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MiR-126 induces myeloma cell line Karpas707 apoptosis by downregulating anti-apoptotic protein MCL

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Abstract. – OBJECTIVE: Myeloma seriously threats human life and health and needs more efficacy treatment method in the clinic. MiR-126 regulates cell proliferation and apoptosis. This study explores the regulatory role of miR-126 in myeloma and related molecular mechanism.

MATERIALS AND METHODS: MiR-126 and control were synthesized and transfected to myeloma cell line Karpas707 using Lipofectamine. Cell apoptosis was evaluated by MTT assay, caspase-3 activity detection, and flow cytometry. Myeloid cell leukemin (MCL) siRNA and plasmid were transfected to Karpas707 cells to test its impact on cell apoptosis.

RESULTS: MTT assay revealed that miR-126 significantly restrained Karpas707 cell growth (p=0.0017). Cell apoptosis detection showed that miR-126 significantly promoted phosphatidylserine eversion and caspase-3 activation (p=0.031), and downregulated MCL level (p=0.017). MCL siRNA markedly enhanced Karpas707 cell apoptosis induced by miR-126 (p=0.024), while the MCL overexpression apparently inhibited Karpas707 cell apoptosis induced by miR-126 (p=0.0073).

CONCLUSIONS: MiR-126 induces Karpas707 cell apoptosis by downregulating anti-apoptotic protein MCL, which provides a theoretical basis for the target selection of myeloma.

Key Words:
miR-126, Anti-apoptotic protein MCL, MHCC97H cell, Apoptosis.

Introduction

Myeloma critically threatens human life[1,2]. Individualized and comprehensive therapy according to different stages emerge as popular means against myeloma, including surgery, chemotherapy, and radiotherapy[3,4]. Though the comprehensive therapy exhibits good curative effect, it still presents several deficiencies, such as bleeding[5-7].

At present, molecular targeted therapy becomes a hotspot in myeloma treatment[8-10]. There is still a lack of targets for myeloma treatment, current molecular targets against Bcl-2 and AIPs shows limited efficacy[11]. Thus, it is urgently needed to find more effective targets for myeloma[11,12]. MiRNA is a type of small endogenous RNA at about 20-24 nucleotides. It shows multiple important regulatory effects in cells. It is found that miRNA plays a profound role in cancer diagnosis and treatment. For instance, miR-143 can inhibit myeloma cell proliferation, while miR-34a is related to tumor metastasis[13,14]. It is suggested that miRNA may be involved in myeloma occurrence and development[13-15]. The miR-143 level was significantly higher in the myeloma tissue compared with that in adjacent normal control, revealing that miR-143 may participate in myeloma cell development[16,17].

The ideal anti-tumor strategy is characterized to kill tumor cells without affecting normal cells. Cell apoptosis is regulated by anti-apoptotic proteins and pro-apoptotic proteins[18,19]. Apoptosis inhibiting protein myeloid cell leukemia (MCL) is a widely investigated anti-apoptotic molecule[20,21]. Though there are numerous drugs targeting MCL, their effects in reducing MCL protein level are not satisfactory[22]. MiR-126 is involved in regulating multiple cells growth and apoptosis. However, the role of miR-126 in Karpas707 cells remains unclear. The purpose of this study is to investigate the potential regulatory role of miR-126 on myeloma cells and related molecular mechanism.

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Materials and Methods

Reagents and Cell Model

FBS and medium were purchased from Hualan Biological Engineering, Inc (Beijing, China). Lipofectamine was bought from Invitrogen (Carlsbard, CA, USA). Protein extraction kit, bicinchoninic acid (BCA) protein quantification kit, Fluoresceine isothiocyanate (FITC)-annexin detection kit, and caspase-3 detection kit were got from Beyotime (Shanghai, China). 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) was obtained from Dingguo Changsheng Biotechnology co., Ltd (Beijing, China). Horseradish peroxidase (HRP) labeled rabbit anti-mouse IgG, and mouse anti-human myeloid cell leukemia (MCL) and actin monoclonal antibodies were provided by Santa Cruz Biotechnology (Santa Cruz, CA, USA). MiR-126 (5'-AGACTGCATCTCGTCCAAGTAT-3' and 5'-AAGAGTATAGGGACAAAAGCA-3'), control miR-NC (5'-CTTCAAGCTACTCTGAGGGC-3' and 5'-TTATCTTGACACGTCGAAT-3'), MCL siRNA (5'-CTACGCGTGAGCATTACTGC-3' and 5'-TCTCTATCAAGCGATTAGT-3'), and MCL plasmid were derived from Genepharma (Suzhou, Zhejiang, China). Myeloma cell line Karpas707 was purchased from ATCC (Manassas, VA, USA).

Cell Culture

Myeloma cell line Karpas707 was maintained in Dulbecco's Modified Eagle's medium (DMEM) high-glucose medium (Waltham, MA, USA) at 5% CO_2 and 37°C[9].

Transfection

MiR-126 and control miR-NC were transfected to Karpas707 cells using Lipofectamine. Karpas707 cells were seeded in 24-well plate at density 68%. A total of 1 μl miR-126 or miR-NC (1 μg/μl) was suspended in lipo2000 at room temperature for 7 min. Then the mixture was added to the cells for culture[9]. The cell medium was changed after 12 h and the cells were further cultured at 5% CO_2 and 37°C for 20 h.

MTT Assay

MTT assay was applied to test Karpas707 cell viability[10]. Karpas707 cells were seeded in 6-well plate at 1×10^6/well for 8 h. After transfected by miR-126 or miR-NC, the cells were added with MTT at 1 mg/ml. After incubation for 5 h, the cells were treated by Dimethyl sulfoxide (DMSO) to stop the reaction. At last, the plate was read at 420 nm to draw the cell growth curve[11].

Flow Cytometry

Annexin-V-FITC double staining method was used to evaluate cell apoptosis. Karpas707 cells were transfected with miR-126 or miR-NC. After 48 h, the cells were collected and added with 8 μl loading buffer and 0.5 μl Annexin-V-Fluoresceine isothiocyanate (FITC) at room temperature avoid of light for 17 min. Then the cells were analyzed on flow cytometry (BD FACSCalibur) (San Jose, CA, USA) with exciting light and absorption light at 426 nm and 628 nm[14].

Western Blot

The cells were collected to extract protein according to the manual. After quantified by BCA method, the protein was boiled for 7 min and added with five times of loading buffer. Next, the protein was separated by sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membrane at 140 mA for 2 h. After blocking in 8% skim milk at room temperature for 1.5 h, the membrane was incubated in primary antibody (1:800) at 4°C overnight. Then the membrane was washed by Phosphate-Buffered Solution and Tween 20 (PBST) and incubated in secondary antibody at 1:1500 at 37°C for 2 h. At last, the membrane was developed using enhanced chemiluminescence (ECL) reagent. The image was obtained by the imaging system to analyze protein expression[15].

Caspase-3 Activity Detection

Karpas707 cells transfected by miR-126 or miR-NC were collected and resuspended in DMEM. After cracking, the supernatant was collected and added with the chromophoric substrate at room temperature. Next, the mixture was moved to 6-well plate and measured the absorbance value on a microplate reader (GeneTex, Irvine, CA, USA)[17]. Relative caspase-3 activity was calculated by the value obtained from the control group and miR-126 transfection group.

MCL siRNA or Overexpression

Karpas707 cells were seeded in 12-well plate at density 65% before transfection. A total of 2 μl MCL siRNA (1.6 μg/μl) was suspended in lipo2000 and added to the cells for 1.5 h under

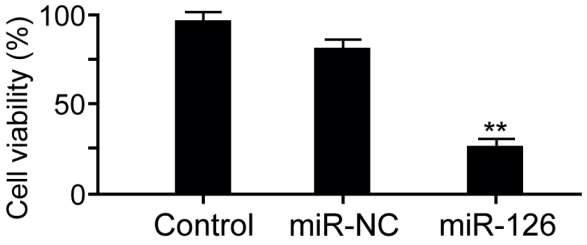

Figure 1. MiR-126 transfection reduced Karpas707 cell viability and inhibited cell growth. $^{**}p < 0.01$, compared with miR-NC.

5% CO_2 and 37°C. Next, 2 µl miR-126 or miR-NC (0.6 µg/µl) was mixed in lipo2000 and added to the cells transfected by MCL plasmid or siRNA. The cell medium[9] was changed after 12 h and the cells were cultured for another 48 h at 5% CO_2 and 37°C.

Statistical Analysis

All data analyses were performed on SPSS14.0 software (SPSS Inc., Chicago, IL, USA). The data were presented as mean ± standard deviation and compared by *t*-test. $p<0.05$ was depicted as statistical significant.

Results

MiR-126 Transfection Reduced Karpas707 Cell Viability and Inhibited Cell Growth – Karpas707 Cell Viability Was Determined by MTT Assay

Karpas707 cell viability was significantly suppressed by 0.5 µg miR-126 compared with miR-NC group (p=0.0017). Since no statistical difference was observed on cell viability between the miR-NC group and untransfected group ($p<0.05$). This study used miR-NC as control in the following experiments (Figure 1).

MiR-126 Transfection Induced Karpas707 Cell Apoptosis

Annexin-V-FITC staining was used to test Karpas707 cell apoptosis. Phosphatidylserine eversion was statistically increased in Karpas707 cells transfected by 1 µg miR-126 compared with control (p=0.031, Figure 2).

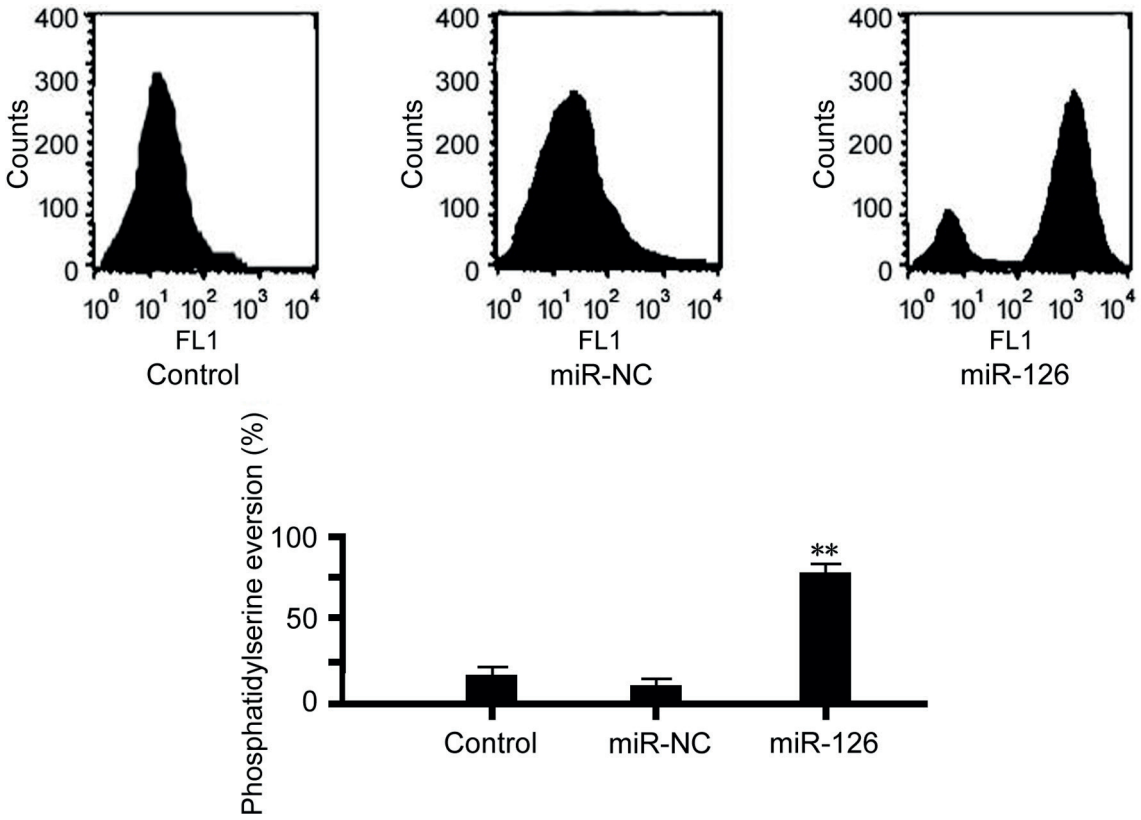

Figure 2. MiR-126 transfection induced Karpas707 cell apoptosis. $^{**}p < 0.01$, compared with miR-NC.

MiR-126 Transfection Activated Caspase-3 in Karpas707 Cells

As shown in Figure 3, caspase-3 activity was significantly enhanced in Karpas707 cells transfected by 0.5 μg miR-126 compared with control (p=0.0042).

MiR-126 Transfection Downregulated MCL Protein Expression in Karpas707 Cells

Western blot was applied to test MCL protein expression in Karpas707 cells. As shown in Figure 4, MCL protein level was significantly downregulated in Karpas707 cells transfected by 0.5 μg miR-126 compared with control (p=0.0036).

MCL siRNA Enhanced Karpas707 Cell Apoptosis Induced by miR-126

We then determined the impact of MCL on Karpas707 cell apoptosis induced by miR-126, through the transfection of MCL siRNA to Karpas707 cells before miR-126 treatment. As shown in Figure 5A, MCL protein level was significantly reduced in Karpas707 cells transfected by MCL siRNA. It exhibited statistical difference in MCL siRNA + miR-126 group compared with MCL siRNA group. As shown in Figure 5B, caspase-3 activity was markedly enhanced in miR-126 transfection group compared with the transfection with miR-NC (p=0.014), while it was apparently higher in MCL siRNA + miR-126 transfection group compared with miR-126 group (p=0.0024).

MCL Overexpression Inhibited Karpas707 Cell Apoptosis Induced by miR-126

We also tested the effect of MCL on Karpas707 cell apoptosis induced by miR-126. MCL was transfected into Karpas707 cells before miR-126 transfection. As shown in Figure 6A, MCL protein level was significantly upregulated in Karpas707 cells transfected by MCL plasmid. The level was remarkably increased in MCL + miR-126 group compared with that in MCL group. As shown in Figure 6B, caspase-3 activity was significantly attenuated in miR-126 transfection group (p=0.0034), while it was apparently lower in MCL + miR-126 transfection group compared with miR-126 group (p=0.0073).

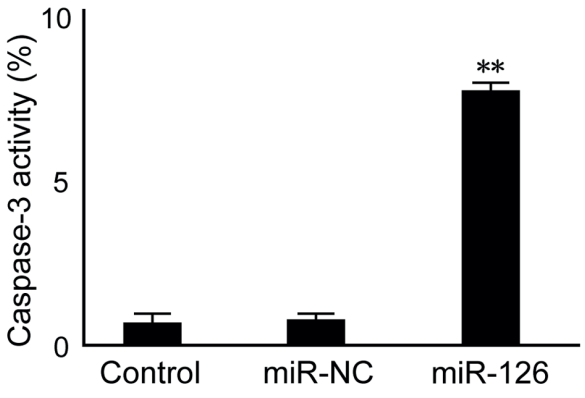

Figure 3. MiR-126 transfection activated caspase-3 in Karpas707 cells. **p < 0.01, compared with miR-NC.

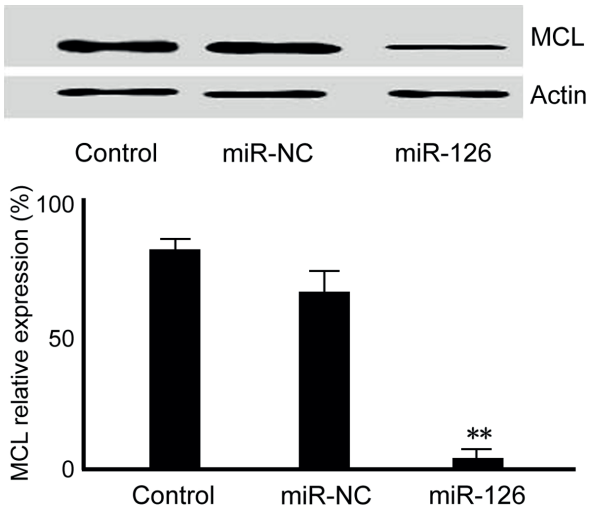

Figure 4. MiR-126 transfection downregulated MCL protein expression in Karpas707 cells.

Discussion

This study used myeloma cell line Karpas707 to explore the regulatory role of miR-126 and related mechanism. Our results showed that miR-126 transfection declined Karpas707 cell viability and growth, and induced cell apoptosis, suggesting that miRNA is involved in cell growth and survival[3]. There is still a lack of investigation about the impact of miRNA in myeloma[3]. It has been shown that miR-143 can inhibit myeloma cell proliferation, while miR-34a is related to tumor metastasis[13,14]. It suggested that miRNA may be involved in myeloma occurrence and development[13-15]. MCL is a type of anti-apoptotic protein[23]. It remains to be elucidated on whether MCL is regulated by miR-126 to affect myeloma cell line Karpas707 growth[24,25]. Our results showed that miR-126 transfection reduced MCL protein level. Karpas707 cell apoptosis was even enhanced

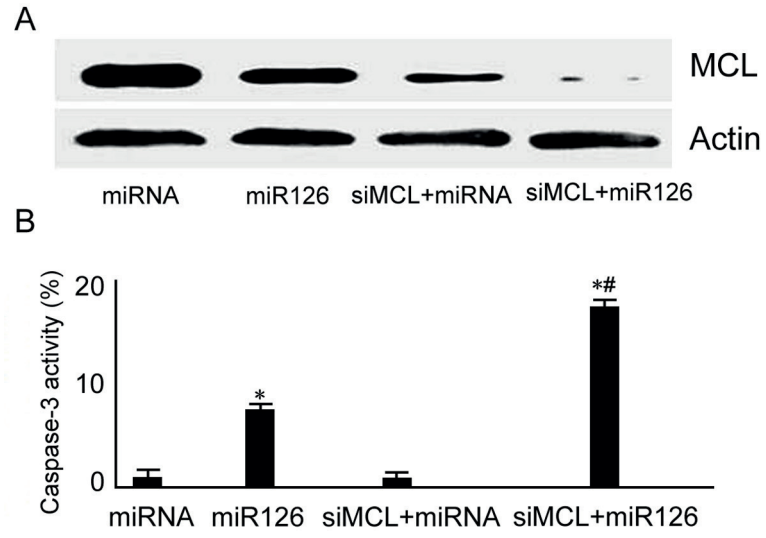

Figure 5. MCL siRNA enhanced Karpas707 cell apoptosis induced by miR-126. *$p < 0.05$, compared with miR-NC. #$p < 0.05$, compared with miR-126.

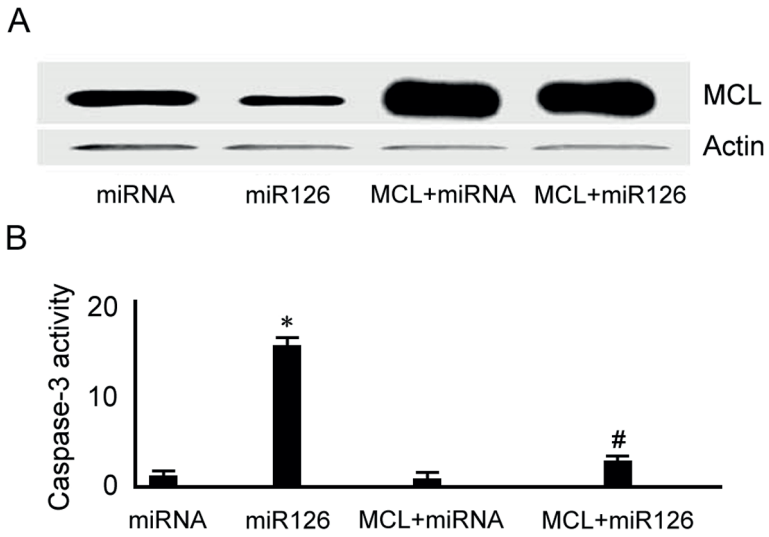

Figure 6. MCL overexpression attenuated Karpas707 cell apoptosis induced by miR-126. *$p < 0.05$, compared with miR-NC. #$p < 0.05$, compared with miR-126.

transfected by miR-126 and MCL siRNA. Zhou et al[26] revealed that a variety of factors, such as epigallocatechin gallate, inhibited the proliferation of myeloma cells via certain mechanism. Interestingly, this study demonstrated the role of MCL in Karpas707 cell apoptosis induced by miR-126, among which, our data on Western blot showed that MCL protein significantly downregulated in Karpas707 transfected by miR-126. Importantly, the inhibition of MCL by specific siRNA enhanced Karpas707 cell apoptosis induced by miR-126. In contrast, MCL plasmid restrained Karpas707 cell apoptosis induced by miR-126. Our result indicates that MCL plays a crucial role in Karpas707 cell apoptosis induced by miR-126. MCL protein may be a new strategy for the molecular targeted treatment of myeloma[27]. Currently, MCL also plays an anti-apoptosis role in other types of cancer[22,25,28,29]. There are still several drawbacks in this study. *In vivo* test by compar-

ing myeloma tissue and adjacent normal control ought to be performed to further determine the relationship between MCL and myeloma.

Conclusions

We demonstrated that miR-126 restrained Karpas707 cell viability by downregulating anti-apoptotic protein MCL. MCL may be a therapeutic target for myeloma, which provides theoretical support for the treatment of myeloma.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Liu T, Krysiak K, Shirai CL, Kim S, Shao J, Ndonwi M, Walter MJ. Knockdown of HSPA9 induces TP53-dependent apoptosis in human hematopoietic progenitor cells. PLoS One 2017; 12: e0170470.
2) Mehrzadi S, Safa M, Kamrava SK, Darabi R, Hayat P, Motevalian M. Protective mechanisms of melatonin against hydrogen peroxide induced toxicity in human bone-marrow derived mesenchymal stem cells. Can J Physiol Pharmacol 2017; 95: 773-786.
3) Liu C, Tsai AL, Li PC, Huang CW, Wu CC. Endothelial differentiation of bone marrow mesenchyme stem cells applicable to hypoxia and increased migration through Akt and NFkB signals. Stem Cell Res Ther 2017; 8: 29.
4) Lertsuwan K, Choe LH, Marwa IR, Lee K, Sikes RA. Identification of Fibulin-1 as a human bone marrow stromal (HS-5) cell-derived factor that induces human prostate cancer cell death. Prostate 2017; 77: 729-742.
5) Linz BM, Neely CJ, Kartchner LB, Mendoza AE, Khoury AL, Truax A, Sempowski G, Eitas T, Brickey J, Ting JP, Cairns BA, Maile R. Innate immune cell recovery is positively regulated by NLRP12 during emergency hematopoiesis. J Immunol 2017; 198: 2426-2433.
6) Furukawa M, Ohkawara H, Ogawa K, Ikeda K, Ueda K, Shichishima-Nakamura A, Ito E, Imai JI, Yanagisawa Y, Honma R, Watanabe S, Waguri S, Ikezoe T, Takeishi Y. Autocrine and paracrine interactions between multiple myeloma cells and bone marrow stromal cells by growth arrest-specific gene 6 cross-talk with Interleukin-6. J Biol Chem 2017; 292: 4280-4292.
7) Yu Y, Liao L, Shao B, Su X, Shuai Y, Wang H, Shang F, Zhou Z, Yang D, Jin Y. Knockdown of MicroRNA Let-7a Improves the functionality of bone marrow-derived mesenchymal stem cells in immunotherapy. Mol Ther 2017; 25: 480-493.
8) Jiang M, Yu Y, Luo J, Gao Q, Zhang L, Wang Q, Zhao J. Bone marrow-derived mesenchymal stem cells expressing thioredoxin 1 attenuate bleomycin-induced skin fibrosis and oxidative stress in scleroderma. J Invest Dermatol 2017; 137: 1223-1233.
9) Zhaleh M, Azadbakht M, Bidmeshki Pour A. Inhibitory effects of mouse bone marrow mesenchymal stem cell soup on staurospurine-induced cell death in MCF-7 and AGS. Bratisl Lek Listy 2017; 118: 34-43.
10) Bahrami E, Witzel M, Racek T, Puchalka J, Hollizeck S, Greif-Kohistani N, Kotlarz D, Horny HP, Feederle R, Schmidt H, Sherkat R, Steinemann D, Gohring G, Schlegelbeger B, Albert MH, Al-Herz W, Klein C. Myb-like, SWIRM, and MPN domains 1 (MYSM1) deficiency: Genotoxic stress-associated bone marrow failure and developmental aberrations. J Allergy Clin Immunol 2017; 140: 1112-1119.
11) Ma Y, Ge S, Zhou D, Zhang H, Sun L, Wang X, Su J. [Rat bone marrow mesenchymal stem cells promote apoptosis and inhibit proliferation and migration of RSC96 cells]. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2017; 33: 190-195.
12) Tabe Y, Yamamoto S, Saitoh K, Sekihara K, Monma N, Ikeo K, Mogushi K, Shikami M, Ruvolo V, Ishizawa J, Hail N, Jr., Kazuno S, Igarashi M, Matsushita H, Yamanaka Y, Arai H, Nagaoka I, Miida T, Hayashizaki Y, Konopleva M, Andreeff M. Bone marrow adipocytes facilitate fatty acid oxidation activating AMPK and a transcriptional network supporting survival of acute monocytic leukemia cells. Cancer Res 2017; 77: 1453-1464.
13) Zhang W, Zhao L, Liu J, Du J, Wang Z, Ruan C, Dai K. Cisplatin induces platelet apoptosis through the ERK signaling pathway. Thromb Res 2012; 130: 81-91.
14) Zhang W, Liu J, Sun R, Zhao L, Du J, Ruan C, Dai K. Calpain activator dibucaine induces platelet apoptosis. Int J Mol Sci 2011; 12: 2125-2137.
15) Bar-Natan M, Stroopinsky D, Luptakova K, Coll MD, Apel A, Rajabi H, Pyzer AR, Palmer K, Reagan MR, Nahas MR, Karp Leaf R, Jain S, Arnason J, Ghobrial IM, Anderson KC, Kufe D, Rosenblatt J, Avigan D. Bone marrow stroma protects myeloma cells from cytotoxic damage via induction of the oncoprotein MUC1. Br J Haematol 2017; 176: 929-938.
16) Li W, Liu Y, Wang B, Luo Y, Hu N, Chen D, Zhang X, Xiong Y. Protective effect of berberine against oxidative stress-induced apoptosis in rat bone marrow-derived mesenchymal stem cells. Exp Ther Med 2016; 12: 4041-4048.
17) Mete M, Aydemir I, Unlu Unsal U, Duransoy YK, Tuglu IM, Selcuki M. Neuroprotective effects of bone marrow-derived mesenchymal stem cellsand conditioned medium in mechanically injured neuroblastoma cells. Turk J Med Sci 2016; 46: 1900-1907.
18) Oben KZ, Gachuki BW, Alhakeem SS, McKenna MK, Liang Y, St Clair DK, Rangnekar VM, Bondada S. Radiation induced apoptosis of murine bone marrow cells is independent of Early Growth Response 1 (EGR1). PLoS One 2017; 12: e0169767.

19) Shabestari RM, Safa M, Alikarami F, Banan M, Kazemi A. CREB knockdown inhibits growth and induces apoptosis in human pre-B acute lymphoblastic leukemia cells through inhibition of prosurvival signals. Biomed Pharmacother 2017; 87: 274-279.

20) Wang DM, Li J, Chen WM, Long XP, Shi B, Zhao RZ. [The effect and mechanism investigation of intermedin on the apoptosis of bone marrow derived mesenchymal stem cells induced by hydrogen peroxide]. Zhonghua Yi Xue Za Zhi 2016; 96: 3888-3892.

21) Baligar P, Kochat V, Arindkar SK, Equbal Z, Mukherjee S, Patel S, Nagarajan P, Mohanty S, Teckman JH, Mukhopadhyay A. Bone marrow stem cell therapy partially ameliorates pathological consequences in livers of mice expressing mutant human alpha1-antitrypsin. Hepatology 2017; 65: 1319-1335.

22) Zhang W, Ren H, Xu C, Zhu C, Wu H, Liu D, Wang J, Liu L, Li W, Ma Q, Du L, Zheng M, Zhang C, Liu J, Chen Q. Hypoxic mitophagy regulates mitochondrial quality and platelet activation and determines severity of I/R heart injury. Elife 2016; 5: e21407.

23) Tan MY, Zhang CD, Xia B, Guo J, Fan ZW, Wu TH, Wang S, Liu SF, Deng L, Guo X, Huang YC. The expression of HMGB1 in bone marrow MSCs is upregulated by hypoxia with regulatory effects on the apoptosis and adhesion. Biomed Res Int 2016; 2016: 4598927.

24) Lv B, Li F, Fang J, Xu L, Sun C, Han J, Hua T, Zhang Z, Feng Z, Wang Q, Jiang X. Activated microglia induce bone marrow mesenchymal stem cells to produce glial cell-derived neurotrophic factor and protect neurons against oxygen-glucose deprivation injury. Front Cell Neurosci 2016; 10: 283.

25) Salvestrini V, Orecchioni S, Talarico G, Reggiani F, Mazzetti C, Bertolini F, Orioli E, Adinolfi E, Di Virgilio F, Pezzi A, Cavo M, Lemoli RM, Curti A. Extracellular ATP induces apoptosis through P2X7R activation in acute myeloid leukemia cells but not in normal hematopoietic stem cells. Oncotarget 2017; 8: 5895-5908.

26) Zhou CG, Hui LM, Luo JM. Epigallocatechin gallate inhibits the proliferation and induces apoptosis of multiple myeloma cells via inactivating EZH2. Eur Rev Med Pharmacol Sci 2018; 22: 2093-2098.

27) Jiang S, Naito M, Kiyonari H, Fujii M. USP10 is an essential deubiquitinase for hematopoiesis and inhibits apoptosis of long-term hematopoietic stem cells. Stem Cell Reports 2016; 7: 1116-1129.

28) Kim DR, Lee JE, Shim KJ, Cho JH, Lee HC, Park SK, Chang MS. Effects of herbal Epimedium on the improvement of bone metabolic disorder through the induction of osteogenic differentiation from bone marrow-derived mesenchymal stem cells. Mol Med Rep 2017; 15: 125-130.

29) Wang L, Zhang HY, Gao B, Shi J, Huang Q, Han YH, Hu YQ, Lu WG, Zhao ZJ, Liu BH, Jie Q, Yang L, Luo ZJ. Tetramethylpyrazine protects against glucocorticoid-induced apoptosis by promoting autophagy in mesenchymal stem cells and improves bone mass in glucocorticoid-induced osteoporosis rats. Stem Cells Dev 2017; 26: 419-430.