

# Prolyl oligopeptidase inhibitor suppresses the upregulation of ACSDKP in patients with acute myeloid leukemia

HAIWU CAO, XIAOHONG ZHAO, SHIYUN LU and ZHI WANG

Department of Hematology, Wuxi Second People's Hospital, Nanjing Medical University, Wuxi, Jiangsu 214002, P.R. China

Received August 18, 2017; Accepted February 2, 2018

DOI: 10.3892/etm.2018.6111

**Abstract.** The aim of the current study was to measure the expression of acetyl-N-Ser-Asp-Lys-Pro (ACSDKP) in patients with acute myeloid leukemia (AML) and the effect of prolyl oligopeptidase inhibitor (POPi) on the bone marrow stromal cells of these patients. Serum and bone marrow stromal cell samples were collected from 33 patients with AML admitted to Wuxi Second People's Hospital, Nanjing Medical University between September 2011 and August 2016. ACSDKP levels were measured using a highly specific competitive enzyme immunoassay (EIA). Bone marrow stromal cells were treated with synthetic ACSDKP (10  $\mu$ M/ml) or different concentrations of POPi S17092 (25, 50 and 100  $\mu$ g/ml). Cells that received no treatment were used as control. An MTT assay was conducted to measure the proliferation of bone marrow stromal cells. The results demonstrated that serum levels of ACSDKP in patients with AML were significantly higher than those of controls ( $P < 0.05$ ). Following treatment with ACSDKP, cell proliferation was significantly increased compared with untreated cells ( $P < 0.05$ ). However, following treatment with different concentrations of POPi, the expression of ACSDKP was significantly decreased in a dose-dependent manner ( $P < 0.05$ ). Furthermore, the proliferation of bone marrow stromal cells was also decreased in a dose-dependent manner. Therefore, the present study demonstrated that ACSDKP levels were increased in the serum and bone marrow stromal cells of patients with AML and that ACSDKP promoted the proliferation of bone marrow stromal cells of these patients, which was inhibited by POPi. These results may identify a novel target for the treatment of AML.

## Introduction

Acute myeloid leukemia (AML), is the most common type of leukemia in adults, is a disease that leads to impaired

hematopoiesis and bone marrow failure induced by clonal expansion of undifferentiated myeloid precursors (1). Epidemiological studies demonstrate that in 2017 there were 19,950 cases of AML in the USA, (11,130 males and 8,820 females) and 10,430 individuals succumbed (2). In China, the incidence and mortality rates of all types of leukemia have markedly increased between 2011 and 2015 (3). There were ~18,000 new cases in 2011 and ~75,300 new cases in 2015, while 24,526 people succumbed to the disease in 2011 compared with 53,400 in 2015 (3). As the population of China is ageing rapidly, the incidence of AML is expected to increase by ~50% by 2030 (4).

The etiology of most cases of AML is unknown. Environmental and genetic factors are considered to be the most possible risks for AML (5,6). It is likely that many different mutations, epigenetic aberrations and downstream abnormalities are involved in development of AML (7). The results of whole-genome sequencing have indicated that AML is a complex, dynamic disease (8,9). Despite the improvement of chemotherapy for AML, refractory disease is common and relapse is the primary cause of treatment failure (10,11). The exact etiology of AML remains unknown and current treatments of AML are not as effective as they could be; therefore, it is important to determine the molecular biological mechanisms of AML and identify novel potential treatment targets for AML in order to develop novel potential treatments for AML.

The natural tetrapeptide acetyl-N-Ser-Asp-Lys-Pro (ACSDKP) is generated from the N-terminus of thymosin- $\beta$ 4 via enzymatic cleavage by prolyl oligopeptidase (POP) (12). It has been demonstrated that ACSDKP is associated with endothelial cell proliferation (13), the promotion of angiogenesis (14) and the inhibition of myofibroblast differentiation (15). ACSDKP is also considered to be abnormally expressed in some human malignant tumors, including tumors of the thyroid gland (16), breast, colon, head and neck, kidney, lung, skin, ovary and prostate (17,18). It has been demonstrated that ACSDKP expression varies during chemotherapy to treat patients with AML, in certain patients the ACSDKP level increased sharply during treatment, whereas in others it did not change or decreased (19); however, few studies investigating ACSDKP have been conducted since. It has been demonstrated that ACSDKP is upregulated in human leukemia cells and may enhance the proliferation of U87-MG glioblastoma cells via the phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit  $\alpha$  (PI3KCA)/protein kinase B (Akt) signaling

---

*Correspondence to:* Professor Zhi Wang, Department of Hematology, Wuxi Second People's Hospital, Nanjing Medical University, 68 Zhongshan Road, Wuxi, Jiangsu 214002, P.R. China  
E-mail: wangzhi\_zl@126.com

**Key words:** acute myeloid leukemia, acetyl-N-Ser-Asp-Lys-Pro, prolyl oligopeptidase inhibitor

pathway (17). Furthermore, it was indicated that prolyl oligopeptidase inhibitor (POPi) may inhibit the stimulation of U87-MG glioblastoma cell proliferation by ADSCKP (20). Therefore, the present study focused on the expression of ADSCKP and its effects on AML.

In present study, the serum level of ADSCKP in patients with AML was determined and the effect of POPi on ADSCKP expression and the proliferation of bone marrow stromal cells in patients with AML was assessed. The aim of the present study may identify a novel target for the treatment of patients with AML.

## Materials and methods

**Patients.** Serum and bone marrow stromal cell samples were collected from 33 patients with AML admitted to the Wuxi Second People's Hospital, Nanjing Medical University (Wuxi, China) between September 2011 and August 2016. All patients were diagnosed with AML according to the World Health Organization criteria and no patients received chemotherapy prior to the study (21). The mean age of patients was  $57.33 \pm 9.32$  (range, 18-77) years, and patients consisted of 23 males and 10 females. Patients were classified according to the French, American and British (FAB) classification system for AML (22). Sera samples were obtained from 25 healthy individuals who underwent a physical examination at the Wuxi Second People's Hospital over the same time period. The control patients had a mean age of  $53.48 \pm 12.50$  (range, 18-75) years and consisted of 17 males and 8 females. A total of 5 ml blood was obtained from the ulnar vein of each participant. To obtain the sera the blood samples were centrifuged for 10 min at room temperature at  $2,795 \times g$  and stored at  $-20^{\circ}\text{C}$ . Written informed consent was obtained from all participants and the present study was approved by the Research Committee of Wuxi Second People's Hospital (Wuxi, China). Baseline clinical data from the patients and healthy volunteers is presented in Table I.

**ACSDKP measurement.** ACSCKP concentration was measured using a highly specific ACSCKP ELISA kit (cat. no. 69-99161; MSKBio, Ltd.; Merck KGaA, Darmstadt, Germany) with acetylcholinesterase conjugate as a tracer (Bertin Pharma, Montigny le Brettonneux, France), as described previously (19,20). ACSCKF levels were measured using ELISA on the serum fraction following extraction by methanol, evaporation and reconstitution in saline buffer. The extracts were then added to 0.5 ml buffer and used to determine ACSCKP concentration, according to the manufacturer's protocol.

**Bone marrow stromal cell culture and treatment.** Bone marrow stromal cell samples were collected from all patients with AML. Briefly, 3-5 ml marrow fluid was extracted using a sterile injector with heparin sodium (20 IU/ml) anticoagulant (Sigma Aldrich; Merck KGaA). Bone marrow fluid was diluted with RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and centrifuged for 10 min at room temperature at  $2,795 \times g$  and the cell supernatant was extracted. Subsequently, the cell fluid was cultured with RPMI 1620 medium in a humidified incubator with 5%  $\text{CO}_2$  at  $37^{\circ}\text{C}$ . Following 72 h culture, all supernatant

and suspension cells were discarded and the complete culture medium of the aforementioned system was added. When the adherent cells were spread across  $>90\%$  of the bottom of the culture flask, cells were collected and stored at  $-20^{\circ}\text{C}$  prior to further investigation.

The cells were subsequently treated with  $10 \mu\text{M}/\text{ml}$  synthetic ACSCKP (Genepep, Saint-Jean de Védas, France) or different concentrations of POPi S17092 (25, 50 and  $100 \mu\text{g}/\text{ml}$ ; Institut de Recherche Servier, Croissy, France) and cultured for 72 h. Untreated cells were used as control.

**MTT assay.** An MTT assay was then conducted to measure the proliferation of bone marrow stromal cells, following a previously described protocol in which the formazan crystals were dissolved in 0.2 ml dimethyl sulfoxide for 30 min at  $37^{\circ}\text{C}$  (23). The optical density (OD) of each well was measured at 590 nm using a BioTek microplate reader (BioTek Instruments, Winooski, VT, USA). Values are expressed as the percentage of the OD of the control cells.

**Statistical analysis.** All data are expressed as the mean  $\pm$  standard deviation, apart from the particular ADSCKP values of each patient. All analyses were conducted using SPSS 18.0 (SPSS, Inc., Chicago, IL, USA). A  $\chi^2$  test was performed to analyze characteristics, such as the gender ratio. A Student's t-test was used to compare differences between continuous variables, including the ages of patients and controls. A Kruskal-Wallis test followed by Turkey's post hoc test was used to compare levels of ADSCKP in patients with different FAB stages of AML. The Mann-Whitney U test was used in the *in vitro* study to compare differences between the groups.  $P < 0.05$  was considered to indicate a significant difference.

## Results

**Serum levels of ACSCKP are upregulated in patients with AML.** The baseline clinical characteristics of all participants are presented in Table I. Serum levels of ACSCKP in patients with AML and healthy controls were measured and compared. The results demonstrated that serum levels of ACSCKP in patients with AML were significantly higher than those of control group ( $P < 0.05$ ; Fig. 1A). This indicates that serum ACSCKP levels are upregulated in patients with AML. Subsequently, serum levels of ACSCKP were analyzed in patients with different FAB stages of AML; however, no significant differences were observed among these groups (Fig. 1B).

**ACSDKP promotes the proliferation of bone marrow stromal cells of patients with AML.** To determine the effect of ACSCKP on bone marrow stromal cell proliferation in patients with AML,  $10 \mu\text{M}/\text{ml}$  ACSCKP was used to treat cells and proliferation was measured using MTT. The results demonstrated that following treatment with ACSCKP, proliferation was significantly promoted compared with untreated cells ( $P < 0.05$ ; Fig. 2), indicating that ACSCKP stimulates the progression of AML.

**POPi reverses the effects of ACSCKP on bone marrow stromal cells taken from patients with AML.** Finally the

Table I. Basic clinical data of all participants.

Characteristic	Patients (n=33)	Healthy controls (n=25)	P-value
Mean age $\pm$ SD, years	57.30 $\pm$ 15.1	55.36 $\pm$ 12.57	0.198
Age range, years	(18-77)	(18-73)	
Sex (male:female)	23:10	18:7	0.720
FAB group			
0	1	N/A	N/A
1	8	N/A	N/A
2	6	N/A	N/A
3	4	N/A	N/A
4	4	N/A	N/A
5	3	N/A	N/A
6	2	N/A	N/A
7	1	N/A	N/A
Unc	4	N/A	N/A

Unc, unclassified; N/A, not applicable; SD, standard deviation; FAB, French, American and British classification system.

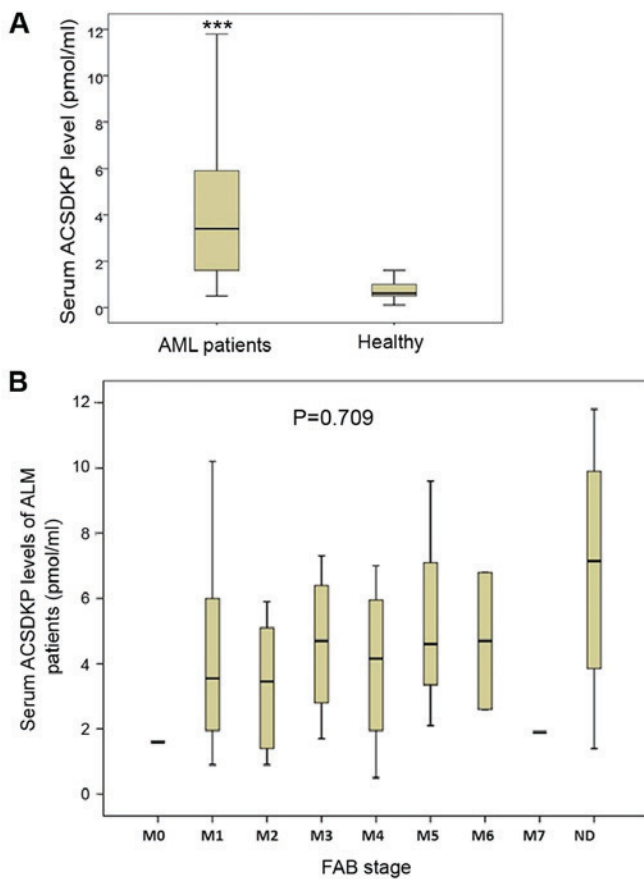


Figure 1. (A) Serum ACSDKP levels of patients with ALM and healthy controls. (B) Serum ACSDKP levels of patients with ALM with different FAB stages. \*\*\* $P < 0.05$  vs. Healthy controls. ALM, acute myeloid leukemia; ACSDKP, acetyl-N-Ser-Asp-Lys-Pro; FAB, French, American and British classification system.

effects of POpi on ACSDKP expression and the proliferation of bone marrow stromal cells of patients with AML were determined. The results indicated that cell proliferation was

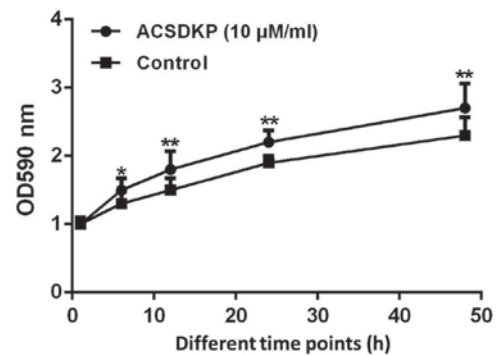


Figure 2. The proliferation of bone marrow stromal cells taken from patients with acute myeloid leukemia following treatment with ACSDKP. \* $P < 0.05$  and \*\* $P < 0.01$  vs. control. OD, optical density; ACSDKP, acetyl-N-Ser-Asp-Lys-Pro.

significantly inhibited following POpi treatment ( $P < 0.05$ ; Fig. 3A). Furthermore, treatment of bone marrow stromal cells with different concentrations of POpi (25, 50 and 100 µg/ml) decreased the expression of ACSDKP in a dose-dependent manner ( $P < 0.05$ ; Fig. 3B).

## Discussion

High-risk AML is characterized primarily by cytogenetic features of the blast population and less often by immunophenotypic abnormalities (24). Patients with AML usually respond to induction therapy but may also experience secondary disease manifestation following myelodysplastic syndrome or cytotoxic treatment for another malignant disease (25). Numerous studies have focused on the pathogenesis of AML; however, the mechanisms by which AML develops remain unknown.

The function of ACSDKP in angiogenesis and its association with leukemia has been characterized (19,26). However the precise role that ACSDKP serves in leukemia has not yet been elucidated. It was identified that ACSDKP expression is upregulated in human leukemia (17) and it was demonstrated

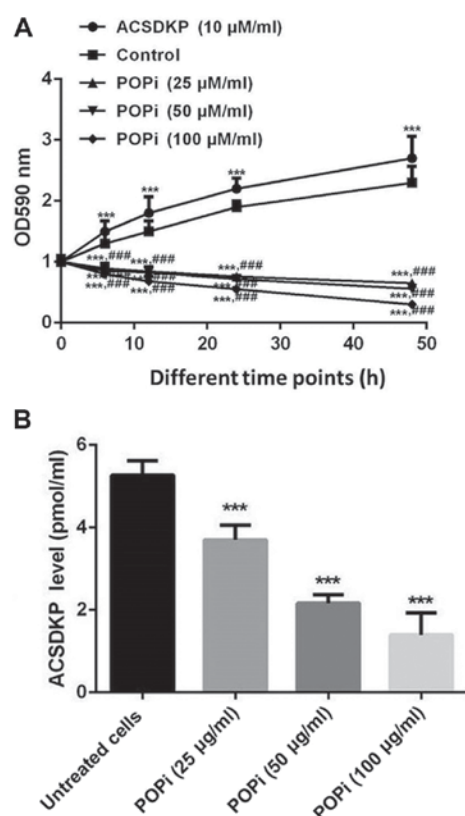


Figure 3. (A) The proliferation of bone marrow stromal cells taken from patients with acute myeloid leukemia following treatment with ACSDKP or different concentrations of POPi. (B) ACSDKP levels of bone marrow stromal cells of patients with AML following treatment with ACSDKP or different concentrations of POPi. \*\*\* $P < 0.001$  vs. the control. ### $P < 0.001$  vs. the ACSDKP group. OD, optical density; ACSDKP, acetyl-N-Ser-Asp-Lys-Pro; POPi, prolyl oligopeptidase inhibitor.

that ACSDKP could enhance the proliferation of U87-MG glioblastoma cells via the PI3KCA/Akt signaling pathway (20). Therefore, the current study aimed to determine the role of ACSDKP in AML.

In the present study, the serum level of ACSDKP in patients with AML and healthy controls were assessed and compared and it was demonstrated that ACSDKP expression was significantly increased in patients with AML. Subsequently, the proliferation of bone marrow stromal cells of patients with AML was determined and the results indicated that ACSDKP promoted bone marrow stromal cell proliferation. Finally it was determined that POPi inhibits ACSDKP expression and the proliferation of bone marrow stromal cells from patients with AML in a dose-dependent manner.

Hu *et al* (20) identified that ACSDKP expression was significantly upregulated in U87-MG glioblastoma cells; furthermore, the expression of ACSDKP in K562 human leukemia cells was not particularly high. Other studies obtained different results; for example, Liozon *et al* (19) indicated that serum levels of ACSDKP varied between 0.6 and 13.0 pmol/ml in patients with AML. Liu *et al* (17) demonstrated that serum levels of ACSDKP were significantly increased in a mouse model of leukemia and in patients with leukemia. In the present study, it was demonstrated that ACSDKP levels were enhanced in the serum and bone marrow stromal cells of patients with AML.

There were certain limitations of the current study. The molecular mechanisms underlying the ACSDKP-mediated increase in bone marrow stromal cell proliferation remain unclear. Furthermore, the mechanism by which POPi suppresses the ACSDKP-induced increase in bone marrow stromal cell proliferation remains unknown. Further studies are required to elucidate these mechanisms of action.

In conclusion, the current study measured the serum levels of ACSDKP in patients with AML and determined the effect of POPi on ACSDKP expression and the proliferation of bone marrow stromal cells of patients with AML. The results indicated that ACSDKP levels were enhanced in the serum and bone marrow stromal cells of patients with AML and indicated that ACSDKP may promote the proliferation of bone marrow stromal cells. Treatment with POPi reversed this increase in bone marrow stromal cell proliferation. Therefore, the current study indicated that POPi treatment may present a novel therapeutic strategy for the treatment of AML.

## Acknowledgements

Not applicable.

## Funding

No funding was received.

## Availability of data and materials

All data generated or analyzed during this study are included in this published article.

## Authors' contributions

HC wrote the manuscript and conducted the experiments. XZ and SL collected and analyzed the data. ZW designed the study and approved the final version of the manuscript.

## Ethics approval and consent to participate

Written informed consent was obtained from all participants and the present study was approved by the Research Committee of Wuxi Second People's Hospital (Wuxi, China).

## Consent for publication

Written informed consent was obtained from all patients for the publication of their data.

## Competing interests

The authors declare that they have no competing interests.

## References

1. Stelljes M, Beelen DW, Braess J, Sauerland MC, Heinecke A, Berning B, Kolb HJ, Holler E, Schwerdtfeger R, Arnold R, *et al*: Allogeneic transplantation as post-remission therapy for cytogenetically high-risk acute myeloid leukemia: Landmark analysis from a single prospective multicenter trial. *Haematologica* 96: 972-979, 2011.



2. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2016. *CA Cancer J Clin* 66: 7-30, 2016.
3. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J: Cancer statistics in China, 2015. *CA Cancer J Clin* 66: 115-132, 2016.
4. Medeiros BC, Othus M, Estey EH, Fang M and Appelbaum FR: Impact of body-mass index on the outcome of adult patients with acute myeloid leukemia. *Haematologica* 97: 1401-1404, 2012.
5. Tsai RJ, Luckhaupt SE, Schumacher P, Cress RD, Deapen DM and Calvert GM: Acute myeloid leukemia risk by industry and occupation. *Leuk Lymphoma* 55: 2584-2591, 2014.
6. Wang ML and Bailey NG: Acute myeloid leukemia genetics: Risk stratification and implications. *Arch Pathol Lab Med* 139: 1215-1223, 2015.
7. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, Potter NE, Heuser M, Thol F, Bolli N, *et al*: Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med* 374: 2209-2221, 2016.
8. The Cancer Genome Atlas Research Network: Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature* 507: 315-322, 2014.
9. Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, Kennedy JA, Schimmer AD, Schuh AC, Yee KW, *et al*: Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature* 506: 328-333, 2014.
10. Döhner H, Weisdorf DJ and Bloomfield CD: Acute myeloid leukemia. *N Engl J Med* 373: 1136-1152, 2015.
11. Ritchie DS, Neeson PJ, Khot A, Peinert S, Tai T, Tainton K, Chen K, Shin M, Wall DM, Hönnemann D, *et al*: Persistence and efficacy of second generation CAR T cell against the LeY antigen in acute myeloid leukemia. *Mol Ther* 21: 2122-2129, 2013.
12. Ding G, Zhang Z, Chopp M, Li L, Zhang L, Li Q, Wei M and Jiang Q: MRI evaluation of BBB disruption after adjuvant AcSDKP treatment of stroke with tPA in rat. *Neuroscience* 271: 1-8, 2014.
13. Huang WQ, Wang BH and Wang QR: Thymosin beta4 and AcSDKP inhibit the proliferation of HL-60 cells and induce their differentiation and apoptosis. *Cell Biol Int* 30: 514-520, 2006.
14. Liu JM, Lawrence F, Kovacevic M, Bignon J, Papadimitriou E, Lallemand JY, Katsoris P, Potier P, Fromes Y and Wdzieczak-Bakala J: The tetrapeptide AcSDKP, an inhibitor of primitive hematopoietic cell proliferation, induces angiogenesis in vitro and in vivo. *Blood* 101: 3014-3020, 2003.
15. Xu H, Yang F, Sun Y, Yuan Y, Cheng H, Wei Z, Li S, Cheng T, Brann D and Wang R: A new antifibrotic target of Ac-SDKP: Inhibition of myofibroblast differentiation in rat lung with silicosis. *PLoS One* 7: e40301, 2012.
16. Kusinski M, Wdzieczak-Bakala J, Liu JM, Bignon J and Kuzdak K: Acsdkp: A new potential marker of malignancy of the thyroid gland. *Langenbecks Arch Surg* 391: 9-12, 2006.
17. Liu JM, Garcia-Alvarez MC, Bignon J, Kusinski M, Kuzdak K, Riches A and Wdzieczak-Bakala J: Overexpression of the natural tetrapeptide acetyl-N-ser-asp-lys-pro derived from thymosin beta4 in neoplastic diseases. *Ann N Y Acad Sci* 1194: 53-59, 2010.
18. Liu JM, Kusinski M, Ilic V, Bignon J, Hajem N, Komorowski J, Bordessoule D, Frindel E and Praloran V: Acsdkp serum concentrations of the angiogenic tetrapeptide acsdkp in human malignant tumors. *Anticancer Res* 28: 2813-2817, 2008.
19. Liozon E, Volkov L, Comte L, Trimoreau F, Pradelles P, Bordessoule D, Frindel E and Praloran V: Acsdkp serum concentrations vary during chemotherapy in patients with acute myeloid leukaemia. *Br J Haematol* 89: 917-920, 1995.
20. Hu P, Li B, Zhang W, Li Y, Li G, Jiang X, Wdzieczak-Bakala J and Liu J: AcSDKP regulates cell proliferation through the PI3KCA/Akt signaling pathway. *PLoS One* 8: e79321, 2013.
21. Vardiman JW, Harris NL and Brunning RD: The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood* 100: 2292-2302, 2002.
22. Drexler HG: Classification of acute myeloid leukemias-a comparison of FAB and immunophenotyping. *Leukemia* 1: 697-705, 1987.
23. Lv H, Che T, Tang X, Liu L and Cheng J: Puerarin enhances proliferation and osteoblastic differentiation of human bone marrow stromal cells via a nitric oxide/cyclic guanosine monophosphate signaling pathway. *Mol Med Rep* 12: 2283-2290, 2015.
24. Juopperi TA, Bienzle D, Bernreuter DC, Vernau W, Thrall MA and McManus PM: Prognostic markers for myeloid neoplasms: A comparative review of the literature and goals for future investigation. *Vet Pathol* 48: 182-197, 2011.
25. Cîrstea M, Coliță A, Ionescu B, Ghiaur A, Vasilescu D, Dobrea C, Jordan C, Dragomir M, Gheorghe A, Várady Z and Lupu AR: Therapy-related myelodysplastic syndrome after successful treatment of acute promyelocytic leukemia: Case report and literature review. *Revista Romana De Medicina De Laborator* 25: 165-179, 2017.
26. Frindel E, Masse A, Pradelles P, Volkov L and Rigaud M: Correlation of endogenous acetyl-ser-asp-lys-pro plasma levels in mice and the kinetics of pluripotent hemopoietic stem cells entry into the cycle after cytosine arabinoside treatment: Fundamental and clinical aspects. *Leukemia* 6: 599-601, 1992.