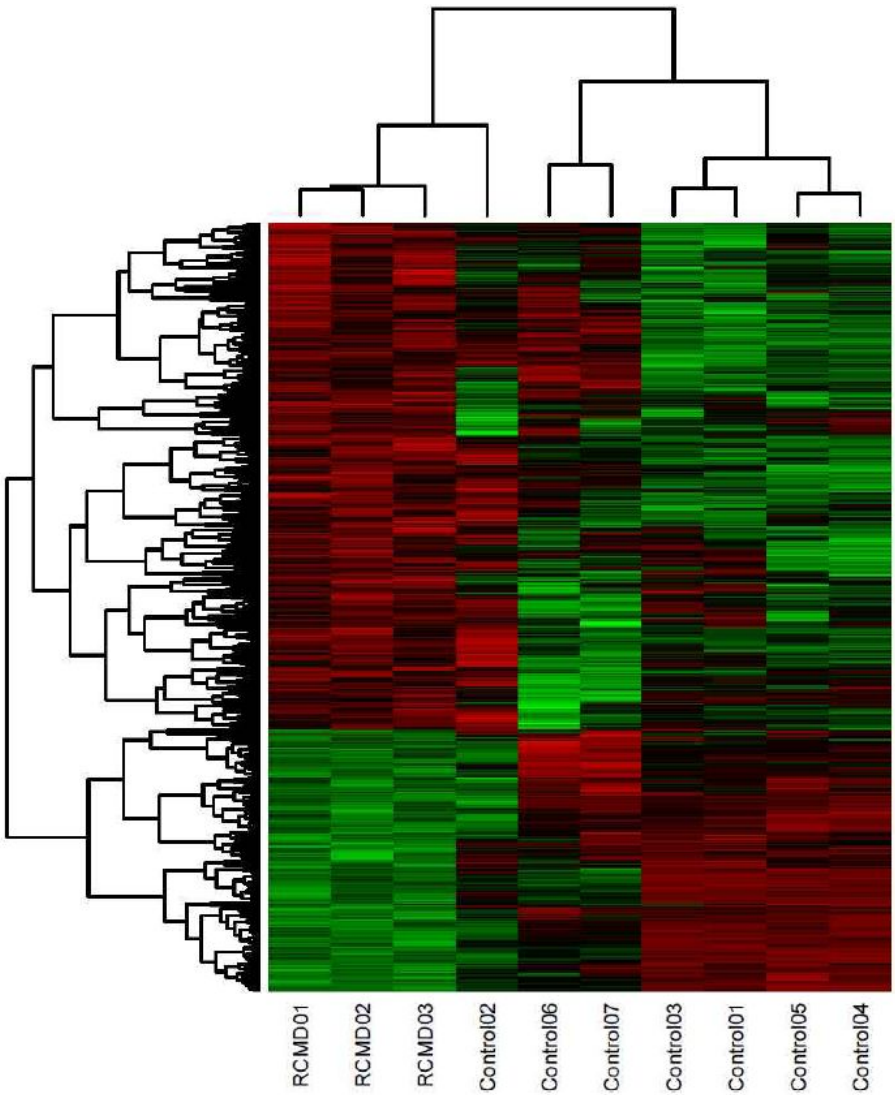


Figure 5. Hierarchical clustering was generated for genes differentially expressed in the comparison between RAEB and normal (control) bone marrow microenvironment. The gene expression profile does not clearly distinguish one group from the other. Color intensity is scaled within each row so that the highest expression value corresponds to bright red and the lowest to bright green.

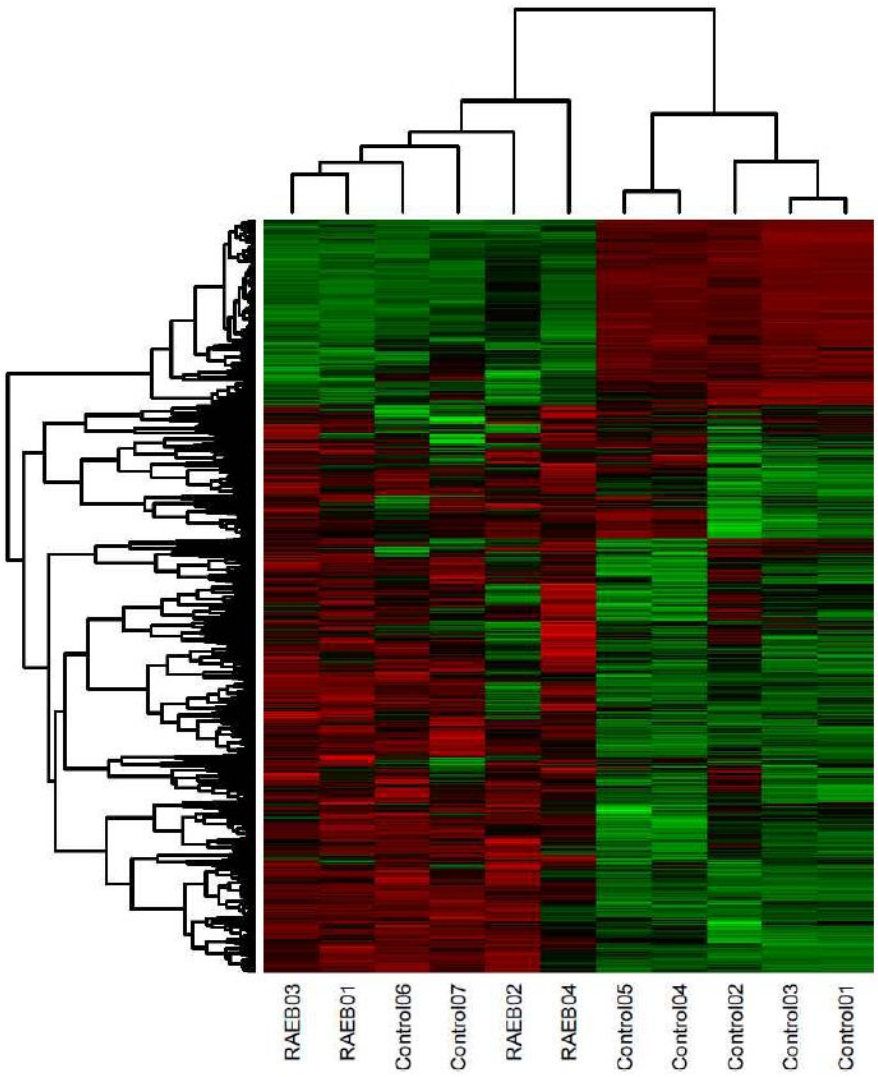


Figure 6. Hierarchical clustering was generated for genes differentially expressed in the comparison between RCMD and RAEB bone marrow microenvironment. The gene expression profile does not clearly distinguish one group from the other. Color intensity is scaled within each row so that the highest expression value corresponds to bright red and the lowest to bright green.

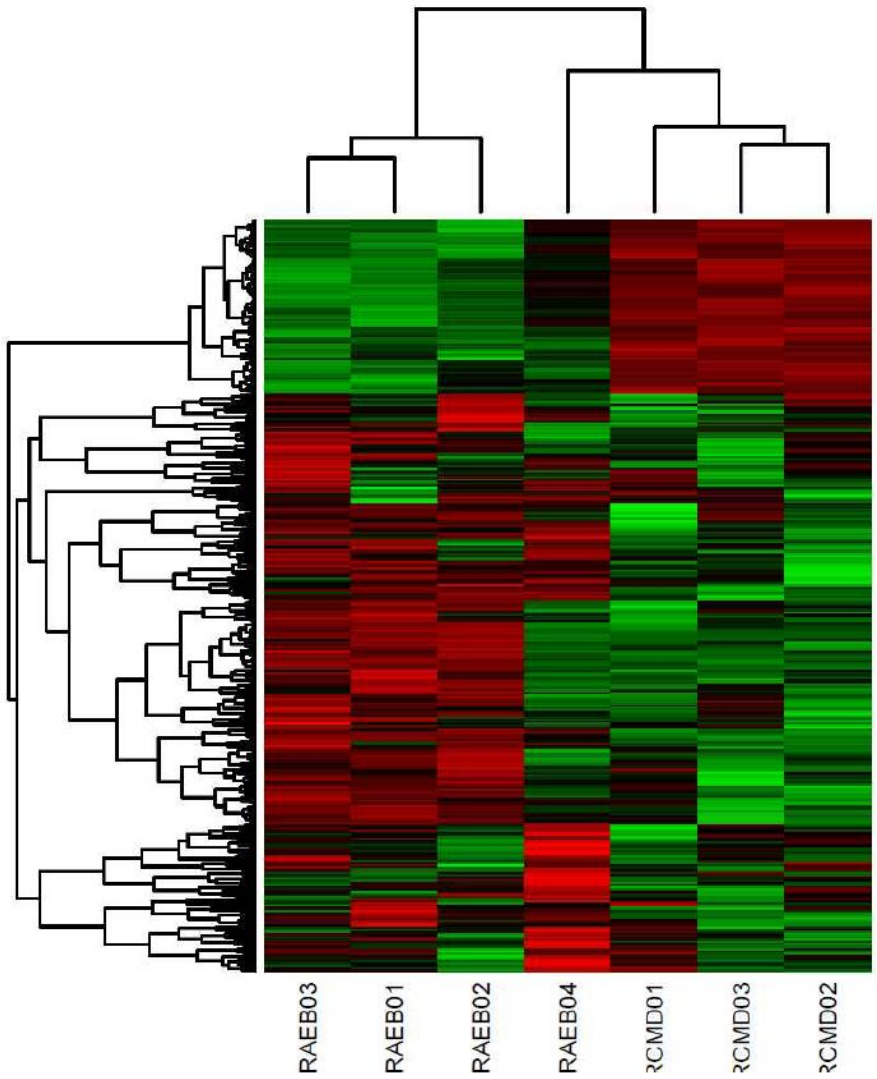
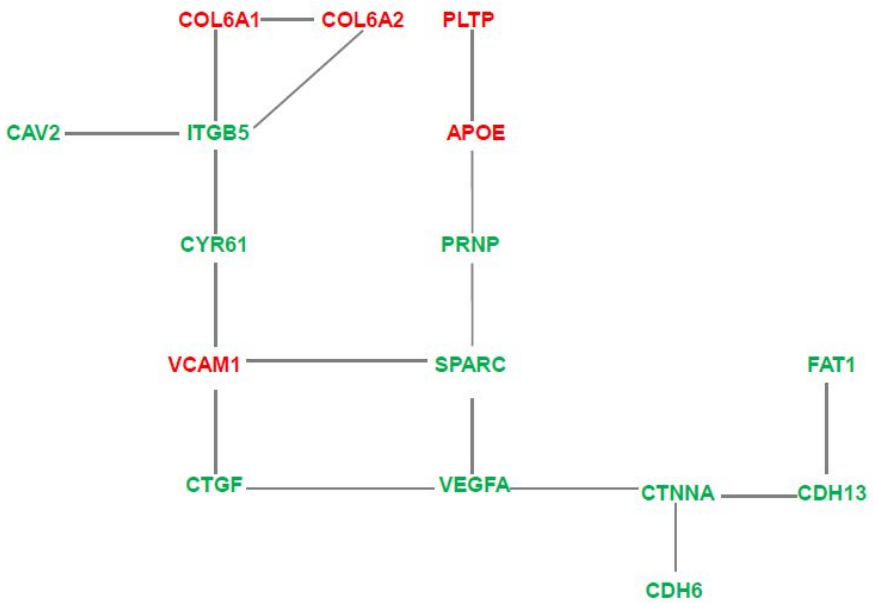


Figure 7. Significantly differentially expressed genes between RCMD and normal controls are mapped to the pathway. Overexpressed genes were written in red and underexpressed genes were written in green. The figure is modified from networks generated by Ingenuity Pathway Analysis program and Cytoscape. (a) ECM and cytoskeleton related genes (b) transcriptional regulation related genes.

(a)



(b)

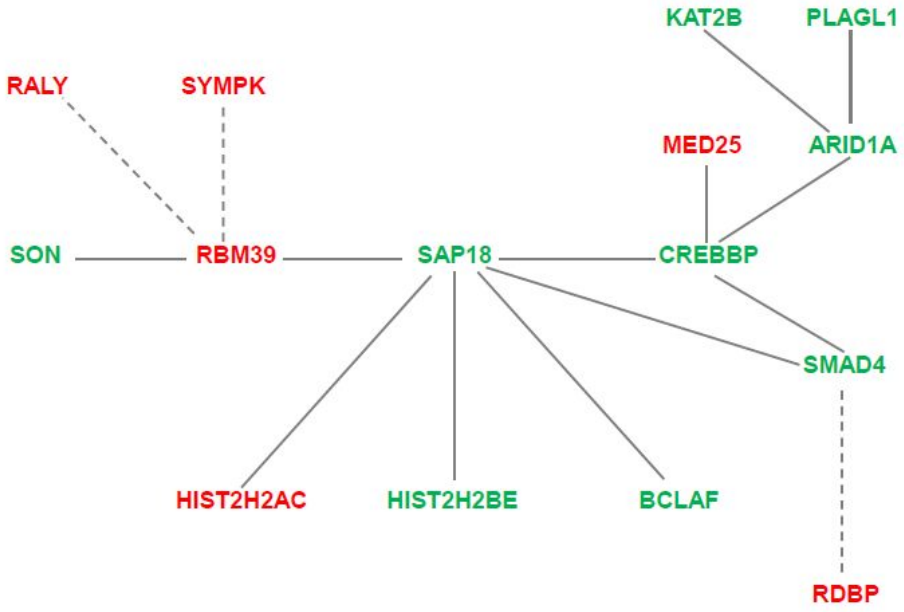

KAT2B
PLAGL1
RALY
SYMPK
MED25
ARID1A
SON
RBM39
SAP18
CREBBP
SMAD4
HIST2H2AC
HIST2H2BE
BCLAF
RDBP

Figure 8. Significantly differentially expressed genes between RAEB and normal controls are mapped to the pathway. Overexpressed genes were written in red and underexpressed genes were written in green. The figure is modified from networks generated by Ingenuity Pathway Analysis program and Cytoscape.

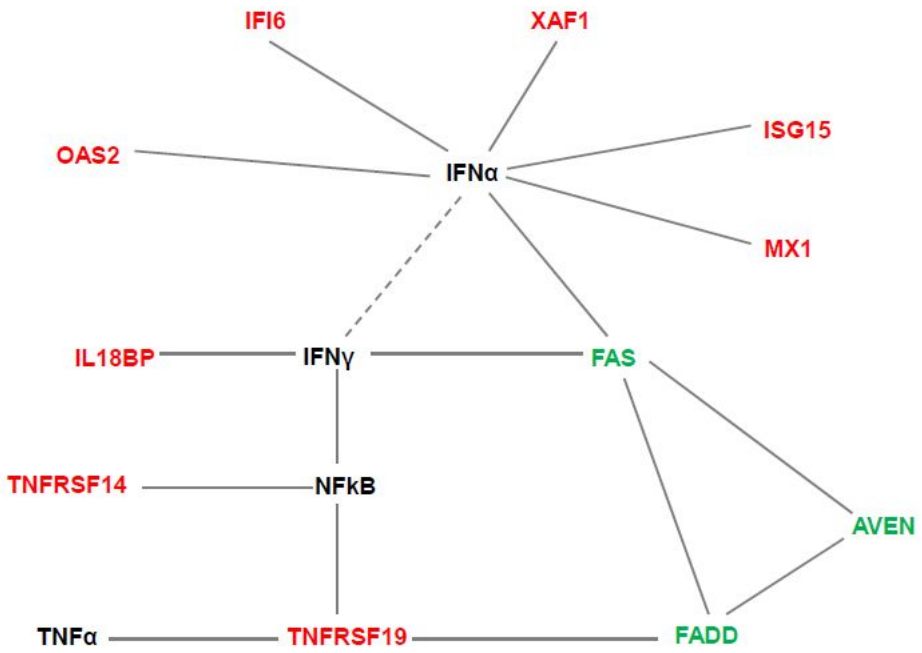


Figure 9. Confirmation of gene expression levels by real-time RT-PCR. The fold difference expression of each target gene relative to an internal control gene (GAPDH) was studied using the $2^{-\Delta\Delta C}{}_{T}$ method. RT-PCR results were consistent with those determined by cDNA microarray showing a similar tendency of gene expression.

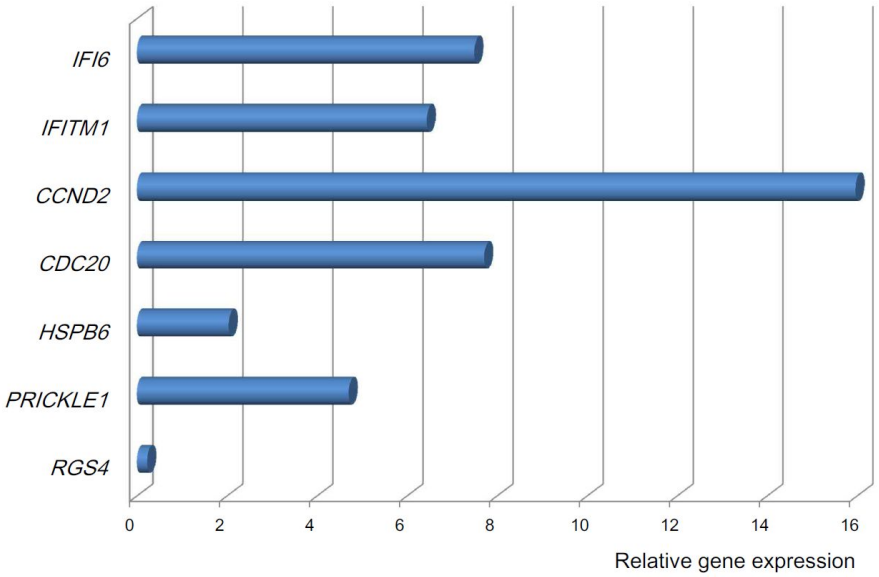


Table 1. Characteristics of MDS patients and normal controls

Case No. (Sex/Age)	Hb (g/dL) - WBC (/uL) - PLT ($X10^3$/uL)	Diagnosis	Cellularity (%)	Karyotype
RAEB01 (M/73)	7.2-8300-108	MDS, RAEB-2	70-80	46,XY,del(20)(q11.2q13.1)[19]/46,XY[1]
RCMD01 (M/41)	5.8-4320-100	MDS, RCMD	90-100	46,XY[20]
RCMD02 (M/65)	8.6-6800-29	MDS, RCMD	90-100	46,XY[20]
RCMD03 (F/69)	9.1-2200-53	MDS, RCMD	80-90	46,XX,9qh-[20]
RAEB02 (M/73)	8.5-1610-186	MDS, RAEB-1	0-10	45,X,-Y[6]/46,XY,add(3)(q?25),del(5)(q?13),add(16)(q?22)[2]/46,XY[13]
RAEB03 (M/58)	8.0-2650-171	MDS, RAEB-1	70-80	54~57,XY,+Y,+1,+add(4)(q3?),+add(6)(q13),-8,+9,+9,del(11)(p11.2),-13,add(15)(p10),-

				17,add(19)(q13.3),+20,+21,+21,+22,+3~6mar,inc[cp6]/46,XY[14]
RAEB04 (M/58)	10.1-6600-37	MDS, RAEB-1	90-100	47,XY,+8[4]/46,XY[16]
Control01 (M/43)	17.0-5700-290	Normal marrow	40-50	46,XY[20]
Control02 (M/58)	13.3-6990-374	Normal marrow	50-60	46,XY[20]
Control03 (M/34)	13.7-8600-240	Normal marrow	50-60	46,XY[20]
Control04 (F/29)	10.2-5260-246	Normal marrow	20-30	46,XX[20]
Control05 (M/51)	13.8-3840-184	Normal marrow	30-40	46,XY[25]
Control06 (F/48)	10.6-10207-409	Normal marrow	30-40	46,XX[20]
Control07 (M/57)	15.1-6410-171	Normal marrow	50-60	46,XY[20]

4. Discussion

MDS is an attractive candidate for genomics research. The molecular pathogenesis and pathophysiology are still uncertain. MDS is considered as a preleukemic state, and the genomic research could provide the information about early genetic factors of susceptibility for malignant transformation. Recent advances in geomics technology have moved MDS genetics from the field of conventional and molecular cytogenetics to genome-wide expression profiling and sequence analysis. Many studies have showed that MDS BM hematopoietic cells exibit differential gene expression from normal BM hematopoietic cells, and related to the pathobiology of MDS, even though not all the reported results are consistent with each other. It has also been suggested that the gene expression profile represents the stage progression in MDS and helps to estimate the prognosis of MDS patients [43-45].

On the contrary, few studies have reported global gene expression profile in MDS BM microenvironment [1]. Roela et al. performed cDNA microarray on the mesenchymal cells derived from BM of 4 pediatric MDS, RAEB patients and 2 MDS-AML patients (according to FAB classification). Each group was well separated from another according to the differential gene expression profile. Genes related to regulation of cell cycle, RNA metabolism and vesicle-mediated transport were differentially overexpressed and genes related to protein transport, collagen catabolism and Wnt receptor signaling pathway were underexpressed in BM mesenchymal cells from MDS and MDS-AML. As far as we know, gene expression profile of adult MDS BM

microenvironment has never been reported yet. The most probable reason for the rarity of the gene expression data in adult BM microenvironment is the difficulties in obtaining the cells composing BM microenvironment. Lopez-Villar et al. showed that MDS mesenchymal cells occasionally fail to grow in vitro [46]. Klaus et al. found that the rate of cellular growth of BM mesenchymal cells through passages, represented by the population doubling time, was reduced in MDS patients compared to controls [42]. The difficulties in culture might be due to the primary defect in MDS BM microenvironment or a premature cellular replicative exhaustion secondary to the long-term inflammatory process in MDS BM. In our experiment, we found that the initial seeding of cells obtained from BM aspiration onto the glass coverslip in 6-well plate, and successfully cultured BM mesenchymal cells from 7 MDS patients. The well was small enough to minimize the shaking and to help the initial attachment of the mesenchymal cells. Culture with glass coverslip promoted the attachment of the mesenchymal cells compared with the condition without glass coverslip. We did not use cytokines or growth factors promoting cell growth, because the gene expression of the mesenchymal cells could be affected by them. To eliminate the effect of malignant hematopoietic clone and analyze the true nature of BM microenvironment, we obtained the adherent cell layer (considered as mesenchymal cells) with recurrent washing out, and performed immunophenotypic characterization. The cells of early (4–6) passages were used to reduce the alteration of gene expression during a long period of time in *in vitro* culture.

Hierarchical clustering showed that gene expression profile discriminates

MDS BM microenvironment from normal BM microenvironment, but there exists overlap in between. Similar finding was observed in the comparison between RCMD BM microenvironment and RAEB BM microenvironment. We confirmed that MDS BM microenvironment is different from normal BM microenvironment, and associated with the initiation and stage progression of MDS. Possible explanation for the heterogeneity between MDS BM microenvironment specimens is that MDS is not the status of overt malignancy (leukemia), and the changes in BM microenvironment is not clearly distinguished. Previous functional studies and clonality studies have shown controversial results about the difference between MDS and normal BM microenvironment [3,38,39,42]. Klaus et al. has reported that the number, differentiation potential toward adipocytes/chondrocytes/osteoblasts and immunosuppressive function in terms of inhibition of mitogen-induced T-cell proliferation did not differ significantly between MDS and normal BM mesenchymal cells [42]. Aanei et al. reported BM mesenchymal cells from some MDS patients have reduced clonogenic capacity, while those from the other MDS patients have proliferative advantage [47,48]. Similarly, data regarding the capacity of BM mesenchymal cell of MDS patients in supporting hematopoietic cells vary widely with the inhibition of clonogenicity in some, but no impact or stimulation of clonogenic growth in others [48-52]. In addition to the heterogeneity of MDS, other differences such as culture condition might have affected the result. In a research with BM mesenchymal cells derived from pediatric MDS and pediatric MDS-AML, Roela et al. have suggested that MDS BM microenvironment is clearly

separated from normal BM microenvironment [1]. Further study with larger study population will be helpful in resolving the issue.

Most microarray researches rely on gene ontology software to assign gene functions to known broad categories. Many genes have multiple roles in cellular function, but the annotation by software might be based on the convenience of current experimental assays and may not reflect a physiologic role. We did not use this approach in this study. Instead, we generated gene networks composed of differentially expressed genes using 2 pathway analysis programs and investigated their roles based on the knowledges in the published literature.

The most notable finding is the overexpression of interferon stimulated genes (ISGs), even though the expression of interferon genes themselves was not altered. They are induced by IFNs, and play essential roles in the IFN pathway. For example, *IFI6* and *IFITM* genes play an essential role in the antiproliferative action of IFN [17,53]. *IFITM*, in association with other proteins on the cell surface, forms a complex which relays growth inhibitory and cell adhesion signals. *MX1* and *OAS2* are also induced by IFN-γ, and show antiviral action by inhibiting the endocytosis of virus (*MX1*) and by activating RNase which degradates viral RNA (*OAS2*). *XAF1*, induced by IFN-β counteracts the inhibitory effect of inhibitor of apoptosis protein (IAP), thus promotes apoptosis. Overexpression of ISGs might be resulted from increased IFN secretion from MDS BM hematopoietic cells. MDS is considered as chronic inflammatory status, and the elevation of IFN-γ, the proinflammatory cytokine, along with TNF-α and IL-6 were reported [56,57].

The elevation of theses cytokines show growth inhibitory effect on BM hematopoietic cells, a mechanism suggested to account for the apoptosis of hematopoietic cells [38,57,58]. Thus, the overexpression of those ISGs has also been observed in MDS BM hematopoietic cells: Pellagatti et al. reported the overexpression of *IFITM1* and *MX1* in MDS BM CD34+ cells [17]. Together with BM hematopoietic cells, MDS BM microenvironment cells take parts in the proinflammatory process induced by IFN. IFN-α, β and γ modulates cell signaling by interacting with *PI3K* pathway, and *JAK/STAT* pathway as well.

Mesenchymal cells are the most common cell type of extracellular matrix (ECM), and we observed the alteration of ECM genes (*SPARC*, *VIM* and *LAMA3* encoding osteonectin, vimentin and lamin, α3, respectively), cytoskeletal gene (*ACTN4* encoding spectrin) and genes modulating them. *PARP* positively regulates the expression of vimentin and compensate for DNA damage through posttranscriptional modification. *PODXL* also positively regulates the expression of vimentin. BM ECM not only provides the physical microenvironment to the cells that reside in (BM hematopoietic cells in this study), but also regulate the fate of BM hematopoietic cells. Through integrin binding, BM hematopoietic cells adhere to BM microenvironment, and exchange signals for differentiation, proliferation and death. The adhesion is activated by Rho-family GTPase and affects the *PI3K* pathway as shown in our network. Thus, the changes in ECM genes, cytoskeletal gene and genes modulating them could lead to the abnormal differentiation, proliferation and death of BM hematopoietic cells, which may,

in part, account for the increased apoptosis and selective proliferation CD34+ blasts in MDS.

Activation of *PI3K* pathway reduces apoptosis and promotes cell proliferation through activating *AKT-mTOR*. Our result showed evidence of the disturbance in *PI3K* pathway. *PODXL*, already mentioned as a vimentin activator, activates *PI3K* pathway. *CXCL16* is a chemokine which functions as an adhesion molecule. It is induced by IFN-γ, and activates *PI3K* pathway and *NFkB* pathway. *MDK* roles in angiogenesis, cell growth and cell migration, and activates *PI3K* pathway. *NFkB* pathway is induced by IFN-γ and TNF-α and so on, and plays essential roles in inflammatory response, thus also regulates cell proliferation and survival. *CARD10*, a gene contains a caspase recruitment domain, is positively regulates *NFkB* pathway through *Bcl10*. *SGK1* is suggested to activate *NFkB* pathway by controlling Ca2+ entry. *TNFSF14* inhibits apoptosis triggered by caspase8 and suppresses *NFkB* pathway. *UNC93B1* encodes a protein found in endoplasmic reticulum, and activates *NFkB* pathway by interacting toll-like receptors (*TLRs – TLR3*, *TLR7* and *TLR9*). *PPM1D* is positively regulated by *NFkB* and *p53*, and gives negative feedback to *p53*.

ERK (also called *MAPK*) pathway roles in signal transduction from a receptor on the cell surface to the DNA in the nucleus. An extracellular mitogen binds to the membrane ligand, allowing *Ras* (a GTPase) to swap its GDP for a GTP. It now activates *Raf* which activates *MAPK*, then *MAPK* activates a transcription factor, such as *MYC*. Activation of *ERK* pathway induces cell division and proliferation. Many genes involved in *ERK* pathway and GTPase

showed altered expression. *CNN2* which binds to actin2 and functions in structural organization of a cell, activates *ERK* pathway. *WWOX* is an essential mediator of TNF-α mediated apoptosis, but also activates *ERK* pathway. *PTPRA* activates *ERK* pathway, and roles in cell adhesion by regulating integrin signaling, and promotes cell growth and differentiation. *PDCD10* positively regulates *ERK* pathway by working with *MST4*. *PAQR3* sequestrates *Raf* kinase and downregulates *ERK* pathway. *RGS4*, *BNIP2* and *RAP1GDS1* negatively regulate GTPase activity, while *RP2* positively regulates GTPase activity. *MYC* was underexpressed, suggesting the downregulaton of *ERK* pathway.

Expression of genes related with transcriptional regulations was altered. *ADA*, *KAT8* and *MORF4L1* help histone acetylation, thus positively regulates gene transcription. *DMAP1*, encoding DNA methyltransferase 1-associated protein 1, binds to *DNMT1* and *HDAC2*, and acts as a transcriptional repressor. *CBX1* encodes a nonhistone protein which localizes in heterochromatin site where it mediates transcriptional silencing. *UCHL1* is putatively connected with *CBX1*, and induces *p53* accumulation and upregulated the expression of *p21*, as well as cleavage of *caspase3* and *PARP*. These pathways are interacting with each other as shown in the network.

We compared the genes differentially expressed in RCMD and RAEB BM mesenchymal cells. Among more than 600 genes differentially expressed in RCMD or RAEB BM mesenchymal cells compared with control BM mesenchymal cells, only a small portion of genes (48 genes) were observed in common. Differences in fold changes in the two comparisons were negligible.

This finding suggests that the progression of MDS from RCMD to RAEB does not result from the continuous expression of certain pathologic genes in BM microenvironment, but different gene groups with different effect on BM microenvironment play important roles in the disease progression. The pathway analysis showed ECM and cytoskeleton related genes and transcriptional regulation genes were altered in RCMD BM mesenchymal cells, while interferon stimulated genes were altered in RAEB BM mesenchymal cells. Except for interferon pathway in which all the related genes were overexpressed, the alteration of the other pathways could not be interpreted as either activated or suppressed, but merely as disturbed. It might result in the disturbance in the controlled proliferation and survival of BM mesenchymal cells, which lead to alteration in normal supporting activity for the BM hematopoietic cells, and contribute to the pathogenesis and progression of MDS.

We compared the differentially expressed genes in our study with those in the previous studies with childhood MDS BM mesenchymal cells. Borojevic et al. reported 343 genes out of 4500 cDNA fragments were overexpressed in childhood MDS (MDS, RAEB) BM mesenchymal cells [7]. The list of overexpressed genes was not provided in the literature, but only 4 genes related with the ECM were verified using RT-PCR: *OSF-2, SPARC, COL1A2* and *THBS1*. The expression of those genes were not altered in RAEB BM mesenchymal cells in our study, however, *SPARC* was underexpressed in RCMD BM mesenchymal cells. Not *COL1A2*, but other collagen related genes, *COL6A1* and *COL6A2* were overexpressed in RCMD BM

mesenchymal cells. Roela et al. performed cDNA array using about 4600 probes with 6 childhood MDS (4 MDS, RAEB and 2 MDS-AML) [1]. They reported 145 overexpressed genes and 150 underexpressed genes using > 2.0-fold and FDR < 0.05. Only a small portion of genes were observed in common with our result: 2 overexpressed genes (*P8* and *IRF7*) and 5 underexpressed genes (*TOP2A, PRC1, ACSL4, ECT2* and *SLC39A6*). The discrepancy may result from the composition of cDNA array probes and the difference of characteristics between childhood MDS and adult MDS BM microenvironment.

Using cDNA microarray technology, our study provided an overview of altered gene expression in MDS BM microenvironment. The study showed that MDS BM microenvironment is different from normal BM microenvironment and stage specific to some extent, but do not answer the question whether the cells from the BM microenvironment play a role in the pathogenesis of MDS or they are merely 'bystanders' who are affected by the events evoked by clonal abnormalities in BM hematopoietic cells. Another unresolved issue is whether the unclear separation between MDS and normal BM microenvironment, or RAEB and RCMD BM microenvironment is resulted from the heterogeneity of the disease or mere bias due to the small study population. Further study with gene expression and clonality data of paired BM hematopoietic cell and mesenchymal cells from larger MDS population will be helpful to resolve those issues.

In the present study, we analyzed gene expression profile in BM microenvironment from adult MDS applying cDNA array on BM derived

mesenchymal cells. Overexpression of ISGs attributes to the increased interferon level in MDS, a chronic inflammatory status. Altered expression of genes related with ECM composition, transcription, GTPase, *PI3K* pathway, *ERK* pathway and *NFkB* pathway might lead to the disturbance in the controlled survival-death balance in BM microenvironment. The altered supportive activity might contribute to the increased apoptosis of MDS hematopoietic cells and selective blast proliferation. Our result also suggests that different gene groups with different effect on BM microenvironment play important roles in the disease progression. Gene expression profile does not clearly separate MDS and normal BM microenvironment, or RCMD and RAEB, which might be resulted from the preleukemic status of MDS or the disease heterogeneity.

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국문 초록

서론: 골수형성이상증후군은 비효과조혈과 다양한 정도의 아세포의 증가를 특징으로하는 이질적인 질환의 모임인 증후군으로, 다단계의 질환 발전 단계를 거친다. 골수형성이상증후군의 유전자 변화는 이미 상당히 연구되어 있으나, 골수형성이상증후군의 골수 미세환경의 유전자 변화에 대해서는 거의 알려진 바가 없다. 본 연구에서는, cDNA microarray 기술을 이용하여, 조혈세포 기원이 아닌 골수 기질세포의 유전자 발현 변화를 확인하고, 이를 질환의 특성과 연관하여 분석하였다.

재료 및 방법: 7명의 정상 성인과 7명의 골수형성이상증후군 환자(RCMD 3명, RAEB-1 3명, RAEB-2 1명)의 골수 조혈모세포를 배양하여, 섬유아세포로 생각되는 부착층을 얻었다. Illumina사의 humanHT-12 expression v.4 bead array를 이용하여 cDNA microarray를 시행하고 Illumina GenomeStudio v2009.2를 이용하여 분석하였으며, fold change가 1.5 초과이거나 -1.5 미만이며, p-value가 0.05미만인 유전자를 유의하게 발현의 차이가 있는 유전자(differentially expressed gene)으로 분류하였다. Ingenuity Pathway Analysis (Ingenuity Systems, www.ingenuity.com)을 이용하여, 임상적으로 의의가 있다고 판단되는 pathway의 유전자 network를 생성하고, 이에 대해 조사하였다

결과: 골수형성이상증후군(RCMD, RAEB) 환자의 골수 기질세포에서 669개의 유전자가 유의한 발현 차이를 보였다(246개의 과발현, 423개의 저발현). 인터페론 유도성 유전자의 변화가 유의하였는데, 이들의 대부분은 과발현되어 있었다. 그 외에 extracellular matrix와 cytoskeleton 구성에 관여하는 유전자, transcriptional regulation에 관여하는 유전자, *ERK*-GTPase pathway, *PI3K* pathway와 *NFkB* pathway에 관여하는 유전자의 발현 변화가 특징적이었다. 정상 골수 기질세포에 비하여 RCMD의 골수 기질세포에서는 683개의 유전자가, RAEB의 골수 기질세포에서는 740개의 유전자가 유의한 발현 차이를 보였다. 이 두 비교에서 48개의 유전자만이 공통적으로 발견되었다. RCMD에서는 주로 extracellular matrix와 cytoskeleton 구성에 관여하는 유전자, transcriptional regulation에 관여하는 유전자의 변화가, RAEB에서는 주로 인터페론 유도성 유전자의 변화가 특징적이었다. RCMD 환자와 RAEB 환자의 골수 기질세포를 직접 비교하였을 때, RAEB 환자의 골수 기질세포는 RCMD 환자의 골수 기질세포에 비하여 368개의 유전자가 유의한 발현 차이를 보였다(86개의 과발현, 282개의 저발현). Hierarchical clustering analysis를 시행하였는데, 유전자 발현 차이로는 골수형성이상증후군과 정상인의 골수 기질세포는 대략 구분되나, 일부에서는 그렇지 않았다. RCMD와 RAEB 골수 기질세포도 명확히 구분되지는 않았다.

결론 및 고찰: 본 연구는 성인 골수형성이상증후군 환자의 골수 미

세환경 세포의 유전자 변화를 cDNA array를 이용하여 연구하였으며, 다음과 같은 결론을 얻었다. 골수형성이상증후군 환자의 골수 미세환경에서는 인터페론 유도 유전자의 발현이 증가되어 있었는데, 원인은 골수형성증후군이 일종의 만성 염증상태로 인터페론을 비롯한 염증 사이토카인의 분비가 증가되어 있기 때문일 것이다. 타 pathway 관련 유전자의 발현 변화도 관찰되었는데, 이들은 골수 미세환경 기질세포의 정상적인 생존 – 사멸 균형의 혼란을 가져와 골수 미세환경의 불안정을 초래할 것이며, 골수 조혈세포와의 상호작용으로 질환의 발생과 연관이 있을 것이라 추측된다. 정상과 RCMD, RAEB를 각각 비교하였을 때, 소수의 유전자의 변화만이 공통적으로 관찰된 것은, 질환의 진행에 있어 골수 기질세포의 특정 유전자가 지속적으로 관여한다기보다는, 다른 유전자군이 관여함을 시사한다. 그러나, 유전자 발현 양상 만으로 골수형성이상증후군의 골수 미세환경과 정상인의 그것을 명확히 구분할 수 없고, 또한 RCMD와 RAEB도 명확히 구분되지 않았는데, 원인은 골수형성이상증후군이 전백혈병 단계이며, 질환의 양상 또한 다양한 것이 골수 미세환경의 유전자 발현에 반영되었기 때문일 것이다.

중심어: 골수형성이상증후군, 미세환경, 기질세포, 유전자 발현 변화

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