의학석사 학위논문

# Gene Expression Profiles of Bone Marrow Stromal Cells and Their Correlation with Clinical Characteristics of Patients with Multiple Myeloma

# 다발골수종환자의 골수 기질세포의 유전자발현양상의 특성 및 임상상과의 연관성 연구

2013년 2월

서울대학교 대학원
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**A thesis of the Degree of Master of Science**

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**February 2013**

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# Gene Expression Profiles of Bone Marrow Stromal Cells and Their Correlation with Clinical Characteristics of Patients with Multiple Myeloma

**by**
**Seon Young Kim**

**A thesis submitted to the Department of Medicine in partial fulfillment of the requirements for the Degree of Master of Science in Medicine (Laboratory Medicine) at Seoul National University College of Medicine**

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**Approved by Thesis Committee:**

**Professor _____Chairman**
**Professor _____Vice chairman**
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# ABSTRACT

**Introduction:** Multiple myeloma (MM) is a hematologic malignancy characterized by the clonal proliferation of plasma cells. Recently, increasing evidence supports the hypothesis that bone marrow (BM) microenvironmental cells play important roles in the proliferation, survival, and drug resistance of clonal plasma cells. The aim of this study is to investigate expression profiles of BM stromal cells cultured from MM patients and to explore their relationship with clinical characteristics of patients.

**Methods:** BM stromal cells were cultured from BM aspirates of 12 patients newly diagnosed with MM. As the control groups, BM stromal cells were cultured from 8 B-cell lymphoma patients without BM involvement, and 5 patients with cytopenia with no apparent evidence of hematologic malignancies were also analyzed. Fluorescence in situ hybridization (FISH) studies for *IGH*, *RB1*, 1q, *p16*, *IGH/FGFR3*, *IGH/MAF*, *TP53* were performed. RNA from BM stromal cells was extracted and gene expression profiles were analyzed using HumanHT-12 Expression v4 BeadChips (Illumina, Inc., San Diego, CA, USA).

**Results:** The growth rates of BM stromal cells from MM patients were variable between patients, and showed no apparent differences compared with control groups. Flow cytometric analysis of cultured BM stromal cells showed no expression of hematopoietic lineage antigens such as CD45 and positive expression of CD90, CD105, and CD44, which was consistent with a

mesenchymal stem cell phenotype. The stromal cells were devoid any contamination by CD138-positive plasma cells. FISH study using probes for abnormalities frequently found in MM did not show any abnormalities in BM stromal cells from MM patients. In unsupervised clustering using the results of gene expression profiles, MM and control groups did not form clearly separated clusters. Although the gene expression profiles of BM stromal cells of MM patients were heterogeneous, they showed preferential grouping into clusters reflecting characteristic clinical presentation. Many of differentially expressed genes of BM stromal cells from MM patients with multiple lytic bone lesions were associated with cell to cell interactions and formation of extracellular matrix. Differentially expressed genes of BM stromal cells from MM patients with renal failure were associated with cell proliferation. BM stromal cells from an MM patient with amyloidosis demonstrated significant higher expression levels of the lambda light chain gene.

**Conclusions:** Because BM stromal cells from MM patients did not show clonal markers identified in myeloma cells, it is unclear whether BM microenvironmental cells in MM patients are primarily tumoral. The gene expression profiles of BM stromal cells in MM patients were different between patients with different clinical presentations, and we could suggest that these genes may play important roles in MM pathogenesis and manifestation of clinical symptoms. Further study is needed to investigate the expression levels of these genes in a larger number of MM patients and to define their pathogenetic roles and prognostic significance in MM.

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**Keywords: Multiple myeloma, Microenvironment, Stromal cell, Mesenchymal stem cell, Gene expression profiling**

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| BM | bone marrow |
| FISH | fluorescence in situ hybridization |
| GO | gene ontology |
| GSEA | gene set enrichment analysis |
| ISS | International staging system |
| MGUS | monoclonal gammopathy of undetermined significance |
| MM | multiple myeloma |
| MSC | mesenchymal stem cell |
| WHO | World Health Organization |

# INTRODUCTION

Plasma cell neoplasms are hematologic malignancies characterized by the clonal proliferation of plasma cells ultimately resulting in the organ damage [1]. The plasma cell noeplasms span a broad spectrum of diseases include a precursor disorder, monoclonal gammopathy of undetermined significance (MGUS), symptomatic plasma cell myeloma or multiple myeloma (MM), asymptomatic smoldering myeloma, plasmacytoma, and the diseases characterized by the tissue immunoglobulin deposition, primary amyloidosis and light and heavy chain deposition diseases. Severity and characteristics of clinical symptoms and prognosis are variable between patients, from asymptomatic to aggressive diseases, with symptoms associated with bone lesions, bone marrow (BM) suppressions, and/or organ damages due to deposition of abnormal monoclonal immunoglobulins. Like many other malignancies, the pathogenetic roles of genetic and epigenetic abnormalities in clonal plasma cells have been extensively studied for plasma cell neoplasms. It has been known that during the development of MM, multistep genetic changes are accumulated and the early stages of disease progresses to more advanced disease, from MGUS to smoldering myeloma, and finally to symptomatic myeloma [2].

However, recently increasing evidence supports the hypothesis that microenvironmental cells play important roles in the proliferation, survival, and drug resistance of clonal plasma cells [3]. Unlike other hematopoietic malignancies, plasma cell neoplasms are unique in that malignant plasma cells

maintain dormant growth among normal hematopoietic cells for a time, and then progressively become predominant over other hematopoietic cells, through close intercommunication between the microenvironmental cells [3]. The growth privilege of myeloma cells over hematopoietic cells is the result of the concerted action of myeloma cells and BM microenvironmental cells, which eventually results in bone destruction and increasing myeloma cell burden with depletion of hematopoiesis. Myeloma cells per se produce several cytokines for their own survival, that can also stimulate myeloma growth, while microenvironmental cells actively support the growth of myeloma cells through the production of cytokines. Despite recent advance in the treatment of MM, this disease still remains incurable, largely because of the emergence of drug-resistant malignant cells [4]. There is increasing evidence supporting the role of environmental factors in the initial escapes of the malignant plasma cells from cytotoxic agents [4].

Several studies have been performed to demonstrate the distinct gene expression profiles of microenvironmental cells associated with pathogenesis of plasma cell neoplasms and development of characteristic clinical symptoms of myeloma, such as bone lesions [5-7]. The aim of this study is to investigate gene expression profiles of stromal cells cultured from BM aspirates of MM patients and to explorer their relationships with the clinical characteristics of patients.

# MATERIALS AND METHODS

## 1. Patients

Study data were collected from a total of 12 newly diagnosed MM patients (8 males and 4 females) at Seoul National University Hospital between October 2010 to August 2011. The median age of the patients was 63 years, ranging from 40 to 77 years. As control groups, 8 B-cell lymphoma patients with no evidence of BM involvement (median age 51 years, range 30-70 years) and 5 patients with mild to moderate cytopenia with no evidence of hematologic malignancies (median age 50 years, range 23-65 years) were included in this study. For all the patients BM aspirates were obtained at the time of initial diagnosis. The following information was gathered for each patient: date of diagnosis and start of therapy; age; sex; ethnicity; hemoglobin level; level and type of paraprotein; and serum levels of albumin, creatinine, calcium, lactate dehydrogenase (LDH), and β2-microglobulin. Stage was assessed according to the International Staging System (ISS) [8], and the Durie-Salmon system [9]. Furthermore, we recorded the percentage of bone marrow plasma cell infiltration, conventional cytogenetic results of BM cells by G-banding, and the number of osteolytic lesions. The BM samples were all collected with informed consent and the study was reviewed and approved by the Institutional Review Board of Seoul National University College of Medicine.

## 2. Culture and characterization of BM stromal cells

Buffy coat cells were obtained from BM aspirate samples after centrifugation and cultured in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum. After 7 to 10 days in culture non-adherent cells were removed and BM stromal cells were visible to adherent to the plasticware (this was considered as passage 0, P0) [5]. The culture medium was replaced three times a week until BM stromal cells were approximately 70% confluent. When sufficient confluency was reached cells were trypsinized (0.05% trypsin-EDTA) and expanded in a 1:2 ratio (P1). Cells were cultured until the $5.0\times10^5$ cells were obtained up to the passage 8. The cultures were maintained at 37°C and 5% $CO_2$.

Selected cultured BM stromal cells from both MM patients (n = 4) and healthy donors (n = 5) at final harvesting time were tested for surface antigen expression using flow cytometry. Combinations of monoclonal antibodies for detection of hematopoietic cells include anti-CD45-APC (allophycocyanine) (Beckman Coulter, Miami, Florida, USA), anti-CD34-PerCPCy5.5 (Beckman Coulter); for detection of plasma cells include anti-CD138-PerCPCy5.5 (Beckman Coulter); for characterization of a mesenchymal stem cell (MSC) phenotype include anti-CD44-FITC (fluorescein isothiocyanate), anti-CD29-FITC, anti-CD105-PE (phycoerythrin), anti-CD144-PE, and anti-CD90-PE (all from Becton Dickinson Biosciences Pharmingen, San Jose, CA, USA) were used. Antibody-labelled cells were acquired using a Navios flow cytometer (Beckman Coulter) and analyzed using Kaluza software (Beckman Coulter).

### 3. Fluorescence in situ hybridization (FISH)

Interphase FISH was performed on BM aspiration specimens and cultured BM stromal cells to investigate common chromosomal abnormalities known to be frequently found in MM. Commercial FISH probes: an LSI dual-color, break-apart probe for *IGH* translocations; a dual color, dual-fusion translocation probe for t(14;16)(q13;q32)/*IGH-MAF*; a dual color, dual-fusion translocation probe for t(4;14)(p16;q32) /*IGH-FGFR3*; LSI 13 (*RB1*) 13q14 probe; LSI *p53* (17p13.1); Vysis LSI *p16* (9p21) SpectrumOrange/CEP 9 SpectrumGreen probe; and LSI 1p36/1q25 probe (all from Abott Molecular/Vysis, Des Plaines, IL) were used. Plasma cells in BM aspirates were separately tested using modification of the simultaneous κ/λ immunoglobulin light chain cytoplasmic staining method (cIg FISH) according to the procedure described elsewhere [10]. Five microliters of 1:20 dilution of 1:1 mixture of polyclonal anti-human κ (Clone F0198; DakoCytomation, Glostrup, Denmark), and anti-human λ (Clone F0199; Dako) was added with incubation for 40 minutes in the dark humidified chamber. Slides were washed 2 times in phosphate-buffered saline (PBS) and dried. Slides were stained with FISH probes and counter-stained with DAPI and then fluorescence signals were analyzed by use of a fluorescence microscope (Zeiss, Göttingen, Germany). Interphase FISH signals were evaluated in 20 plasma cells. The results of the FISH studies were recorded according to the Standing Committee on Human Cytogenetic Nomenclature's International System for Human Cytogenetic Nomenclature (ISCN) [11].

## 4. RNA preparation

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

## 5. Labeling and purification

Total RNA was amplified and purified using the Ambion Illumina RNA amplification kit (Ambion, Austin, 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, USA).

## 6. Hybridization and data export

A total of 750 ng of labeled cRNA samples were hybridized to each HumanHT-12 Expression v4 BeadChips (Illumina, Inc., San Diego, CA, USA) for 16-18 h at 58°C, according to the manufacturer's instructions. 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 beadarray Reader confocal scanner according to the manufacturer's instructions.

## 7. 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 scanned data. Raw data were extracted using the software provided by the manufacturer [Illumina GenomeStudio v2009.2 (Gene Expression Module v1.5.4)].

Array data were filtered by detection p-value < 0.05 (similar to signal to noise) in at least 50% samples (we applied a filtering criterion for data analysis; higher signal value was required to obtain a detection p-value < 0.05). Selected gene signal value was transformed by logarithm and normalized by robust spline normalization (RSN) algorithm using a lumi package in Bioconductor software [12]. Statistical significance of the expression data was determined using the Significant Analysis of Microarray (SAM) test and Fold change in which the null hypothesis was that no difference exists among 2 groups [13]. Hierarchical cluster analysis was performed using complete linkage and Euclidean distance as a measure of similarity. Cancer Outlier Profile Analysis (COPA) [14] were used to detect genes showing over-expression in a subgroup of disease samples based on a cut-off threshold is defined as an outlier. Gene Set Enrichment Analysis (GSEA, Version 2.08) was used to identify gene set collection of biolocial

processes from online database available at the GSEA Web site (http://www.broadinstitute.org/gsea/) (c2.all.v3.1.symbols; curated gene sets) [15]. To define the enrichment of correlated pathways or functional program at the individual sample level, we performed the analysis of individual sample GSEA (iGSEA) to generate enrichment scores for different gene sets for individual samples, which has been described elsewhere [16]. All data analysis and visualization of differentially expressed genes was conducted using R 2.15.2 (www.r-project.org) and using SPSS version 12.0 (SPSS Inc, Chicago, IL, USA). P values $< 0.05$ were considered statistically significant.

# RESULTS

## 1. Patient characteristics

The baseline characteristics of the MM patients are summarized in Table 1. Eight males and 4 females were included in the study, with a median age of 63 years. Most of the patients had advanced disease, with 8 of them had stage III disease in ISS staging system, one of them had stage II disease, and 3 of them had stage I disease. The clinical presentations were variable among patients. Five patients presented multiple lytic bone lesions in axial bones. Two patients (patient 6 and 8) did not show bone lesions, however, they presented renal failure requiring hemodialysis. One patient (patient 3) presented mild renal impairment which do not require hemodialysis, and spinal bone fracture without evidence of multiple lytic bone lesions. Another patient (patient 10) had multiple plasmacytoma in bones along with increase in BM plasma cells. There was one patient presenting as amyloidosis (patient 12), with cardiomyopathy due to deposition of amyloid proteins. Patient 11 presented marked increase of plasma cells in BM, however, she did not presented bone lesions or renal symptoms. Another patient (patient 2) did not presented clinical symptoms, therefore he classified as asymptomatic MM.

**Table 1. Characteristics of MM patients.**

| Patients | Age | Sex | Clinical presentation | ISS | Ig | M-protein (g/L) | Cr (mg/dL) | Ca (mg/dL) | Hgb (g/dL) | Alb (g/dL) | β2m (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 73 | M | Bone lesions | III | Gκ | 87.5 | 1.65 | 10.0 | 7.1 | 2.6 | 9.12 |
| 2 | 55 | M | Asymptomatic | I | Gλ | 12.5 | 0.93 | 9.7 | 14.3 | 4.5 | 2.05 |
| 3 | 68 | F | Bone lesions, Renal impairment | III | κ | 2.7 | 2.55 | 8.8 | 7.3 | 3.2 | 11.89 |
| 4 | 62 | F | Bone lesions | III | Aκ | 68.9 | 1.04 | 11.9 | 7.4 | 2.9 | 6.58 |
| 5 | 54 | F | Bone lesions | III | Gλ | 65.6 | 0.80 | 9.0 | 7.4 | 10.7 | 6.08 |
| 6 | 71 | M | Renal impairment | III | λ | 5.3 | 6.91 | 9.7 | 8.1 | 4.1 | 18.36 |
| 7 | 59 | M | Bone lesions | III | λ | 32.7 | 1.80 | 10.2 | 9.0 | 4.4 | 5.95 |
| 8 | 40 | M | Renal impairment | III | λ | 9.2 | 11.10 | 9.0 | 9.4 | 3.7 | 23.35 |
| 9 | 73 | M | Bone lesions | I | Gκ | 0.3 | 0.78 | 10.1 | 14.4 | 4.1 | 2.52 |
| 10 | 61 | M | Plasmacytoma | I | Aκ | 2.7 | 0.84 | 9.1 | 10.8 | 4.1 | 1.59 |
| 11 | 77 | F | Anemia | III | Gκ | 51.4 | 2.25 | 10.4 | 8.2 | 3.3 | 9.87 |
| 12 | 63 | M | Amyloidosis | II | Gλ | 16.8 | 0.98 | 9.7 | 11.8 | 3.5 | 3.68 |

Abbreviations: Alb, albumin; BM, bone marrow; β2m, β2 microglobulin; Cr, creatinine; Ca, calcium; F, female, Hgb, hemoglobin; Ig, immunoglobulin; ISS, international staging system for multiple myeloma; M, male

## 2. Growth and phenotypic characteristics of BM stromal cells in MM patients

The growth rates of stromal cells from MM patients were variable between patients, and showed no apparent differences compared with control groups. Flow cytometric analysis of BM stromal cells showed no expression of hematopoietic lineage antigens such as CD45 and positive expression of CD90, CD105, and CD44 , which was consistent with a MSC phenotype. The stromal cells were devoid any contamination by CD138-positive plasma cells.

**Table 2. Culture characteristics of BM stromal cells.**

| | Multiple myeloma (n = 12) | Control -1* (n = 8) | Control -2† (n = 5) |
|---|---|---|---|
| Culture days | 53 (22-110) | 45 (24-64) | 61 (53-62) |
| Passages | 6 (5-8) | 6 (5-8) | 7 (4-7) |
| Fianl cell counts ($\times 10^5$ cells) | 10.1 (1.3-91.8) | 13.5 (11.3-1002.6) | 8.4 (3.0-11.6) |

*Control-1: B-cell lymphoma patients without bone marrow involvement.

†Control-2: Patients with cytopenia without hematologic malignancies

Data are presented as median (range).

### 3. Cytogenetic characteristics of BM plasma cells and BM stromal cells in MM patients

Cultured BM stromal cells were tested using interphase FISH to examine the presence of specific cytogenetic alterations found in the matching BM malignant plasma cells. Among a total of 12 MM patients, 5 patients presented complex abnormalities using conventional cytogentic testing, and 7 patients showed abnormalities in FISH results, including *IGH* translocations (n = 6), *RB1* deletions (n = 5), and 1q abnormalities (n = 5) (Table 3). We tested for BM stromal cells from 10 MM patients, however, none of them presented the cytogenetic abnormalities present in the corresponding malignant plasma cells. In addition, BM stromal cells from control groups also showed no abnormalities in the interphase FISH studies.

**Table 3. Cytogenetic characteristics of BM plasma cells and BM stromal cells.**

| | BM plasma cells | | BM stromal cells |
|---|---|---|---|
| Patients | G-banding | FISH | FISH |
| 1 | 46,XY[20] | Normal | Normal |
| 2 | 46,XY[20] | Trisomy 9: 90% | Normal |
| 3 | 46,XX[7] | del *RB1*: 100% | Normal |
| 4 | 46,XX,+1,dic(1;22)(p11;p11.1),der(2)t(2;8)(p12;q24),der(8)del(8)(p21)t(2;8),+9,-11,,del(11)(q23),-12,-13,+15,der(17)t(1;17)(q12;p13),der(20)t(19;20)(p13.1;p13),der(?)t(?;11)(?;q13)[7]/46,XX[2] | t(4;14): 95%; del *RB1*: 100%; Trisomy 9: 100%; dup(1q): 100% | Normal |
| 5 | 46,XX[8] | t(4;14): 88%; del *RB1*: 100%; dup(1q): 100% | Normal |
| 6 | 46,XY[8] | *IGH* rearrnagment: 100%; del *RB1*: 98%; dup(1q): 100% | Normal* |
| 7 | 46,X,der(?Y;6)(q10;p10),t(2;17)(p10;p10),t(3;10)(p13;p13),der(6)del(6)(q15q21)?inv(6)(q21q25),t(11;14)(q13;q32)[8]/47,sl,+der(3)t(3;10)[2]/47,sl,+18[3]/46,XY[4] | *IGH* rearrnagment: 100%; del *TP53*: 100% | Normal |
| 8 | 46,XY,+1,der(1;16)(q10;p10),t(11;14)(q13;q32),add(19)(q13.3)[8]/46,XY[12] | *IGH* rearrnagment: 100%; dup(1q): 100% | Normal |
| 9 | 46,XY[20] | Normal | Normal |
| 10 | 47,XY,+der(1;19)(q10;p10)X2,add(6)(q13),add(11)(p15),-13,add(19)(q13.3)[4]/46,XY[16] | *IGH* rearrnagment: 50%; del *RB1*: 60%; dup(1q): 50% | Normal |
| 11 | 46,XX,1cenh+,der(3)t(3;19)(q21;p13.1),-14,?del(16)(q24),-19,+22,der(?)t(?;3)(?;q21)[6]/46,XX[16] | Normal | NA |
| 12 | 46,XX[20] | Normal | NA |

Abbreviations: BM, bone marrow; FISH, fluorescence in situ hybridization; NA, not assessed

*For this sample, minor population of tetraploidy (5%) were found in chromosome 1, however, this finding was considered as a non-specific finding.

## 4. Gene expression profiles of BM stromal cells from MM patients

The gene expression profiles of BM stromal cells from 12 MM patients were compared with 13 BM stromal cells from control groups of no apparent malignant cells in BM. An unsupervised analysis of the probe sets with high variation between samples (variance ≥ 50%) was first performed on MM and control BM stromal cells. MM and control groups did not form clearly separated clusters. Meanwhile, BM stromal cells from MM patients showed preferential grouping into several main clusters according to characteristic clinical presentations. BM stromal cells from patients with multiple lytic bone lesions and those from patients with renal failure were separated as different clusters. In addition, BM stromal cells from an MM patient with amyloidosis presented differential gene expression profiles separated from other clusters. We investigated the genes differentially expressed between MM and normal BM stromal cells, and between MM samples with different clinical presentations using supervised analysis. It was impossible to select genes which were universally overexpressed or underexpressed for all BM stromal cells from MM patients. Most of the genes were overexpressed in some of MM samples as compared to control groups. Many of differentially expressed genes in BM stromal cells from MM patients with multiple bone results are involved in cell to cell interactions and formation of extracellular matrix. Differentially expressed genes in BM stromal cells from MM patients with renal failure was associated with cell proliferation. BM stromal cells from an MM patient with amyloidosis demonstrated significant higher expression levels of the lambda light chain gene.

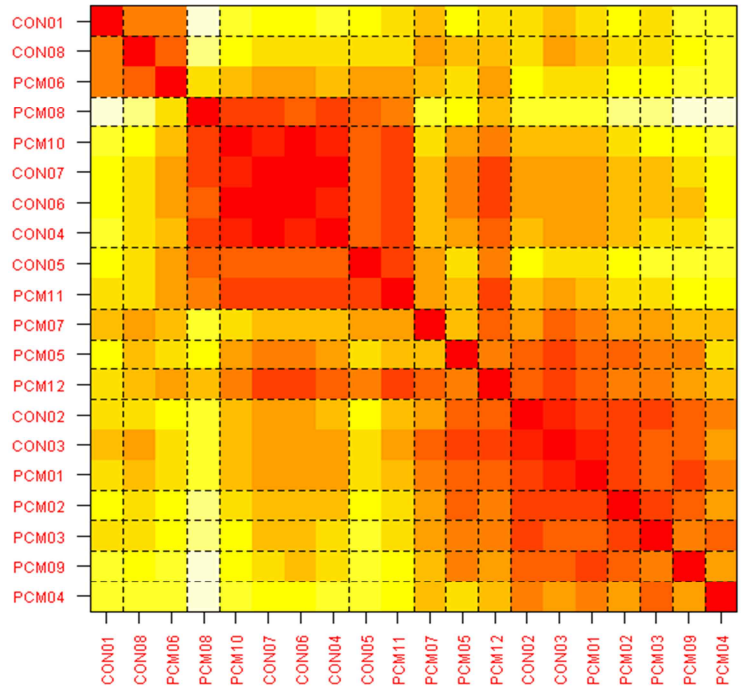



**Figure 1. Unsupervised analysis of gene expression profiles of BM stromal cells from MM patients and control samples.**

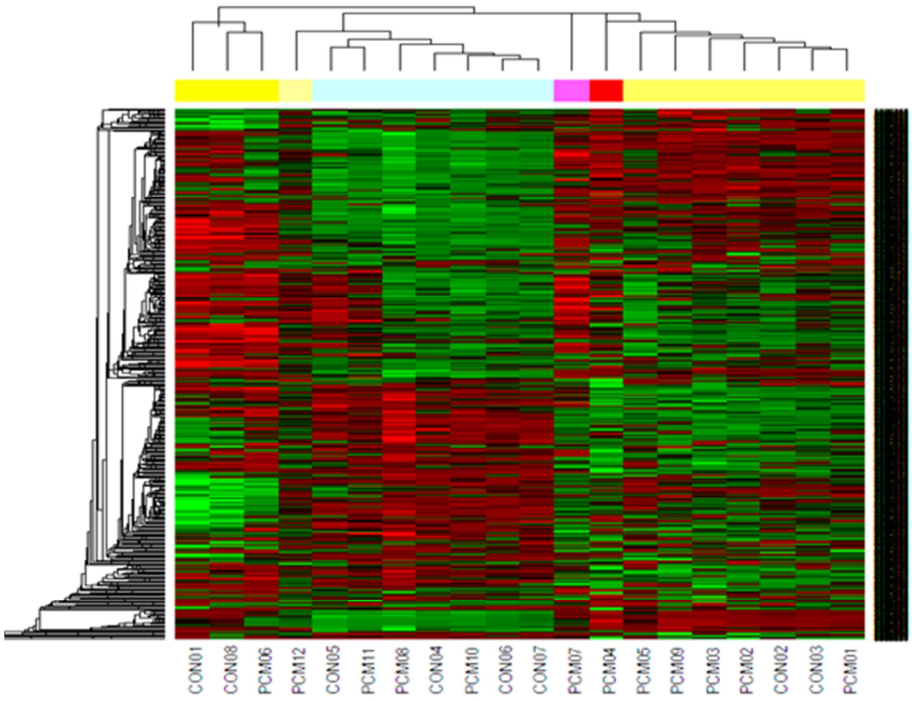



**Figure 2. Gene expression profiles of BM stromal cells from MM patients and control patients.**

**Table 4. Differentially expressed genes of BM stromal cells from MM patients with lytic bone lesions.**

| Gene symbol | Entrez id | Score (*d*) | Fold change | Chromosome | Description |
|---|---|---|---|---|---|
| **Overexpressed** | | | | | |
| COL4A4 | 1286 | 4.671 | 1.92 | 2q36.3 | collagen, type IV, alpha 4 |
| VCAM1 | 7412 | 3.715 | 3.29 | 1p21.2 | vascular cell adhesion molecule 1 |
| SEMA3C | 10512 | 3.417 | 2.18 | 7q21.11 | sema domain, immunoglobulin domain, short basic domain, secreted, (semaphorin) 3C |
| POSTN | 10631 | 3.410 | 2.89 | 13q13.3 | periostin, osteoblast specific factor |
| RGS5 | 8490 | 3.401 | 1.43 | 1q23.3 | regulator of G-protein signaling 5 |
| FOXS1 | 2307 | 3.399 | 2.08 | 20q11.21 | forkhead box S1 |
| KCTD16 | 57528 | 3.352 | 1.44 | 5q32 | potassium channel tetramerisation domain containing 16 |
| PPFIBP2 | 8495 | 3.316 | 2.10 | 11p15.4 | PTPRF interacting protein, binding protein 2 (liprin beta 2) |
| 11-Sep | 55752 | 3.315 | 1.83 | 4q21.1 | septin 11 |
| FBLN5 | 10516 | 3.296 | 2.11 | 14q32.12 | fibulin 5 |
| PLEKHA1 | 59338 | 3.175 | 1.60 | 10q26.13 | pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 1 |
| RALGAPA1 | 253959 | 3.173 | 1.28 | 14q13.2 | Ral GTPase activating protein, alpha subunit 1 |
| FRY | 10129 | 3.109 | 1.37 | 13q13.1 | furry homolog (Drosophila) |
| GDF6 | 392255 | 3.104 | 1.47 | 8q22.1 | growth differentiation factor 6 |
| MARVELD2 | 153562 | 3.057 | 2.61 | 5q13.2 | MARVEL domain containing 2 |
| PLAG1 | 5324 | 3.027 | 1.47 | 8q12.1 | pleiomorphic adenoma gene 1 |
| STX6 | 10228 | 2.994 | 1.34 | 1q25.3 | syntaxin 6 |
| SEMA5A | 9037 | 2.943 | 1.69 | 5p15.2 | sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5A |
| PGRMC2 | 10424 | 2.942 | 1.55 | 4q28.2 | progesterone receptor membrane component 2 |
| NUAK1 | 9891 | 2.941 | 1.67 | 12q23.3 | NUAK family, SNF1-like kinase, 1 |
| RECK | 8434 | 2.910 | 2.13 | 9p13.3 | reversion-inducing-cysteine-rich protein with kazal motifs |
| OSBPL2 | 9885 | 2.901 | 1.39 | 20q13.33 | oxysterol binding protein-like 2 |
| FBN1 | 2200 | 2.892 | 1.64 | 15q21.1 | fibrillin 1 |
| BTLA | 151888 | 2.892 | 1.32 | 3q13.2 | B and T lymphocyte associated |
| TRPC6 | 7225 | 2.826 | 1.40 | 11q22.1 | transient receptor potential cation channel, subfamily C, member 6 |
| NCOA3 | 8202 | 2.815 | 1.29 | 20q13.12 | nuclear receptor coactivator 3 |

**Underexpressed**

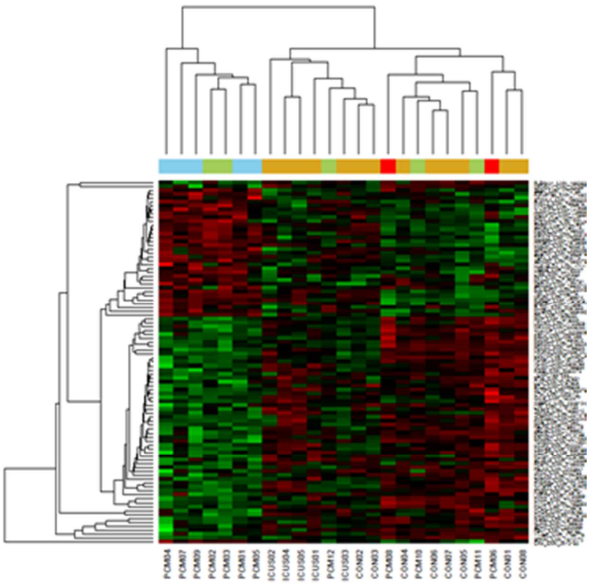
| | | | | | |
|---|---|---|---|---|---|
| HMOX1 | 3162 | -3.352 | 0.39 | 22q12.3 | heme oxygenase (decycling) 1 |
| LY96 | 23643 | -3.155 | 0.60 | 8q21.11 | lymphocyte antigen 96 |
| PANX2 | 56666 | -3.105 | 0.65 | 22q13.33 | pannexin 2 |
| PODXL | 5420 | -3.058 | 0.46 | 7q32.3 | podocalyxin-like |
| LYN | 4067 | -2.965 | 0.63 | 8q12.1 | v-yes-1 Yamaguchi sarcoma viral related oncogene homolog |
| NPC2 | 10577 | -2.947 | 0.76 | 14q24.3 | Niemann-Pick disease, type C2 |
| FOXD2 | 2306 | -2.898 | 0.58 | 1p33 | forkhead box D2 |
| NMB | 4828 | -2.887 | 0.66 | 15q25.2-q25.3 | neuromedin B |
| SDHC | 6391 | -2.860 | 0.81 | 1q23.3 | succinate dehydrogenase complex, subunit C, integral membrane protein, 15kDa, nuclear gene encoding mitochondrial protein |
| B4GALNT1 | 2583 | -2.825 | 0.73 | 12q14.1 | beta-1,4-N-acetyl-galactosaminyl transferase 1 |
| DUSP23 | 54935 | -2.796 | 0.79 | 1q23.2 | dual specificity phosphatase 23 |
| SBSN | 374897 | -2.787 | 0.52 | 19q13.12 | suprabasin |
| SCARA3 | 51435 | -2.776 | 0.57 | 8p21.1 | scavenger receptor class A, member 3 |
| RPL26L1 | 51121 | -2.764 | 0.80 | 5q35.2 | ribosomal protein L26-like 1 |
| CHCHD5 | 84269 | -2.750 | 0.79 | 2q13 | coiled-coil-helix-coiled-coil-helix domain containing 5 |
| ICAM2 | 3384 | -2.729 | 0.62 | 17q23.3 | intercellular adhesion molecule 2 |
| TXN | 7295 | -2.719 | 0.71 | 9q31.3 | thioredoxin |
| STOML2 | 30968 | -2.643 | 0.79 | 9p13.3 | stomatin (EPB72)-like 2 |
| FMNL3 | 91010 | -2.628 | 0.83 | 12q13.12 | formin-like 3 |
| MIR423 | 494335 | -2.607 | 0.80 | 17q11.2 | microRNA 423, microRNA. |

**Table 5. Differentially expressed genes of BM stromal cells from MM patients with renal impairment.**

| Gene symbol | Entrez id | Score (*d*) | Fold change | Chromosome | Description |
|---|---|---|---|---|---|
| **Overexpressed** | | | | | |
| NPTX1 | 4884 | 6.200 | 2.28 | 17q25.3 | neuronal pentraxin I |
| RGS17 | 26575 | 4.682 | 1.90 | 6q25.2 | regulator of G-protein signaling 17 |
| KCNJ8 | 3764 | 3.947 | 1.48 | 12p12.1 | potassium inwardly-rectifying channel, subfamily J, member 8 |
| SRGAP3 | 9901 | 3.853 | 1.56 | 3p25.3 | SLIT-ROBO Rho GTPase activating protein 3 |
| HSPA6 | 3310 | 3.756 | 2.03 | 1q23.3 | heat shock 70kDa protein 6 (HSP70B') |
| IGF2BP3 | 10643 | 3.677 | 2.90 | 7p15.3 | insulin-like growth factor 2 mRNA binding protein 3 |
| EIF6 | 3692 | 3.670 | 1.54 | 20q11.22 | eukaryotic translation initiation factor 6, transcript variant 4 |
| UBE2E3 | 10477 | 3.558 | 1.96 | 2q31.3 | ubiquitin-conjugating enzyme E2E 3 (UBC4/5 homolog, yeast) |
| LOC728312 | 728312 | 3.444 | 1.39 | | PREDICTED: miscRNA. |
| GSTCD | 79807 | 3.406 | 1.41 | 4q24 | glutathione S-transferase, C-terminal domain containing |
| MMP3 | 4314 | 3.276 | 2.27 | 11q22.2 | matrix metallopeptidase 3 (stromelysin 1, progelatinase) |
| CCDC15 | 80071 | 3.264 | 1.37 | 11q24.2 | coiled-coil domain containing 15 |
| NUDT6 | 11162 | 3.262 | 1.43 | 4q28.1 | nudix (nucleoside diphosphate linked moiety X)-type motif 6 (NUDT6), transcript variant 2 |
| MUC5AC | 4586 | 3.131 | 1.86 | 11p15.5 | PREDICTED: mucin 5AC, oligomeric mucus/gel-forming |
| TH1L | 51497 | 3.123 | 1.31 | 20q13.32 | TH1-like (Drosophila) |
| SNORA7B | 677797 | 3.113 | 1.51 | 3q21.3 | small nucleolar RNA, H/ACA box 7B (SNORA7B), small nucleolar RNA. |
| PTGR1 | 22949 | 3.010 | 2.53 | 9q31.3 | prostaglandin reductase 1 |
| MRPL11 | 65003 | 3.001 | 1.52 | 11q13.1 | mitochondrial ribosomal protein L11, nuclear gene encoding mitochondrial protein |
| AP1S2 | 8905 | 3.000 | 1.93 | Xp22.2 | adaptor-related protein complex 1, sigma 2 subunit |
| LOC100128765 | 100128765 | 2.989 | 1.40 | 13q12.11 | PREDICTED: similar to hCG2041516 |
| C6orf1 | 221491 | 2.975 | 1.23 | 6p21.31 | chromosome 6 open reading frame 1 |
| OPRM1 | 4988 | 2.930 | 1.28 | 6q25.2-q25.2 | opioid receptor, mu 1, transcript variant MOR-1X |
| PHLDA1 | 22822 | 2.924 | 2.44 | 12q21.2 | pleckstrin homology-like domain, family A, member 1 |

| | | | | | |
|---|---|---|---|---|---|
| MRPL20 | 55052 | 2.921 | 1.44 | 1p36.33 | mitochondrial ribosomal protein L20, nuclear gene encoding mitochondrial protein |
| CKS1B | 1163 | 2.915 | 3.01 | 1q21.3 | CDC28 protein kinase regulatory subunit 1B |
| H2AFX | 3014 | 2.886 | 1.53 | 11q23.3 | H2A histone family, member X |
| **Underexpressed** | | | | | |
| COL1A1 | 1277 | -4.338 | 0.44 | 17q21.33 | collagen, type I, alpha 1 |
| COL5A1 | 1289 | -4.060 | 0.52 | 9q34.3 | collagen, type V, alpha 1 |
| RBMS3 | 27303 | -3.994 | 0.71 | 3p24.1-p24.1 | RNA binding motif, single stranded interacting protein |
| ARHGEF10 | 9639 | -3.842 | 0.63 | 8p23.3 | Rho guanine nucleotide exchange factor (GEF) 10 |
| LOX | 4015 | -3.463 | 0.50 | 5q23.1 | lysyl oxidase |
| PLOD1 | 5351 | -3.454 | 0.48 | 1p36.22 | procollagen-lysine 1, 2-oxoglutarate 5-dioxygenase 1 |
| ASNS | 440 | -3.413 | 0.41 | 7q21.3 | asparagine synthetase |
| COL12A1 | 1303 | -3.388 | 0.69 | 6q13-q14.1 | collagen, type XII, alpha 1 |
| PSAT1 | 29968 | -3.292 | 0.36 | 9q21.2 | phosphoserine aminotransferase 1 |
| PCK2 | 5106 | -3.266 | 0.35 | 14q12 | phosphoenolpyruvate carboxykinase 2 (mitochondrial), nuclear gene encoding mitochondrial protein, transcript variant 1 |
| TRIB3 | 57761 | -3.253 | 0.26 | 20p13 | tribbles homolog 3 |
| MFAP5 | 8076 | -3.206 | 0.28 | 12p13.31 | microfibrillar associated protein 5 |
| SLC7A5 | 8140 | -3.110 | 0.43 | 16q24.2 | solute carrier family 7 (cationic amino acid transporter, y+ system), member 5 |
| EPHX2 | 2053 | -3.086 | 0.79 | 8p21.1 | epoxide hydrolase 2, cytoplasmic |
| SLC6A9 | 6536 | -3.081 | 0.39 | 1p34.1 | solute carrier family 6 (neurotransmitter transporter, glycine), member 9 |
| GARS | 2617 | -3.046 | 0.62 | 7p15.1 | glycyl-tRNA synthetase |
| FLG | 2312 | -3.036 | 0.13 | 1q21.3 | filaggrin |
| PGCP | 10404 | -3.035 | 0.49 | 8q22.1-q22.1 | plasma glutamate carboxypeptidase |
| KIAA1598 | 57698 | -3.034 | 0.53 | 10q25.3 | KIAA1598 |
| EFEMP1 | 2202 | -3.014 | 0.32 | 2p16.1 | EGF-containing fibulin-like extracellular matrix protein 1 |
| CD81 | 975 | -3.005 | 0.64 | 11p15.5 | CD81 molecule |

**(A)**



**(B)**

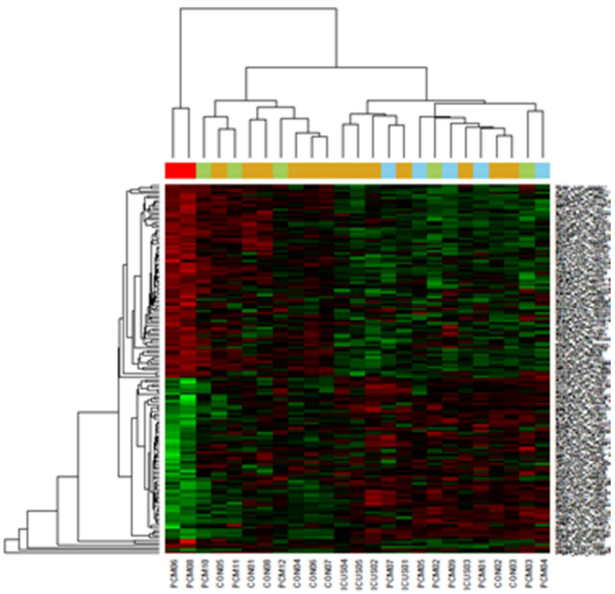


**Figure 3. Differentially expressed genes of BM stromal cells from (A) MM patients with bone lesions and from (B) patients with renal impairment.**

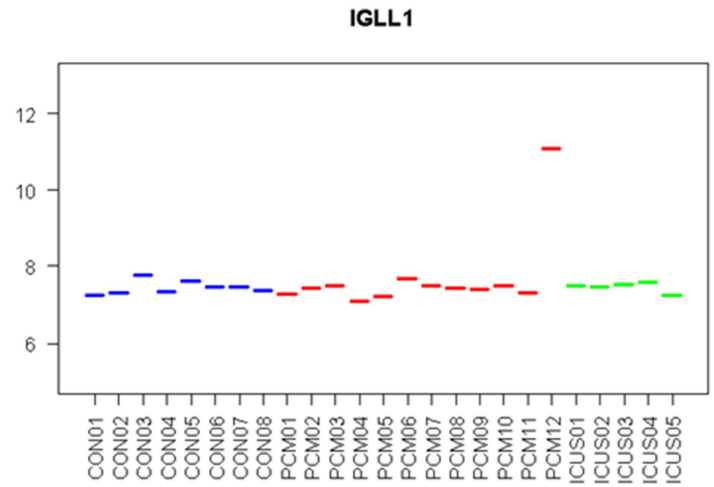



**Figure 4. Expression levels of immunoglubulin lambda light chain genes in all samples.**

## 5. Gene set enrichment analysis using gene expression data

To investigate biologic features shared by BM stromal cells from MM patients with characteristic clinical presentations, we performed GSEA. This algorithm identified a number of significant gene sets elevated according to different groups (Table 6 and Table 7). To identify which gene sets are significantly associated with each patient, we analyse the individual sample GSEA and several gene sets were clustered together (Figure 5). The enriched gene sets of BM stromal cells from MM patients with bone lesions were involved in the biologic processes of extracellular matrix formation and organization, cell adhesion, skeletal system development, and TGFβ signaling pathways. On the other hand, the enriched gene sets of BM stromal cells from MM patients with renal impairment were associated with regulation of cell cycles, mitosis, regulation of transcription and translation.

**Table 6. Top gene sets of BM stromal cells associated with bone lesions.**

| Name | NES | NOM p-val | FDR q-val |
|---|---|---|---|
| ZHAN_MULTIPLE_MYELOMA_PR_DN | 1.787 | 0.002 | 1.000 |
| WENDT_COHESIN_TARGETS_UP | 1.768 | 0.000 | 0.895 |
| SOTIRIOU_BREAST_CANCER_GRADE_1_VS_3_DN | 1.757 | 0.004 | 0.711 |
| KEGG_SELENOAMINO_ACID_METABOLISM | 1.710 | 0.000 | 1.000 |
| GARGALOVIC_RESPONSE_TO_OXIDIZED_PHOSPHOLIPIDS_ MAGENTA_DN | 1.707 | 0.006 | 0.897 |
| GENTLES_LEUKEMIC_STEM_CELL_UP | 1.701 | 0.006 | 0.810 |
| NIKOLSKY_BREAST_CANCER_19Q13.1_AMPLICON | 1.700 | 0.019 | 0.706 |
| KIM_MYC_AMPLIFICATION_TARGETS_DN | 1.691 | 0.000 | 0.698 |
| PEPPER_CHRONIC_LYMPHOCYTIC_LEUKEMIA_UP | 1.691 | 0.010 | 0.623 |
| BROWNE_HCMV_INFECTION_24HR_DN | 1.691 | 0.000 | 0.562 |
| BIOCARTA_PPARA_PATHWAY | 1.674 | 0.000 | 0.640 |
| BIOCARTA_PAR1_PATHWAY | 1.669 | 0.008 | 0.628 |
| PID_IL6_7PATHWAY | 1.659 | 0.015 | 0.663 |
| PID_ENDOTHELINPATHWAY | 1.655 | 0.002 | 0.645 |
| CAIRO_PML_TARGETS_BOUND_BY_MYC_DN | 1.653 | 0.006 | 0.617 |
| ZHAN_MULTIPLE_MYELOMA_CD2_UP | 1.647 | 0.010 | 0.628 |
| SARTIPY_BLUNTED_BY_INSULIN_RESISTANCE_DN | 1.647 | 0.004 | 0.592 |
| VANHARANTA_UTERINE_FIBROID_DN | 1.642 | 0.004 | 0.590 |
| REACTOME_TRAF6_MEDIATED_NFKB_ACTIVATION | 1.639 | 0.002 | 0.585 |
| ABDELMOHSEN_ELAVL4_TARGETS | 1.637 | 0.012 | 0.568 |
| REACTOME_TIGHT_JUNCTION_INTERACTIONS | 1.634 | 0.012 | 0.562 |
| CADWELL_ATG16L1_TARGETS_UP | 1.633 | 0.004 | 0.546 |

Abbreviations: NES, normalized enrichment score; NOM p-value, nomial p-value; FDR, false discovery rate

**Table 7. Top gene sets of BM stromal cells associated with renal impairment.**

| Name | NES | NOM p-val | FDR q-val |
|---|---|---|---|
| CARDOSO_RESPONSE_TO_GAMMA_RADIATION_AND_3AB | 1.616 | 0.004 | 1.000 |
| MIPS_MLL1_WDR5_COMPLEX | 1.616 | 0.031 | 1.000 |
| GAZIN_EPIGENETIC_SILENCING_BY_KRAS | 1.611 | 0.000 | 1.000 |
| REACTOME_INHIBITION_OF_INSULIN_SECRETION_BY_ADRENALINE_NORADRENALINE | 1.600 | 0.028 | 1.000 |
| HEDENFALK_BREAST_CANCER_BRACX_DN | 1.596 | 0.007 | 1.000 |
| REACTOME_PYRIMIDINE_METABOLISM | 1.581 | 0.000 | 1.000 |
| MIPS_LARGE_DROSHA_COMPLEX | 1.571 | 0.034 | 1.000 |
| WIERENGA_PML_INTERACTOME | 1.567 | 0.000 | 1.000 |
| LIANG_HEMATOPOIESIS_STEM_CELL_NUMBER_LARGE_VS_TINY_DN | 1.557 | 0.014 | 1.000 |
| MOOTHA_FFA_OXYDATION | 1.555 | 0.019 | 1.000 |
| MATTHEWS_AP1_TARGETS | 1.536 | 0.002 | 1.000 |
| REACTOME_TRANSPORT_OF_MATURE_MRNA_DERIVED_FROM_AN_INTRONLESS_TRANSCRIPT | 1.536 | 0.072 | 1.000 |
| HOLLEMAN_VINCRISTINE_RESISTANCE_ALL_DN | 1.516 | 0.058 | 1.000 |
| REACTOME_DOUBLE_STRAND_BREAK_REPAIR | 1.513 | 0.011 | 1.000 |
| MIPS_RNA_POLYMERASE_II_COMPLEX_CHROMATIN_STRUCTURE_MODIFYING | 1.506 | 0.004 | 1.000 |

Abbreviations: NES, normalized enrichment score; NOM p-value, nomial p-value; FDR, false discovery rate

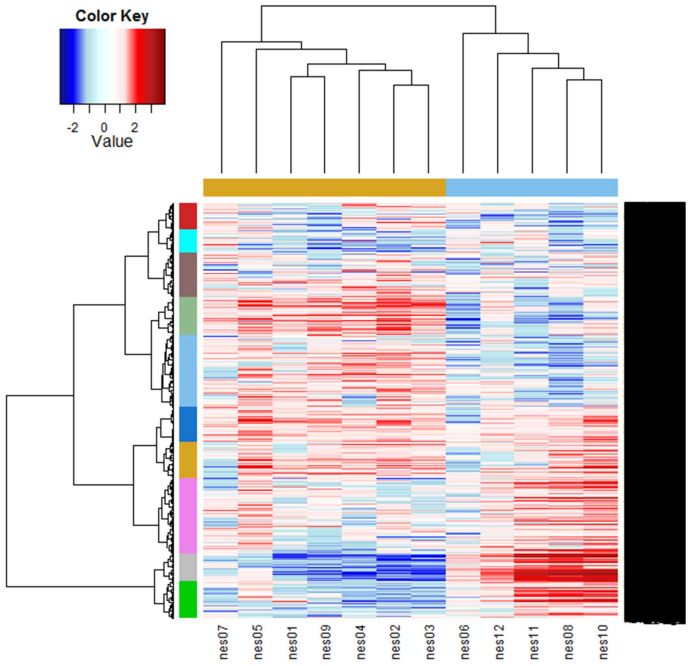



**Figure 5. Clustering analysis of enriched gene sets.** Each row is a gene set and the color coding in the heatmap is based on enrichment score of each gene sets. The clusters of gene sets are indicated and dendrogram on the left and the different colors on the side bar.

# DISCUSSION

This study aimed to evaluate the gene expression characteristics of BM stromal cells from MM patients. These cells presented no clearly different patterns in gene expression compared with control samples, and the differentially expressed genes were heterogeneous among patients. The characteristics of gene expression profiles were different according to the clinical presentations of MM patients.

The importance of BM microenvironment in MM [3, 17] and that of the surrounding microenvironment in solid tumors as key determinants in the progression of cancer is widely accepted. Several studies on BM MSCs from MM patients were previously reported. In one study of MSCs from 11 MM patients, transient atypical surface antigen expression were observed, however the proliferative capacity appeared normal [18]. Many studies demonstrated that an abnormal production of cytokines was thought to dependent on close contact with malignant plasma cells [19, 20]. However, it is still unclear that whether particular genetic or epigenetic abnormalities in BM microenvironmental cells are involved in MM pathogenesis or particular genetic pathways play critical roles in some course of myeloma progression.

There were a few studies investigated the differences of gene expression in BM MSCs from normal samples and from MM patients. In one study, BM MSCs from MM patients presented distinctive gene expression profiles using microarray analysis compared with those of normal donors [21]. In this study,

unsupervised methods classified genes in BM MSCs from MM patients into distinct a distinctive cluster, and reported that more than 140 genes were differentially expressed between MM and normal MSCs. In another study, in the investigation of gene expression profiles associated with bone lesions, MSCs but not osteoblasts have different transcriptional patterns depending on bone lesions [7].

In our results, BM stromal cells from MM patients did not demonstrate clear and universal differences in gene expression compared with those of control samples. Because the gene expression characteristics of BM stromal cells were variable between specimens, we suggest that genetic abnormalities with very strong effects to determine particular sample as MM do not exist in our study population. We postulate that it is not unrational, because BM stromal cells are not likely to primarily tumoral, which means that it is less likely to demonstrate very distinctive deviation from control population. In addition, the intrinsic heterogeneity of BM microenvironmental cells between individuals and different reactions after in vitro culture made the interpretation of gene expression profiles difficult. However, in our results, BM stromal cells from MM patients with different symtptoms presented different gene expression patterns. We postulated that microenvironmental cells may play important roles to determine the presentation of MM. Man of the overexpressed genes in BM stromal cells from MM patients with bone lesions were associated with formation and organization of extracellular matrix proteins and cell adhesion processes. This finding suggested that BM stromal cells play important roles for manifestation of symptoms of the

disease. On the contrary, BM stromal cells from MM patients with renal failure and without bone lesions presented overexpression of genes associated with cell cycle and mitosis. This gene expression pattern could not be associated to the disease presentation as simply and directly as the gene expression patterns associated with bone lesions. However, it can provide interesting points to design further investigation.

One of the interesting findings of this study was that BM stromal cells from a patient presented as amyloidosis demonstrated distinctly higher expression of the immunoglobulin lambda light chain gene. It was an unexpected finding because we regarded microenvironmental cells are not primarily tumoral cells. We cannot clearly the underlying mechanisms of this phenomenon, we can suggest several hypotheses. It can be just a false positive results or due to subtle plasma cell contamination. However, it can have more significant pathogenic events, such as plasma cell transformation or direct involvement of microenvironment cells themselves in the production and deposition of immunoglobulin light chains. Because the pathogenic mechanisms leading to heavy light chain deposition in various tissues in immunoglobulin deposition diseases have not thoroughly determined [22], the findings of this study may provide interesting insights for future studies.

Because BM stromal cells from MM patients did not show clonal markers identified in myeloma cells, it is unclear whether BM microenvironment cells in MM patients are primarily tumoral. The gene expression profiles of BM stromal cells in MM patients were different between patients with different clinical presentations, and we could suggest that these genes may play

important roles in MM pathogenesis and manifestation of clinical symptoms. Further study is needed to investigate the expression levels of these genes in larger number of MM patients and to define their pathogenetic roles and prognostic significance in MM.

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# 국문 초록

**서론:** 다발골수종은 형질세포의 단클론성 증식에 의하여 발생하는 혈액종양 질환이다. 최근 골수미세환경이 종양의 증식과 생존 및 약물 저항성에 중요한 역할을 하고 있다는 증거가 널리 알려 지고 있다. 이 연구에서는 다발골수종 환자의 골수 흡입액으로부터 배양한 골수 기질세포의 유전자 발현 양상에 대하여 조사하였고, 유전자 발현 양상과 환자의 임상적 특성 사이의 관계를 분석하였다.

**방법:** 12 명의 다발골수종 환자 및 8 명의 골수 침범이 없는 림프종 환자와 혈액종양의 증거 없이 범혈구감소증을 보이는 5 명의 환자에서 얻은 골수흡입액에서 기질세포를 배양하였다. 배양한 세포에 대하여 형광동소교잡법을 이용하여 *IGH*, *RB1*, 1q, *p16*, *IGH/FGFR3*, *IGH/MAF*, *TP53* 유전자 이상에 대한 검사를 시행하였다. 배양된 골수 기질세포에서 RNA 를 추출하여 cDNA microarray (HumanHT-12 Expression v4 BeadChips, Illumina, Inc., San Diego, CA, USA) 분석을 시행하였다.

**결과:** 배양 시 골수 기질세포의 배양 속도는 다발골수종 환자들 간에도 다소 변이를 보였으나 대조군과 비교하였을 때는 유의한 차이를 보이지 않았다. 배양한 골수 기질세포에 대하여 유세포 분석을 시행하였을 때 CD45 등의 혈액세포에서 발현하는 항원의

발현은 보이지 않았고, 중간엽 줄기세포에서 발현하는 CD90, CD105 와 CD44 항원의 발현을 확인할 수 있었다. CD138 양성 형질세포 또한 발견되지 않았다. 다발골수종에서 흔히 발견되는 유전적 이상에 대한 형광동소교잡검사에서 골수 기질세포의 경우 악성 형질세포에서 발견되었던 유전적 이상이 관찰되지 않았고 모두 정상 결과를 나타내었다. cDNA microarray 결과를 분석하였을 때, 골수 기질세포의 유전자 발현 양상은 환자에 따라 다양하였고, 정상세포의 유전자 발현 양상이 명확히 구분되지 않았으나, 환자의 임상상에 따라 구분될 수 있는 분리된 군으로 구분할 수 있었다. 골용해성 병변을 가진 환자에서 얻은 골수 중간엽 줄기 세포의 경우 세포간 상호작용이나 세포 외 기질 형성과 관여된 유전자에서 발현 차이를 나타내었고, 신부전을 나타내는 환자의 경우 주로 세포 분열과 관계된 유전자에서 발현 양상에 차이를 나타내었다. 아밀로이드증을 발현한 환자의 경우 골수 기질세포에서 람다 면역글로불린 경쇄의 유의한 발현 증가를 나타내었다.

**결론:** 다발골수종 환자의 골수 기질세포에서는 형질세포에서 발견되는 클론성 유전자 변이가 관찰되지 않았으므로 이들 세포가 일차적으로 종양세포라는 증거는 뚜렷하지 않다. 골수 기질세포의 유전자 발현을 조사하였을 때, 다발골수종 환자의 경우 임상상에 따라 다른 유전자 발현 양상을 보임을 관찰할 수 있었으며, 이러한

유전자들이 다발골수종의 병태생리와 증상 발현에 중요한 역할을 할 것으로 생각된다. 이러한 유전자들의 질환 발생에 있어서의 역할 및 예후와의 상관성을 밝히기 위하여 더 많은 환자군들을 대상으로 하여 향후 더 연구가 필요하다.

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**주요어 : 다발골수종, 골수미세환경, 기질세포, 중간엽줄기세포, 유전자 발현**

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