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Sirtuin 6 promotes cell aging of myeloma cell line KM-HM_(31) by via Hippo signal pathway

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Abstract. – OBJECTIVE: Myeloma poses a serious risk for people's health and life quality. Molecular targeted treatment of myeloma emerges as a promising therapy. This study aimed to determine the effect of Sirtuin 6 on myeloma KM-HM_(31) cell aging and provide evidence for clinical treatment.

MATERIALS AND METHODS: Myeloma KM-HM_(31) cell aging model induced by Carbamide peroxide (CP) was generated. Cells were transfected with Sirtuin 6 over-expression plasmid and specific siRNA. Western blot was used to study Sirtuin 6 expression, P53, P16, and Hippo in KM-HM_(31) cells. β-galactosidase assay was applied to measure cell aging. Verteporfin inhibited Hippo signal pathway and measured aging of KM-HM_(31) cells.

RESULTS: The levels of Sirtuin 6, aging protein P53, and P16 were remarkably elevated while Hippo expression was significantly inhibited in CP-induced KM-HM_(31) cells. Transfection of Sirtuin 6 over-expression plasmid enhanced Sirtuin 6 expression in KM-HM_(31) cells and potentiated cell aging with downregulation of Hippo protein. In contrast, a block of Sirtuin 6 resulted in the opposite effect. Moreover, Verteporfin inhibited Hippo signal pathway and enhanced CP-induced KM-HM_(31) cell aging, which contributed similar effect as Sirtuin 6 did.

CONCLUSIONS: We showed that sirtuin 6 facilitates CP-induced myeloma cell KM-HM_(31) aging via suppressing Hippo.

Key Words:
Sirtuin 6, Hippo, Myeloma cells, Cell aging.

Introduction

Myeloma and other hematological diseases emerge as important factors of death, with higher incidence[1]. However, the pathogenesis mechanism of myeloma is still unknown yet. It was shown that myeloma cell growth and proliferation potentiation, decreased cell apoptosis or aging are important reasons[2]. Cell aging has been paid attention in the treatment strategy for myeloma recently[3,4].

Cells aging leads to the decreasing potency of cell growth, proliferation or differentiation with cell cycle progresses[5,6]. The growth and activity of cancer cells, however, reverses the cell aging process by over-proliferation of cells to cause cancer[7,8]. Current researches[9,10] showed that Sir-related enzymes (Sirtuin) family members play critical roles in cell aging process. Sirtuin 6 family members have highly conserved amino acid sequence and share similar structures and functions. However, the exact functions in different Sirtuin family members vary to some extent[11,12]. For instance, Sirtuin 2 decreases cell proliferation velocity, whilst Sirtuin 1 is closely correlated with lung cancer metastasis[13,14], indicating the possible involvement of Sirtuin 6 in myeloma occurrence and progression[15]. Therefore, this research utilized myeloma cell line KM-HM_(31) as an *in vitro* model, to investigate the possible mechanism of Sirtuin 6 in myeloma cells, in order to provide evidence for selection of treatment targets of myeloma.

Materials and Methods

Myeloma Cell Model and Reagents

Myeloma cell model KM-HM_(31) cell line was purchased from ATCC (Manassas, VA, USA). Cell aging assay reagent was obtained from Solarbio (Beijing, China). Liposome transfection reagent was collected from Invitrogen (Waltham, MA, USA). Cell culture medium related antibiotics, culture medium and fetal bovine serum (FBS) were bought from Beyotime (Beijing, China). The antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). siRNA of Sirtuin 6 (5'-UGCUGACUCCAAAGCUCUG-3',

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and 5'-CAGAGCUUUGGAGUCAGCA-3'), and Sirtuin 6 over-expression plasmid were purchased from Gimma (Shanghai, China).

Cell Culture and Generation of Carbamide Peroxide (CP) Induced Myeloma Cell Line KM-HM_(31) Aging Model

Myeloma KM-HM_(31) cells were resuscitated and re-suspended into normal Dulbecco's Modified Eagle's Medium (DMEM) medium for continuous culture[16]. CP-induced myeloma KM-HM_(31) aging model was generated by adding 1 μl (10 μg/μl) CP into the culture medium for further experiments.

Liposome Transfection

Liposome reagent was used to transfect small interfering ribonucleic acid (siRNA) Sirtuin 6 and controlled siRNA, or Sirtuin 6 over-expression plasmid into myeloma cell line KM-HM_(31) as previously reported[17]. In brief, KM-HM_(31) cells were cultured at 90% confluence, followed by the addition of 1 μl (1 μg/μl) siRNA Sirtuin 6 or controlled siRNA. Sirtuin 6 over-expression plasmid was re-suspended in liposome transfection reagent lipo2000. Culture medium was changed after 48 h for further experiments.

Cell Aging Assay

Liposome reagent was used to transfect siRNA Sirtuin 6 or controlled siRNA, or Sirtuin 6 over-expression plasmid into myeloma KM-HM_(31) cells. β-galactosidase assay measured cell aging, and results were recorded under the microscope for analysis, as previously recorded[18].

Western Blot Assay

Protein concentration in the cell lysate was quantified for Western blot. Electrophoresis was performed under 60 V for 30 min firstly, followed by 120 V for 120 min. After electrophoresis, proteins were transferred to NC membrane under 300 mA for 180 min. The membrane was blocked using 5% defatted milk powder for 60 min incubation at room temperature. Mouse anti-human Sirtuin 6 and Hippo antibodies (1:1000 dilution for both) were added for 120 min room temperature incubation. After rinsing in Phosphate-Buffered Saline Tween 20 (PBST) for three times, the membrane was developed using electrochemiluminescence (ECL) system for exposure after three times of PBST rinsing. An automatic gel imaging system was used to analyze expression level of target proteins Sirtuin 6 and Hippo for comparing levels between all groups of KM-HM_(31) cells (Bio-Rad, Hercules, CA, USA).

To test the effect of Sirtuin 6 interference or over-expression on myeloma cell line KM-HM_(31), cells after transfection were collected and analyzed in Western blot as abovementioned[15].

Statistical Analysis

Data were analyzed by SPSS 19.0 software and were presented as mean±standard error of means (SEM). The Student *t*-test was used for between groups while one-way ANOVA was performed in comparison of data among multiple groups followed by the Tukey's post hoc test. A significance level was defined as $p<0.05$.

Results

Enhanced Sirtuin 6 Expression and Suppression of Hippo Signal Pathway in KM-HM_(31) Cell Aging Model

In the myeloma cell KM-HM_(31), CP treated cell and increased levels of aging proteins P53 and P16, indicating the establishment of a successful aging model for further studies (Figure 1).

We further observed that in cell aging model, Sirtuin 6 level was apparently increased, indicating the possible role of Sirtuin 6 to CP-induced myeloma cell KM-HM_(31) aging. Also, the evident reduction of Hippo was found in myeloma cell aging model compared to normal control (Figure 1).

Transfection of Sirtuin 6 Over-Expression Plasmid or siRNA Increased or Decreased Sirtuin 6 Level in KM-HM_(31) Cells, Enhanced or Suppressed Cell Aging, and Weakened or Potentiated Hippo Signal Pathway

As shown in Figure 2, we further determine the effect of Sirtuin 6 in cell aging, through over-expressing and down-regulating level of Sirtuin 6. Of note, the increase of Sirtuin 6 apparently up-regulated the expression of P53, while reduced the level of Hippo. However, the block of Sirtuin 6 contributed to the opposite effect on P53 and Hippo, indicating that Sirtuin 6 participated in CP-induced myeloma cell KM-HM_(31) aging and might regulate the level of Hippo (Figure 2).

Verteporfin Inhibited KM-HM_(31) Cell Aging

To identify the effect of Hippo on cell aging, Verteporfin, the inhibitor of the Hippo signaling

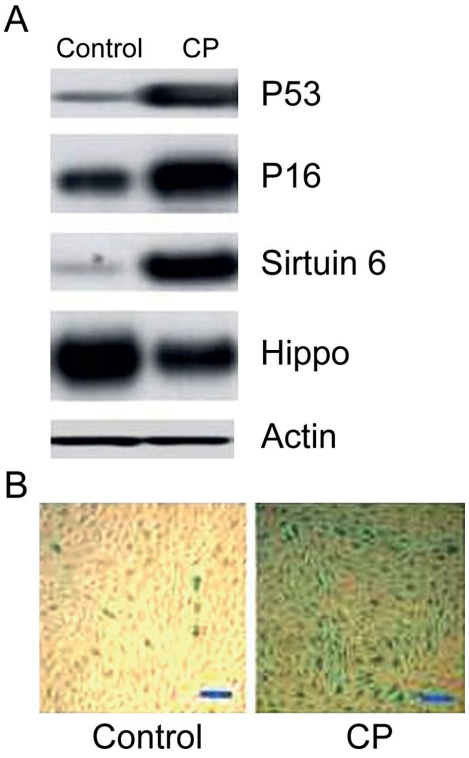

Figure 1. Enhanced Sirtuin 6 expression and suppression of Hippo signal pathway in KM-HM_(31) cell aging model. ***A,*** Western blot test results; ***B,*** β-galactosidase staining for cell aging.

pathway, was used. Importantly, the Hippo was inhibited by Verteporfin, resulting in that rising expression of P53 and suppression of cell aging by the detection of the β-galactosidase assay. Consistently, the overexpression of Sirtuin 6 also served a similar function on the modulation of P53 expression and cell aging as Verteporfin did, indicating that Sirtuin 6 promoted CP-induced myeloma cell KM-HM_(31) aging via suppression of Hippo (Figure 3).

Discussion

Data showed that transfection of Sirtuin 6 enhanced expression level of aging molecules in human myeloma cell line KM-HM_(31), causing cell aging, as consistent with previous results, supporting the participation of Sirtuin 6 in cell growth and aging[19, 20]. However, the exact mechanism of Sirtuin 6 modulates myeloma cell growth, and aging is still unclear yet[3]. Sirtuin 2 decreases tumor cell proliferation, whilst Sirtuin 1 is correlated with tumor metastasis[21,22]. These results indicate that Sirtuin 6 may also regulate myeloma occurrence and progression[23-25]. The previous study[26] showed that Hippo protein served as a cell aging inhibitor. This investigation showed that Sirtuin 6 transfection suppressed Hippo level, and increased the aging rate of myeloma cell line KM-HM_(31). Whilst the inhibitor of Hippo signal pathway enhanced CP-induced KM-HM_(31) cell aging. By over-expression or interference of Sirtuin 6 level, Hippo expression was correspondingly modulated, further suggesting the important role of Hippo in CP-induced myeloma cell KM-HM_(31) aging.

In this work, we found elevated Sirtuin 6 level in CP-induced myeloma cell line KM-HM_(31) aging model, whilst aging protein P53 and P16 expression levels were also increased with suppression of Hippo signal pathway. The change of Sirtuin 6 regulated the cell aging while affected the level of Hippo. Furthermore, verteporfin suppressed Hippo and enhanced CP-induced myeloma cell line KM-HM_(31) aging, confirming the relation of Hippo to cell aging. These results indicated that Sirtuin 6 participated in the regulation of Hippo proteins during the process of CP-induced myeloma cells KM-HM_(31) aging. Targeting Sirtuin 6 or Hippo proteins may be a novel strategy for clinical treatment of myeloma[26]. Currently, Hippo has been shown to suppress cancer cell aging in other malignant tumors[21,23-28]. Previous evidence[29] showed that Yes-Associated Protein (YAP) is a transcriptional co-activator that acts downstream of the Hippo signaling pathway and regulates multiple cellular processes. Hippo-Yap is the novel signaling pathway which plays an important role in gastric cancer tumor development

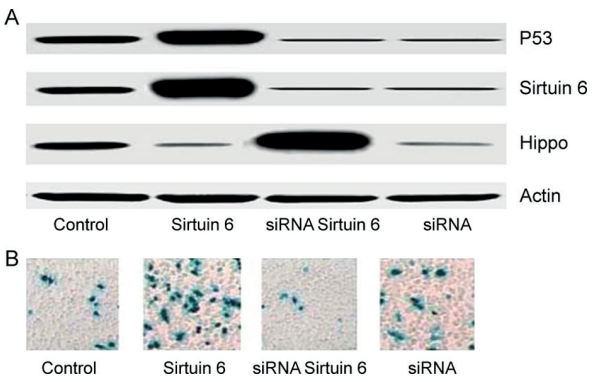

Figure 2. Transfection of Sirtuin 6 over-expression plasmid or siRNA increased or decreased Sirtuin 6 level in KM-HM_(31) cells, enhanced or suppressed cell aging, and weakened or potentiated Hippo signal pathway. ***A,*** Western blot results; ***B,*** β-galactosidase staining for cell aging.

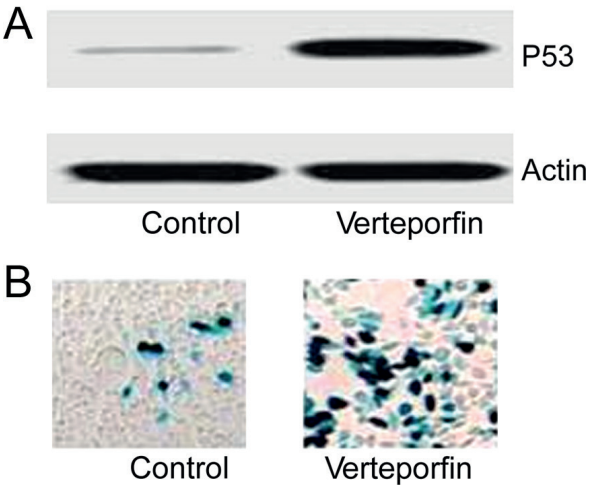

Figure 3. Hippo signal pathway inhibitor verteporfin significantly enhanced CP-induced myeloma cell KM-HM_(31) aging.

and progression. As a tumor promoter, Hippo-Yap preserved Sirtuin 1 (SIRT1) activity and participated in gastric cancer survival and migration via activation of the SIRT1/Mfn2/mitophagy axis[30]. Combined with the previous evidence, our results suggested that Sirtuin 6 induced KM-HM_(31) cell aging via suppressing Hippo pathway. A limitation in our study is that *in vivo* assay with an animal model of myeloma needs to adopted to validate further the efficacy of Sirtuin 6/Hippo on the treatment of myeloma, which provides alternative leads besides recent finding of Epigallocatechin gallate on inhibiting the growth of myeloma cells[31].

Conclusions

Our data unraveled that Sirtuin 6 facilitated CP-induced myeloma cell KM-HM_(31) aging via suppressing Hippo signal pathway, providing a potential novel strategy for treating myeloma.

Acknowledgments

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Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) 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. pii: e21407.
2) Macanas-Pirard P, Broekhuizen R, Gonzalez A, Oyanadel C, Ernst D, Garcia P, Montecinos VP, Court F, Ocqueteau M, Ramirez P, Nervi B. Resistance of leukemia cells to cytarabine chemotherapy is mediated by bone marrow stroma, involves cell-surface equilibrative nucleoside transporter-1 removal and correlates with patient outcome. Oncotarget 2017; 8: 23073-23086.
3) Vatanen A, Hou M, Huang T, Soder O, Jahnukainen T, Kurimo M, Ojala TH, Sarkola T, Turanlahti M, Saarinen-Pihkala UM, Jahnukainen K. Clinical and biological markers of premature aging after autologous SCT in childhood cancer. Bone Marrow Transplant 2017; 52: 600-605.
4) Demaria M, O'Leary MN, Chang J, Shao L, Liu S, Alimirah F, Koenig K, Le C, Mitin N, Deal AM, Alston S, Academia EC, Kilmarx S, Valdovinos A, Wang B, de Bruin A, Kennedy BK, Melov S, Zhou D, Sharpless NE, Muss H, Campisi J. Cellular senescence promotes adverse effects of chemotherapy and cancer relapse. Cancer Discov 2017; 7: 165-176.
5) Kilpinen L, Parmar A, Greco D, Korhonen M, Lehenkari P, Saavalainen P, Laitinen S. Expansion induced microRNA changes in bone marrow mesenchymal stromal cells reveals interplay between immune regulation and cell cycle. Aging (Albany NY) 2016; 8: 2799-2813.
6) Yamaguchi M. The botanical molecule p-hydroxycinnamic acid as a new osteogenic agent: insight into the treatment of cancer bone metastases. Mol Cell Biochem 2016; 421: 193-203.
7) Simon AJ, Lev A, Zhang Y, Weiss B, Rylova A, Eyal E, Kol N, Barel O. Mutations in STN1 cause Coats plus syndrome and are associated with genomic and telomere defects. J Exp Med 2016; 213: 1429-1440.
8) Torano EG, Bayon GF, Del Real A, Sierra MI, Garcia MG, Carella A, Belmonte T, Urdinguio RG, Cubillo I, Garcia-Castro J, Delgado-Calle J, Perez-Campo FM, Riancho JA, Fraga MF, Fernandez AF. Age-associated hydroxymethylation in human bone-marrow mesenchymal stem cells. J Transl Med 2016; 14: 207.
9) Ozcan S, Alessio N, Acar MB, Mert E, Omerli F, Peluso G, Galderisi U. Unbiased analysis of senescence associated secretory phenotype (SASP) to identify common components following different genotoxic stresses. Aging (Albany NY) 2016; 8: 1316-1329.
10) Rundberg Nilsson A, Soneji S, Adolfsson S, Bryder D, Pronk CJ. Human and murine hematopoietic stem cell aging is associated with functional impairments and intrinsic megakaryocytic/erythroid bias. PLoS One 2016; 11: e0158369.

11) Brosh RM, Jr., Bellani M, Liu Y, Seidman MM. Fanconi Anemia: A DNA repair disorder characterized by accelerated decline of the hematopoietic stem cell compartment and other features of aging. Ageing Res Rev 2017; 33: 67-75.

12) Selman C, Sinclair A, Pedroni SM, Irvine EE, Michie AM, Withers DJ. Evidence that hematopoietic stem cell function is preserved during aging in long-lived S6K1 mutant mice. Oncotarget 2016; 7: 29937-29943.

13) Kundrotas G, Gasperskaja E, Slapsyte G, Gudleviciene Z, Krasko J, Stumbryte A, Liudkeviciene R. Identity, proliferation capacity, genomic stability and novel senescence markers of mesenchymal stem cells isolated from low volume of human bone marrow. Oncotarget 2016; 7: 10788-10802.

14) Moehrle BM, Nattamai K, Brown A, Florian MC, Ryan M, Vogel M, Liederhaeuser C, Soller K, Prows DR, Abdollahi A, Schleimer D, Walter D, Milsom MD, Stambrook P, Porteus M, Geiger H. Stem cell-specific mechanisms ensure genomic fidelity within HSCs and upon aging of HSCs. Cell Rep 2015; 13: 2412-2424.

15) Shao L, Wang Y, Chang J, Luo Y, Meng A, Zhou D. Hematopoietic stem cell senescence and cancer therapy-induced long-term bone marrow injury. Transl Cancer Res 2013; 2: 397-411.

16) Luo Y, Li L, Zou P, Wang J, Shao L, Zhou D, Liu L. Rapamycin enhances long-term hematopoietic reconstitution of ex vivo expanded mouse hematopoietic stem cells by inhibiting senescence. Transplantation 2014; 97: 20-29.

17) Chen X, Zhai Y, Yu D, Cui J, Hu JF, Li W. Valproic acid enhances iPSC induction from human bone marrow-derived cells through the suppression of reprogramming-induced senescence. J Cell Physiol 2016; 231: 1719-1727.

18) Tabibian-Keissar H, Hazanov L, Schiby G, Rosenthal N, Rakovsky A, Michaeli M, Shahaf GL, Pickman Y, Rosenblatt K, Melamed D, Dunn-Walters D, Mehr R, Barshack I. Aging affects B-cell antigen receptor repertoire diversity in primary and secondary lymphoid tissues. Eur J Immunol 2016; 46: 480-492.

19) Henry CJ, Casas-Selves M, Kim J, Zaberezhnyy V, Aghili L, Daniel AE, Jimenez L, Azam T, McNamee EN, Clambey ET, Klawitter J, Serkova NJ, Tan AC, Dinarello CA, DeGregori J. Aging-associated inflammation promotes selection for adaptive oncogenic events in B cell progenitors. J Clin Invest 2015; 125: 4666-4680.

20) Stockklausner C, Raffel S, Klermund J, Bandapalli OR, Beier F, Brummendorf TH, Burger F, Sauer SW, Hoffmann GF, Lorenz H, Tagliaferri L, Nowak D, Hofmann WK, Buergermeister R, Kerber C, Rausch T, Korbel JO, Luke B, Trumpp A, Kulozik AE. A novel autosomal recessive TERT T1129P mutation in a dyskeratosis congenita family leads to cellular senescence and loss of CD34+ hematopoietic stem cells not reversible by mTOR-inhibition. Aging (Albany NY) 2015; 7: 911-927.

21) Tourlakis ME, Zhang S, Ball HL, Gandhi R, Liu H, Zhong J, Yuan JS, Guidos CJ, Durie PR, Rommens JM. In vivo senescence in the sbds-deficient murine pancreas: cell-type specific consequences of translation insufficiency. PLoS Genet 2015; 11: e1005288.

22) Zingoni A, Cecere F, Vulpis E, Fionda C, Molfetta R, Soriani A, Petrucci MT, Ricciardi MR, Fuerst D, Amendola MG, Mytilineos J, Cerboni C, Paolini R, Cippitelli M, Santoni A. Genotoxic stress induces senescence-associated ADAM10-dependent release of NKG2D MIC ligands in multiple myeloma cells. J Immunol 2015; 195: 736-748.

23) Alessio N, Del Gaudio S, Capasso S, Di Bernardo G, Cappabianca S, Cipollaro M, Peluso G, Galderisi U. Low dose radiation induced senescence of human mesenchymal stromal cells and impaired the autophagy process. Oncotarget 2015; 6: 8155-8166.

24) Berenstein R, Blau O, Nogai A, Waechter M, Slonova E, Schmidt-Hieber M, Kunitz A, Pezzutto A, Doerken B, Blau IW. Multiple myeloma cells alter the senescence phenotype of bone marrow mesenchymal stromal cells under participation of the DLK1-DIO3 genomic region. BMC Cancer 2015; 15: 68.

25) Kolosa K, Motaln H, Herold-Mende C, Korsic M, Lah TT. Paracrine effects of mesenchymal stem cells induce senescence and differentiation of glioblastoma stem-like cells. Cell Transplant 2015; 24: 631-644.

26) Hilpert M, Legrand C, Bluteau D, Balayn N, Betems A, Bluteau O, Villeval JL, Louache F, Gonin P, Debili N, Plo I, Vainchenker W, Gilles L, Raslova H. p19 INK4d controls hematopoietic stem cells in a cell-autonomous manner during genotoxic stress and through the microenvironment during aging. Stem Cell Reports 2014; 3: 1085-1102.

27) Fu S, Wei J, Wang G, Wang B, Wang Y, Lai X, Huang H. The key role of PML in IFN-alpha induced cellular senescence of human mesenchymal stromal cells. Int J Oncol 2015; 46: 351-359.

28) Liu HY, Huang CF, Lin TC, Tsai CY, Tina Chen SY, Liu A, Chen WH, Wei HJ, Wang MF, Williams DF, Deng WP. Delayed animal aging through the recovery of stem cell senescence by platelet rich plasma. Biomaterials 2014; 35: 9767-9776.

29) Hata S, Hirayama J, Kajiho H, Nakagawa K, Hata Y, Katada T, Furutani-Seiki M, Nishina H. A novel acetylation cycle of transcription co-activator Yes-associated protein that is downstream of Hippo pathway is triggered in response to SN2 alkylating agents. J Biol Chem 2012; 287: 22089-22098.

30) Yan H, Qiu C, Sun W, Gu M, Xiao F, Zou J, Zhang L. Yap regulates gastric cancer survival and migration via SIRT1/Mfn2/mitophagy. Oncol Rep 2018; 39: 1671-1681.

31) 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.