Journal of Biology and Today's World
ISSN 2322-3308
Journal home page: http://journals.lexispublisher.com/jbtw/

Received: 25 August 2015 • Accepted: 02 October 2015

Short C

doi:10.15412/J.JBTW. 01040701

Evaluation of the Effect of MiR-27a Up-regulation on Megakaryopoiesis in Erythroleukemic K562 Cell Line

Farshid Karami[1*], Ahmad Kazemi[1], Fateme Nadali[1], Shaban Alizade[1], Ahmad Faramarzi[2], Sadegh Alijani[1], Neda Minayi[1], Hamidreza Shahmoradi[3], Yadi Farshi[1]

[1] Department of Hematology, Allied Medical School, Tehran University of Medical Sciences, Tehran, Iran

[2] Department of Pathology, Allied Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran

[3] Department of Biochemistry, International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

*correspondence should be addressed to Farshid Karami, Department of Hematology, Allied Medical School, Tehran University of Medical Sciences, Tehran, Iran; Tell: +9883442228565; Fax: +9883442228565; Email: Karami.farshid@yahoo.com.

ABSTRACT

Megakaryopoiesis (platelet production) is a complex process with the potential for regulation at multiple stages. Several studies have indicated that megakaryopoiesis might be regulated post-transcriptionally. This process could be done by small non-coding RNAs called microRNAs which target mRNAs in a sequence-specific manner and lead to translational repression or mRNA decay. The aim of this study is to evaluate the overexpression effects of miR-27a on megakaryocytic differentiation in K562 cell line. K562 cell line was cultured in RPMI 1640 medium, and cloned pre-miR-27a was transfected by lentiviral vector into K562 cells. After RNA extraction and cDNA synthesis in selected days, miRNA up-regulation was confirmed by miRNA real time PCR and then CD41 and CD61 expressions were investigated by RT-PCR. Expression of miR-27a was increased 4.8, 10.3 and 16.8 fold on days 3, 7 and 14 after virus transfect into K562 cell line, in comparison with untransfected cells. Expression level of CD41 and CD61 was positive by RT-PCR technique. The data suggest that miR-27a can be involved in megakaryocytic differentiation and overexpression of megakaryocytic markers in K562 cell line. We suggest that miR-27a may be a significant therapeutic target for increasing both megakaryocytes and platelets in patients with thrombocytopenic diseases.

Key words: miRNA, miR-27a, K562, Megakaryopoiesis

1. INTRODUCTION

MiRNAs are involved in epigenetic modification and first discovered in 1993 in C.elegans by Lee during a study of lin-4 gene. These small RNAs were named miRNA in 2001. More than 1,000 miRNAs have been described in prokaryotes and eukaryotes (1). MiRNAs are single-stranded non-coding RNAs composed of 19 to 24 nucleotides, which regulate gene expression by binding to 3′-untranslated Region (UTR) and maybe to 5′-UTR. They are coded by monocistronic and polycistronic genes. The gene locus responsible for miRNA synthesis is not often related to specific genes. These regions contain fragile sites in human chromosome that are independently expressed from the other genes (2). MiRNAs play important roles in cell processes such as proliferation, aging, apoptosis, metabolism and differentiation. Several miRNAs have been studied in maturation of megakaryocytes. They can be classified into two groups. The first group includes miRNAs that have an inhibitory role in megakaryopoiesis; therefore, their expression is down-regulated in megakaryopoiesis. This group consists of miR-10a, miR-155, miR-126, miR-106, miR-20, miR-17 and miR-10b (1). In contrast, overexpression of the second group of miRNAs in megakaryocyte-erythroid progenitor (MEP) induces megakaryopoiesis and suppresses erythropoiesis. MiR-27a, miR-150 and miR-34 are included in this group (3-5). On the other hand, there are a number of miRNAs that are not included in these two groups. Some of them like miR-125b are overexpressed in both erythroid and megakaryocytic lineages. MicroRNA-27a is highly expressed in cancers and has been identified

as an oncogenic microRNA. MiR-27a was first implicated in breast cancer as an oncomiRNA. Mertens-Talcott et al. found that miR-27a was highly expressed in breast cancer cells. MicroRNA-27a(miR-27a) is found on chromosome 19. The nucleotide sequence of the human mature miR-27a is UUCACAGUGGCUAAGUUCCGC. Megakaryocytes are a small group of progenitor cells in bone marrow responsible for the production of platelets. Platelet production rate in adult human is approximately 1×10^{11} per day and, it can be increased several folds under stressful conditions. Thrombopoietin (TPO) is the major modulator in production of platelets, which is synthesized in liver and kidney. TPO stimulates the proliferation and maturation of megakaryocytes by C-MPL receptor. Many studies have been performed to induce megakaryocytic differentiation and platelet production by the bone marrow of patient as a therapeutic approach. However, each of these inducers is associated with intense cytotoxic complications and even mortality if used for a long time. According to this fact, new therapeutic approaches are needed to induce production of megakaryocytes and platelets. New therapeutic approaches have focused on inducing megakaryocytic and consequently platelet production. These approaches are based on targeting the molecules involved in modulation of megakaryocytic differentiation and platelet production. This therapeutic approach can be used for gene therapy of the patients with platelet disorders in the future as a novel method with lower toxicity and less side effects as well as better efficacy in comparison to current drugs. One of these molecules is miRNAs which has been considered as a novel molecular approach to induce the production of hematopoietic cells. The aim of this study is to evaluate the effect of miR-27a overexpression on the induction of megakaryocytic differentiation in K562 cell line via analysis of expression level of megakaryocytic markers in different days.

2. MATERIALS AND METHODS

K-562 cell line was cultured at 37°*C* under a humidified atmosphere consisting of 95% air and 5% CO2 in RPMI 1640 medium (Gibco.USA), 10% Fetal Bovine Serum (FBS) (Gibco.USA), 50 *units/ml* penicillin and 50 µ*g/ml* streptomycin. The DNA extraction was performed on normal human blood, using DNA extraction kit (VioGene) and the extraction of plasmid DNA from pCDH (511-b1) vector was done using plasmid DNA extraction kit (Vio Gene). PCR product and plasmid DNA were digested with restriction enzymes, XbaI and BamHI (Fermentas). After digestion, ligation of insert fragment and vector were conducted with T4 DNA ligase enzyme (Fermentas). PCDH (511-b1) containing the insert fragment was transformed into the competent bacteria (STBL4) and cultured overnight on LBagar. After 14-16 hours, single random clones were chosen, then cultured and plasmid DNA was extracted. The single positive clones, pCDH containing pre-miR-27a, were confirmed by colony PCR with primary primers, sequencing and digestion with XbaI and BamHI. Lentiviral vectors expressing transgene were produced by transfecting a three plasmid system into producer cells. Packaging plasmid ps-PAX2, envelope plasmid pMD2.G and pCDHCMV-MCS-EF1-copGFP vector plasmids (with insert fragments) were transfected into HEK-293 cells, using the calcium-phosphate method. CD41 mRNA and CD61 mRNA aswell as control GAPDH mRNA were quantified using SYBER GREEN master mix (Bioer) with Bioer's thermal cycler according to manufacturer's protocol (94℃ 2 *min*, 94℃ 10*s*,58℃ 15 *s*, 72℃ 25 *s*) in 40 cycles. For single miRNA analysis, RNA isolated from K562cell culture was used for real time PCR quantification using the high-specificity miRNA QPCR Core Reagent Kit (Stratagene, USA).

3. RESULTS AND DISCUSSION

3.1. PCR reaction results for amplification of Pre-miR-27a

Genomic DNA of peripheral blood mononuclear cells (PBMC) was extracted, and then PCR was done using the designed primer. Amplified PCR products were electrophoresed on 2% agarose gel, and the results are illustrated in Figure 1 (a, b).

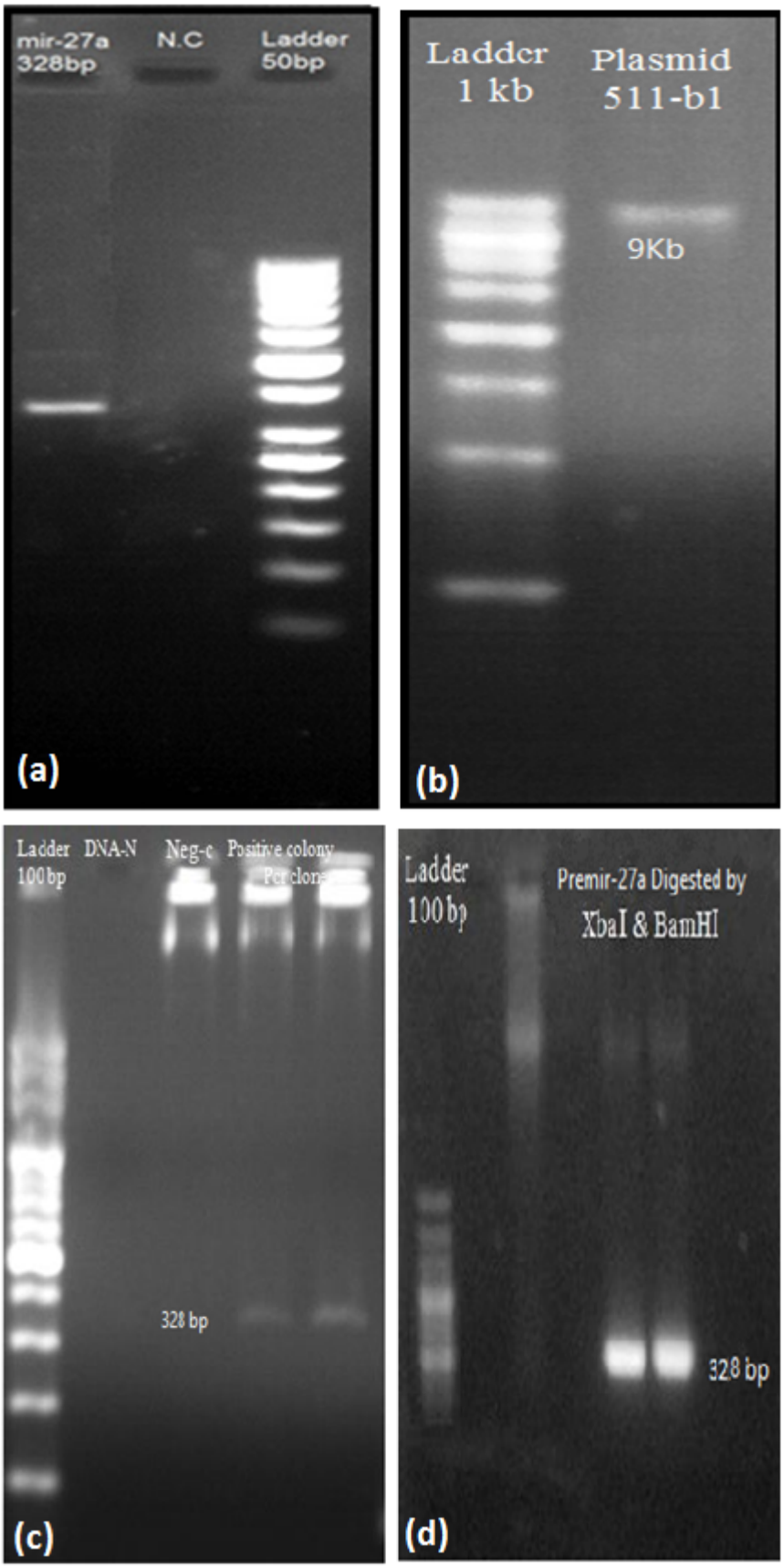

Figure 1. PCR products of miR-27a (a); Extraction products of pCDH plasmid (b); Electrophoresis of PCR product of miR-27a after *cloning* (c); Electrophoresis of positive clone cleavage products for miR-27a with XbaI and BamH (d).

3.2. Cloning validation

After miR-27a and pCDH (511-b1) plasmid ligation, the product was transferred into STBL4 bacteria. Then, it was incubated for 14-16 hours. Next, plasmid DNA was extracted and evaluated by PCR and electrophoresis (Figure 1 (c)) for validation.

3.3. Enzymatic degradation for positive clone validation

If the cloned segment enters into the plasmid, the count of obtained base pairs from enzymatic degradation will be different from non-cloned plasmids. Thus, this can be used as a method for segment validation. The plasmid was degraded by BamHI and XbaI enzymes. Therefore, positive clones appeared as 328bp bands in addition to the main body of plasmid (Figure 1 (d)).

3.4. Evaluation of up-regulation effect of miR-27a on K562 cells

First, Real Time PCR reaction was done by Stratgene kit in order to ensure efficiency of the function of the produced virus and to evaluate the profile of synthesized miR-27a on days 3, 7 and 14 after virus transfection into K562 cell line. Expression of miR-27a was surveyed in comparison to control. Expression level of miR-27a was approximately 4.8, 10.3 and 16.8 fold on days 3, 7 and 14, respectively (Diagram 1).

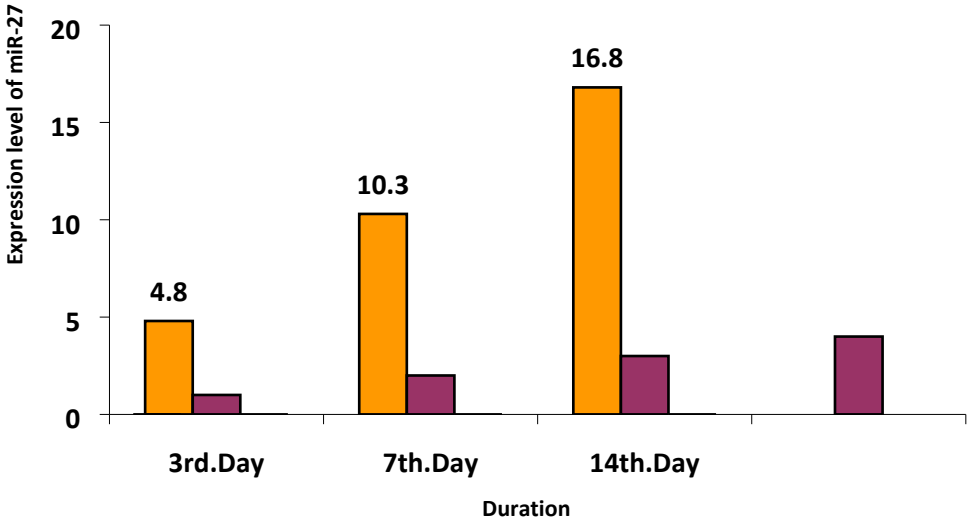

Diagram 1. Expression level of miR-27a on days 3, 7 and 14 after transfer of virus into K562 cell lines (significant P-value)

3.5. Expression Changes in CD41, CD61

RNA was isolated on days 3, 7 and 14 after transfer of virus into K562 cell lines. Then, cDNA was synthesized. The cDNA produced was amplified by RT-PCR, and the results are shown in Figure 2.

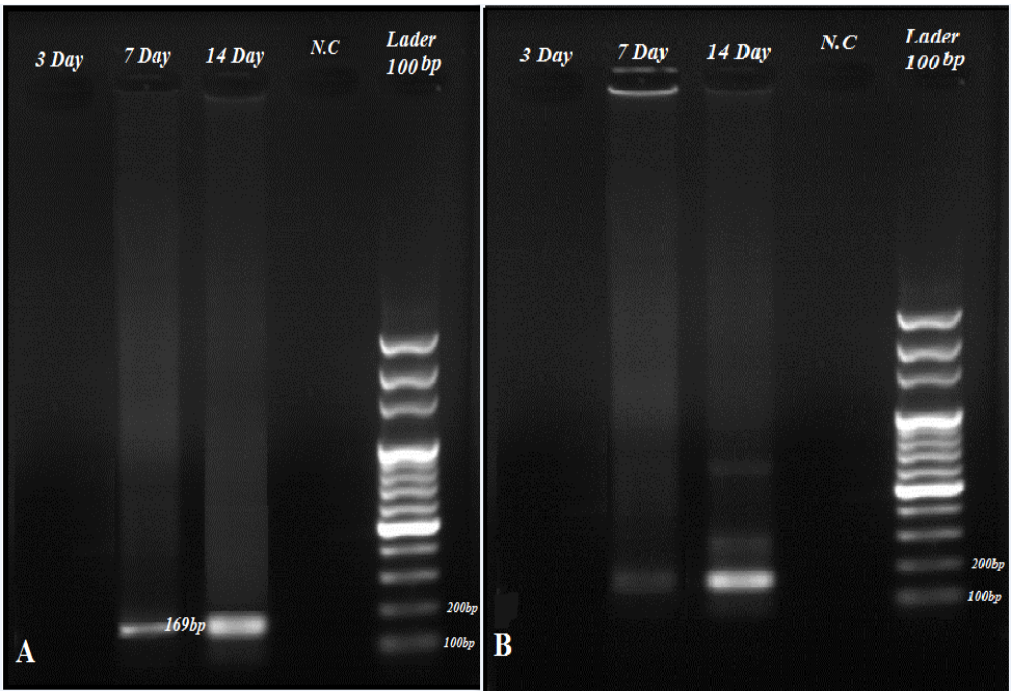

Figure 2. Expression level of CD41 chain on days 3, 7 and 14 after transfer of virus into K562 cell lines (A), the size of PCR product was 196bp; Expression level of CD61 chain on days 3, 7 and 14 after transfer of virus into K562 cell lines (B), the size of PCR product was 164bp.

Many studies have been performed to induce megakaryocytic differentiation and platelet production by bone marrow of the patient as a therapeutic approach. However, each of these inducers involves intense cytotoxic complications if they applied in a long period of time. According to this fact, novel therapeutic approaches are essential to induce megakaryopoiesis and platelet production. Nowadays, drugs that act as inducer are the most common therapeutic approach to induce the production of blood cells. Therefore, new therapeutic approaches are needed to induce megakaryocytes and consequently platelets production. These approaches are based on targeting molecules that play some roles in the regulation of megakaryocytic differentiation and platelet production. This therapeutic approach can be used with lower toxicities and less side effects with better efficacy in comparison to current drugs as gene therapy for treatment of the patients with such disorders in the future. MiRNAs have been the focus of attention as a novel molecular approach to induce the production of hematopoietic cells. MiRNAs are 19 to 24 nucleotides in length, and are single-stranded non-coding RNAs. MiRNAs are bound to sequences in the 3′ UTR on their target mRNAs, and then repress translation and/or destabilize RNA molecules (6, 7). In this study, K562 cell line was evaluated as a CML cell line. Studied K562 cells were divided to control and sample groups with an equal number of cells, and only the test group was treated with miR-27a-containing virus. Cells of both groups were analyzed based on expression of megakaryocytic markers on days 3, 7 and 14 after transfer of virus into K562 cell lines via RT-PCR. First, expression level of Pre-miR-27a was analyzed in the cells of both groups via RT-PCR. Expression of miR-27a was increased in cells of the test group 4.8, 10.3 and 16.8 fold on days 3, 7 and 14, respectively, after virus transfer into K562 cell lines in comparison to cells of control group. These results confirm efficiency of the cloned vector. Expression level of CD41 was not changed in comparison to control group on day 3, but its expression was increased on days 7 and 14 after virus transfer into K562 cell lines. In addition, CD61 was not expressed on day 3, but expression of this marker was seen on days 7 and 14 after transfer of virus into K562 cell line. CD41 (GPIIb) and CD61 (GPIIIa) are two major megakaryocytic markers, and their expression is raised during normal megakaryopoiesis. According to the above mentioned results, during the differentiation, along with the overexpression of miR-27a, expression of CD41 and CD61 is increased in the studied cells. These results are related to the role of miR-27a in megakaryocytic differentiation. Because of lack of studies that directly demonstrate the role of miR-27a in the induction of marker expression and megakaryocytic differentiation, and because of probable mechanisms and target genes involved in these procedures, this study is considered as one of the first studies in this field. In comparison to similar studies, some of the probable mechanisms are implied. Many transcription factors play roles in differentiation of megakaryocyte-erythroid progenitors (MEP). Some of these transcription factors include NF-E2, GABPα, GATA-2, Fli-1, GATA-1, RUNX1, etc. Among them, GABPα, Fli-1 and RUNX1 specifically induce megakaryopoiesis, and others have roles in both megakaryopoiesis and erythropoiesis (8). The correlation between these transcription factors and platelet production has been proven in humans (9). CBF (core binding factor) consists of 3 homologous DNA-binding protein subunits (RUNX1, RUNX2 and RUNX3) and a non-DNA binding subunit (CBFβ). RUNX1 and CBFβ complexes are overexpressed in megakaryopoiesis, and their expression is decreased in erythropoiesis (10). Inactivation of RUNX1 or CBFβ in mouse models leads to suppression of megakaryocytic differentiation (10). RUNX1 cooperates with other transcription factors like Fli-1, GATA-1(10, 11). The RUNX1 expression level is increased in primary stages of megakaryocytic differentiation. This increase leads to induction of miR-27a synthesis. Consequently, miR-27 decreases the expression level of RUNX1, but it never becomes zero. So, RUNX1 factor is the primary target for miR-27a.Transcription factors like EKLF and c-Myc act more specifically for erythroid lineage. According to this fact, mRNAs of these factors are probable targets for miR-27a in megakaryocytic differentiation induction pathway. One of the most probable mechanisms is degradation and translation repression of mRNA of these factors.

4. CONCLUSION

Overall, about the current study, we have to state that our goal like other mentioned studies and researches is only an initial step. With more studies and their development in animal models in later steps and finally in clinical practice in patient with thrombocytopenia and platelet functional disorders, we hope to treat these patients by using miR-27a in the future. Basic studies cannot be certainly compared with clinical studies. However, it should be stated that while clinical studies are first at hypothesis step, they becomes possible by advancement of technology.

Funding/ Support

This study was approved and supported by Tehran University of Medical Sciences (Project Number: 91-03-31-17-148).

AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

ACKNOWLEDGMENT

Not mentioned any acknowledgment by authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

REFERENCES

1. Alizadeh S, Kaviani S, Soleimani M, Kouhkan F, Pourfathollah AA, Amirizadeh N, et al. Mir-155 Downregulation by miRCURY LNA™ microRNA Inhibitor Can Increase alpha Chain Hemoglobins Expression in Erythroleukemic K562 Cell Line. International Journal of Hematology-Oncology and Stem Cell Research. 2010;4(2):4-9.
2. Bartels CL, Tsongalis GJ. MicroRNAs: novel biomarkers for human cancer. Clinical chemistry. 2009;55(4):623-31.
3. Ben-Ami O, Pencovich N, Lotem J, Levanon D, Groner Y. A regulatory interplay between miR-27a and Runx1 during megakaryopoiesis. Proceedings of the National Academy of Sciences. 2009;106(1):238-43.
4. Ichimura A, Ruike Y, Terasawa K, Shimizu K, Tsujimoto G. MicroRNA-34a inhibits cell proliferation by repressing mitogen-activated protein kinase kinase 1 during megakaryocytic differentiation of K562 cells. Molecular pharmacology. 2010;77(6):1016-24.
5. Edelstein LC, Bray PF. MicroRNAs in platelet production and activation. Blood. 2011;117(20):5289-96.
6. Bushati N, Cohen SM. microRNA functions. Annu Rev Cell Dev Biol. 2007;23:175-205.
7. Fanini F, Vannini I, Fabbri M. MicroRNAs: tiny players with a big role in the pathogenesis of leukemias and lymphomas. Hematology Reviews. 2009;1(10
8. Metcalf D, Carpinelli MR, Hyland C, Mifsud S, DiRago L, Nicola NA, et al. Anomalous megakaryocytopoiesis in mice with mutations in the c-Myb gene. Blood. 2005;105(9):3480-7.
9. Goldfarb A. Transcriptional control of megakaryocyte development. Oncogene. 2007;26(47):6795-802.
10. Elagib KE, Racke FK, Mogass M, Khetawat R, Delehanty LL, Goldfarb AN. RUNX1 and GATA-1 coexpression and cooperation in megakaryocytic differentiation. Blood. 2003;101(11):4333-41.
11. Huang H, Yu M, Akie TE, Moran TB, Woo AJ, Tu N, et al. Differentiation-dependent interactions between RUNX-1 and FLI-1 during megakaryocyte development. Molecular and cellular biology. 2009;29(15):4103-15.