IJHOSCR

Original Article

International Journal of Hematology-Oncology and Stem Cell Research

The Involvement of Canonical NFκB Pathway in Megakaryocyte Differentiation Induction by Nanocurcumin

Seyedeh Sahar Mortazavi Farsani[1], Dina Sadeghizadeh[1], Sadegh Babashah[1], Fariba Rad[2], Majid Sadeghizadeh[1,3]

[1]Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
[2]Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran
[3]Alborz Nanomed Technology Company, Tehran, Iran

Corresponding Author: Majid Sadeghizadeh, Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
Tel: +9821 82884409
Fax: +9821 82884717
E-mail: sadeghma@modares.ac.ir

Received: 31, Dec, 2020
Accepted: 31, May, 2021

ABSTRACT

Background: Megakaryopoiesis is characterized by progressive polyploidization and the expression of megakaryocytic markers. Numerous transcription factors and physiological signaling pathways regulate this phenomenon. Megakaryocyte differentiation induction in the K562 cell line and hematopoietic stem cells via nanocurcumin drug has been identified in our previous study. K562 cells are typical Chronic Myelogenous Leukemia (CML) cells that are resistant to apoptosis and express the bcr-abl fusion gene. These cells have the potential to differentiate into erythrocytes and megakaryocytes. Curcumin is well known as a component with strong potential to alter NFκB activity in various cells. NFκB pathway regulates various genes such as apoptotic and immune response genes. The current study attempted to evaluate the possible role of nanocurcumin in NFκB pathway regulation during the megakaryopoiesis process in the K562 cell line.

Materials and Methods: Megakaryocyte markers expression and phenotype alteration of nanocurcumin-treated K562 cells have been detected by flow cytometry and microscopy imaging. The nuclear level of the RelA (p65) subunit of NFκB was determined by western blot test in K562 cells during megakaryopoiesis induction via nanocurcumin treatment at different times. The expression of NFκB target genes including *c-MYC*, *BAX*, *and NQO1* was also analyzed in nanocurcumin-treated K562 cells by quantitative RT-PCR assay at different times.

Results: The study has shown that nanocurcumin causes an increase in NFκB activity transiently during megakaryocyte differentiation, followed by a change in the expression of *c-MYC*, *BAX*, *and NQO1* target genes.

Conclusion: The NFκB pathway can be considered a new pathway for inducing megakaryocyte differentiation by nanocurcumin in vitro and in vivo megakaryopoiesis experiments.

Keywords: Megakaryopoiesis; Nanocurcumin; NFκB pathway; Chronic Myelogenous Leukemia (CML)

INTRODUCTION

Megakaryopoiesis is a complex and important process that produces megakaryocytes cells (MKs) from hematopoietic stem cells in bone marrow[1]. Megakaryocytes are responsible to generate platelet in our body. Decreased platelet levels lead to thrombocytopenia problem. Megakaryocytes have specific properties such as large cell size, polyploidy,

Copyright © 2023 Tehran University of Medical Sciences. This work is licensed under a Creative Commons Attribution-Noncommercial 4.0 International license (http:// creativecommons.org/licenses/by-nc/4.0). Non-commercial uses of the work are permitted, provided the original work is properly cited.

membrane protuberance, and surface MK protein markers [2].

Many of transcription factors, cytokines and chemokines regulate megakaryocyte production. Several pathways are also triggered in this phenomenon. Activity of NFκB (nuclear factor κB) pathway in this process is highly recognized [3-6].

The limitations of current thrombocytopenia treatment strategies encourage scientists to find a safe component to induce megakaryopoiesis [7].

Curcumin is a safe plant natural product that derived from the root of the Curcuma longa. This agent can affect numerous cellular physiological processes such as inflammation and oxidation in a dose-dependent manner [8]. Moreover, curcumin has various molecular targets and can regulate many of transcription factors, cytokines, enzymes, etc[9,10]. Nanocurcumin containing curcumin and nanocarrier has previously synthesized in our laboratory in order to improve solubility, bioavailability, and cellular uptake of curcumin. This nano-carrier was prepared from oleic acid and PEG400[11]. The Significant effect of nanocurcumin on megakaryopoiesis induction in hematopoietic stem cells and K562 cell line was indicated in our previous study[12]. This function of nanocurcumin was associated with intracellular reactive oxygen species alteration and MAPKs pathways modulation [12].

Curcumin has been well known as NFκB pathway modulator[13]. Many of biological processes such as inflammation, cell proliferation, survival, differentiation, and apoptosis can be affected by NFκB activity. Five subunits, including RelA (p65), RelB (p68), c-Rel, p50, and p52 make active homodimers and heterodimers of NFκB in cells. NFκB activity is classified into two “canonical” and “noncanonical” pathways. In the canonical pathway, a dimer of NFκB containing Rel A (p65) is transferred to the cell nucleus[14]. The most abundant dimer of NFκB in mammalian cells is the p50/p65 heterodimer. It has shown that p50 homodimers and p50–p65 heterodimers of NFκB have a fundamental function in the proliferation of megakaryocytes[15]. It has also indicated that the NFκB pathway participates in megakaryocytic differentiation and involves thrombopoietin (TPO) receptor and MPL signaling[16,17]. TPO is a cytokine that is essential for megakaryocyte differentiation regulation in the body. This factor signals through the receptor MPL (myeloproliferative leukemia protein) and is essential for megakaryocyte differentiation [18].

There are some contradictory reports about the effects of NFκB on megakaryocyte differentiation. For example, inhibition of NFκB activity under the influence of Diosgenin in the megakaryocyte production process of HEL cells has been demonstrated [19]. In this study, NFκB activity during megakaryocyte differentiation of the K562 Chronic Myelogenous Leukemia (CML) cell line under the influence of nanocurcumin has been examined. CML is a chronic myeloproliferative disorder with an specific chromosomal abnormality called Philadelphia chromosome that have a translocation between chromosomes 9 and 22 (t(9;22)(q34;q11)). K562 cells have potential to differentiate to erythrocyte and megakaryocyte. These cells have been used in many studies as model cells to recognize the mechanisms of megakaryocyte differentiation mechanisms[20, 21]. The expression of three NFκB downstream genes, including BAX, c-MYC, and NQO has also been evaluated in this study. The outcome of this study showed the nuclear localization increase of the P65 (RelA) subunit of NFκB at the beginning of nanocurcumin treatment and then followed by a decrease in the activity of this factor. Thus, it seems that nanocurcumin can alter the canonical activity of NFκB during megakaryopoiesis transiently. Gene expression analysis has demonstrated the upregulation of NFκB target genes by nanocurcumin treatment in a time-dependent manner.

MTERIALS AND METHODS

Cell culture and reagents

K562 CML cell lines were obtained from the Pasteur Institute of Tehran, Iran. These cells were cultured in RPMI1640 medium (Gibco BRL, Life technology) containing FBS (10% v/v) and penicillin/streptomycin (1% v/v) (Gibco BRL, Life technology). Incubation was performed at 37oC and 5% CO2. Cells were counted by hemocytometer using trypan blue (Sigma-Aldrich, St. Louis, MO) staining. Nanocurcumin, containing Oleoyl chloride and PEG400, was synthesized in Professor Sadeghizadeh’s laboratory[22]. RiboEx was

purchased from GeneAll Biotechnology Company, Korea. May-Grunwald Giemsa was prepared by Sigma Aldrich Company, St. Louis, MO. Antibodies including anti-CD41-FITC, anti-CD61-FITC, and PCNA (Proliferating Cell Nuclear Antigen) were purchased from Miltenyi Biotec Company, Germany. NFκB activation assay kit was purchased from FIVEphoton BiochemicalsTm, San Diego, CA 92117.

Megakaryocyte differentiation analysis

The best concentration of nanocurcumin (15µM) for megakaryopoiesis triggering in K562 cells was identified in our previous study [12]. In the current study, 15µM of nanocurcumin has used for K562 cells treatment in order to NFκB activity evaluation during megakaryocyte differentiation. For this purpose, 2 × 105 cells per well have seeded in a 6-well plate containing RPMI media, FBS (10%), and penicillin/streptomycin (1%). These cells were treated with nanocurcumin for 24, 48, and 72 hours. According to our previous study, 72 hours is the best period time for detection of megakaryocyte differentiation of K562 [12], so phenotype alteration of K562 was evaluated up to 72 hours. In order to evaluate the induction of megakaryocyte differentiation, cell surface megakaryocyte markers including CD41 and CD61 expression was analyzed by flow cytometry (FACS Calibur, BD Biosciences, San Jose, CA 95131) using anti-human FITC-conjugated CD41 and CD61 monoclonal antibodies after 24, 48, and 72 hours. Flow cytometry results were analyed by FlowJo7.6.1 software. The morphology of treated and untreated cells was also determined by May-Grunwald Giemsa 1× staining and visualized via light microscopy.

Nuclear and cytoplasmic protein extraction

After treatment of K562 cells with nanocurcumin for different times (24h, 48h, 72h, and 96h), the cells were harvested by centrifuge. Nuclear protein and cytoplasmic proteins were separated by nuclear fractionation Reagent (NER-1) and cytoplasmic fractionation reagent (CER-1), respectively. Dithiothreitol (DTT from Sigma-Aldrich, St. Louis, MO) and protease inhibitor cocktail (Sigma Aldrich, St. Louis, MO) have been added to the CER-1 buffer. All of these protocols were performed according to the manufacture of the NFκB activation assay kit (FIVEphoton BiochemicalsTm, San Diego, CA 92117). The protein concentration of each sample was determined using Bradford assay [23].

Western blot assay

An equal amount of nuclear protein samples (30 µg) were heated to 60 °C for 5 min in the presence of loading buffer, and vortex was done for 1 min. Proteins were then loaded in SDS-PAGE (SDS–polyacrylamide gel electrophoresis) and transferred to PVDF membrane. The membranes were blocked by 5% BSA (Merk Company, Germany) for 1 hour at room temperature and washed with TBST buffer. The blocked membranes were incubated with P65 primary antibody (1:1000) overnight at 4 °C according to antibody manufacture (FIVEphoton Biochemicals$_{Tm}$, San Diego, CA 92117). After washing with TBST buffer, incubation with anti-rabbit horseradish-conjugated secondary antibody from Santa Cruz Biotechnology, Inc. Texas, U.S.A (1:1000) was performed to detect the primary antibody by enhanced chemiluminescence (ECL) detection system (Cyto Matin Gene Immune Company, Isfahan, Iran). PCNA protein was used as an internal control for nuclear protein and was detected by anti-PCNA antibody (Santa Cruz, Texas, U.S.A, 1:1000) and anti-mouse HRC conjugated antibody (1:1000) as the protocol previously explained. Western blot results were qualified by Image j software (SCR_003070).

Quantitative real time RT-PCR

The expression of c-MYC, BAX, and NQO1 genes was determined by quantitative real-time RT-PCR after 24h, 48h, 72h, and 96h of nanocurcumin treatment. GAPDH gene expression was utilized as an internal control. Total RNA was extracted from samples using RioEx solution (GeneAll Biotechnology company, Korea) and reverse transcribed to cDNA by reverse transcriptase (RT) according to the manufacturer's instructions (from Sina clone Tehran, Iran). Quantitative PCR was done with StepOne real-time PCR equipment. The amplification condition was one cycle at 94° C for 10min, followed by 40 cycles at 94° C for 30 s, 63° C for 30 s and 72° C for 30 s. Primers have been designed via OLIGO Primer Analysis

Software Version 7 and ordered from Macrogen Company of Tehran. Each primer was used at 10 pM concentration and their sequences are listed in Table1. Threshold cycle numbers were determined using Step one manager software (RRID: SCR_014281), and data were analyzed using delta-delta CT method. Statistical analysis was done by GraphPad prism7 software.

Table 1: Primer sequences for real time RT-PCR

Gene	Forward primer	Reverse primer	Product length
c-MYC	CGTCCTCGGATTCTCTGCTCTC	GTTCCTCCTCAGAGTCGCTGC	114
BAX	GCAAACTGGTGCTCAAGG	CAGCCACAAAGATGGTCA	183
GAPDH	CCGAGCCACATCGCACAG	GGCAACAATATCCACTTTACCAG	119
NQO1	CCAATTCAGAGTGGCATTCTGC	GGAAGTTTAGGTCAAAGAGGCTG	190

Statistical analyses

One-way repeated measure analysis of variance (one-way RM ANOVA) was used for the analysis of the difference between groups by GraphPad Prism7 software. Probability values of $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***) were considered statistically significant

RESULTS

Nanocurcumin can increase megakaryocyte markers expression

In order to analyze the rate of megakaryocyte differentiation in K562 cells, the expression level of CD41 and CD61 megakaryocyte markers were detected by flow cytometry after nanocurcumin treatment at 24, 48, and 72 hours. Expression of these markers increased significantly in the presence of nanocurcumin after 72h (67.4% and 49.3% of cells were CD41 and CD61 positive, respectively) (Figure 1). Expression alteration of these markers after 24h and 48h of treatment was not significant (results have not shown). In addition to surface markers, treated cells were stained with May-Grunwald Giemsa and visualized via light microscopy after 48 and 72 hours. Cell imaging demonstrated an increase in the cell size and a protuberance in the cell membrane after nanocurcumin treatment. These alterations indicated specific characteristics of megakaryocyte cells (Figure 2).

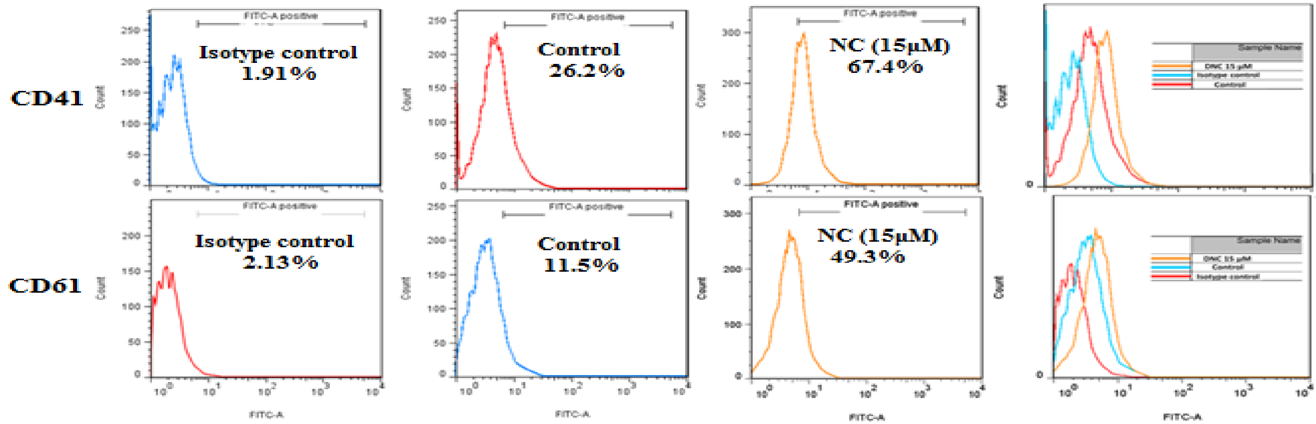
NFκB activity analysis

The involvement of the NFκB pathway in the megakaryocyte differentiation process under the treatment of nanocurcumin was evaluated via western blot analysis of the nuclear p65 subunit. As shown in Figure 3, the nuclear localization of the p65 subunit of NFκB increased 48h after treatment with 15 µM nanocurcumin, then followed by significant decrease after 72 and 96 hours. The amount of nuclear PCNA protein as an internal control was similar in all of the samples. Results were qualified by image j software.

Gene expression alteration under the influence of nanocurcumin

The mRNA expression levels of BAX, c-MYC, NQO1, and GAPDH genes were measured by real-time RT-PCR. The c-MYC gene expression was increased after 48 hours, and NQO1 and BAX were upregulated significantly after 96 hours in nanocurcumin treated K562 cells (Figure 4).

(A)



(B)

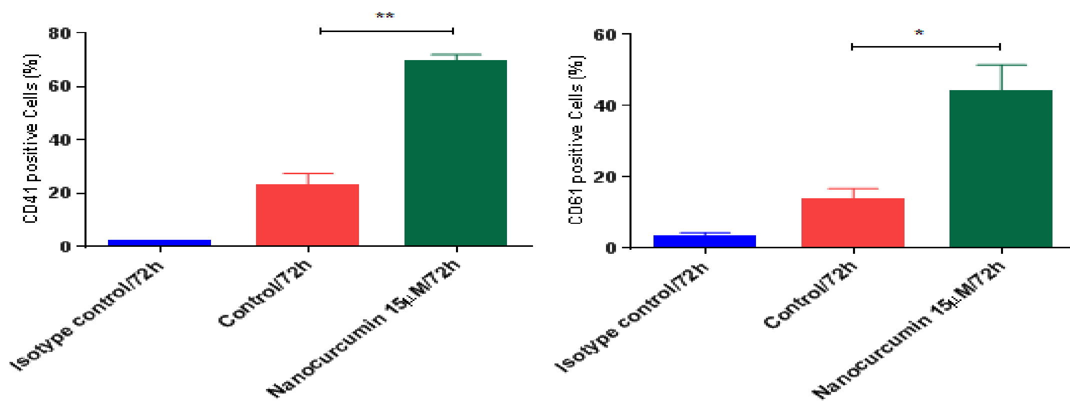


Figure 1. Flowcytometry results of K562 cells stained with FITC-CD41 and FITC-CD61 antibodies. (A) The percentage of CD41 and CD61 positive of control (untreated) and treated cells after 72 hours are shown. (B) The diagrams demonstrated that after 72 hours of nanocurcumin treatment, protein expression levels of CD41 and CD61have increased significantly. Data was not significant for 24 and 48 hours (not shown). (Sample size (n) = 4, *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001, NC: nanocurcumin, CD: cluster of differentiation, FITC: Fluorescein isothiocyanate).

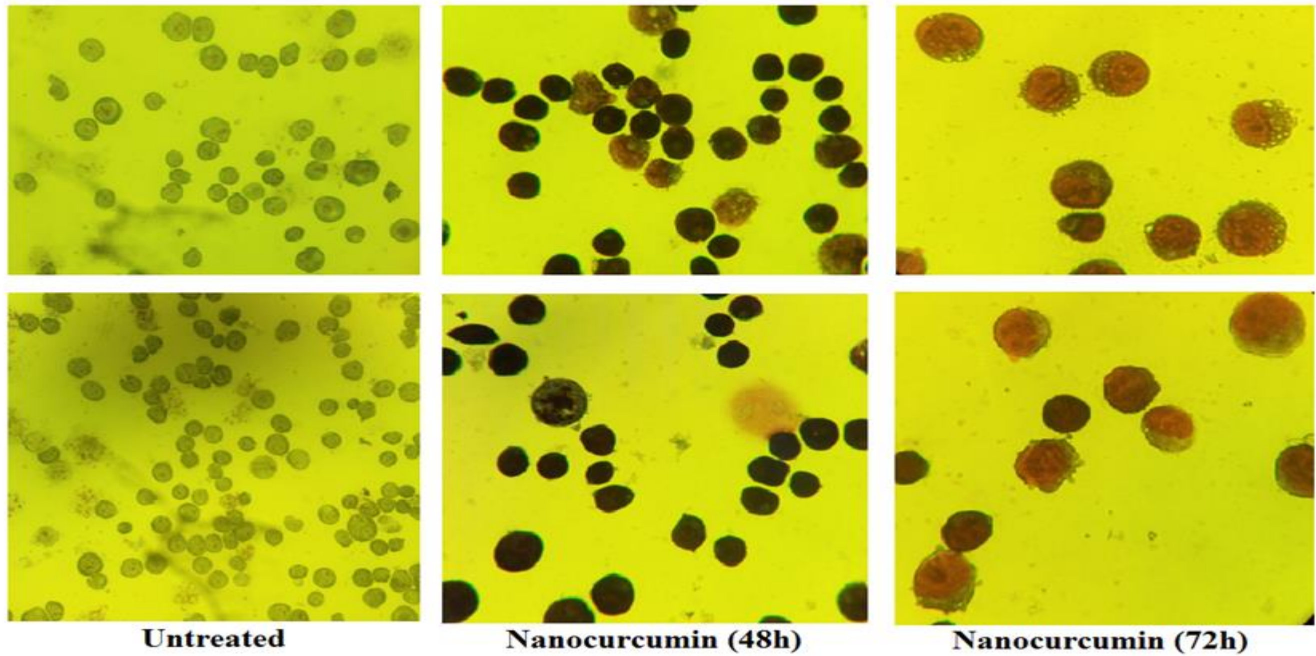


Figure 2. Alteration morphology of k562 cells that treated with nanocurcumin (15μM). Pictures have shown control (untreated) and treated cell with nanocurcumin (15μM) for 48 and 72 hours. Images have been taken by light microscopy after Giemsa staining. As it is evident the cell size increased significantly after 72 hours by nanocurcumin treatment.

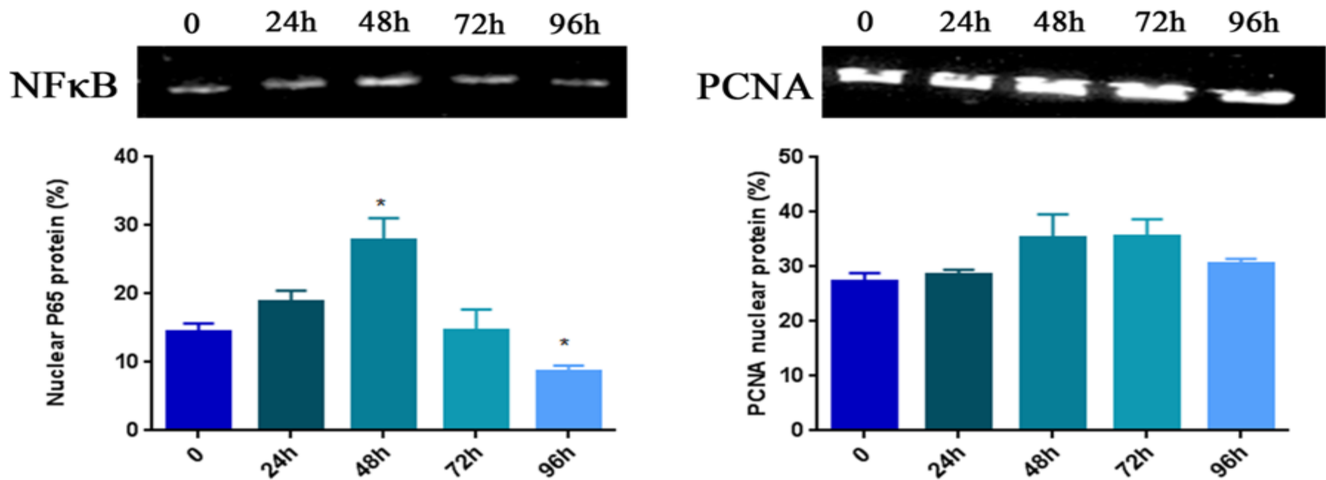


Figure3. Western blot analysis of nuclear NFκB (P65 subunit) in K562 cells treated with nanocurcumin for different time intervals (0, 24, 48, 72 and 96 hours). PCNA protein used as nuclear internal control. Data are expressed as means ± SEM, *P < 0.05 was considered statistically significant.

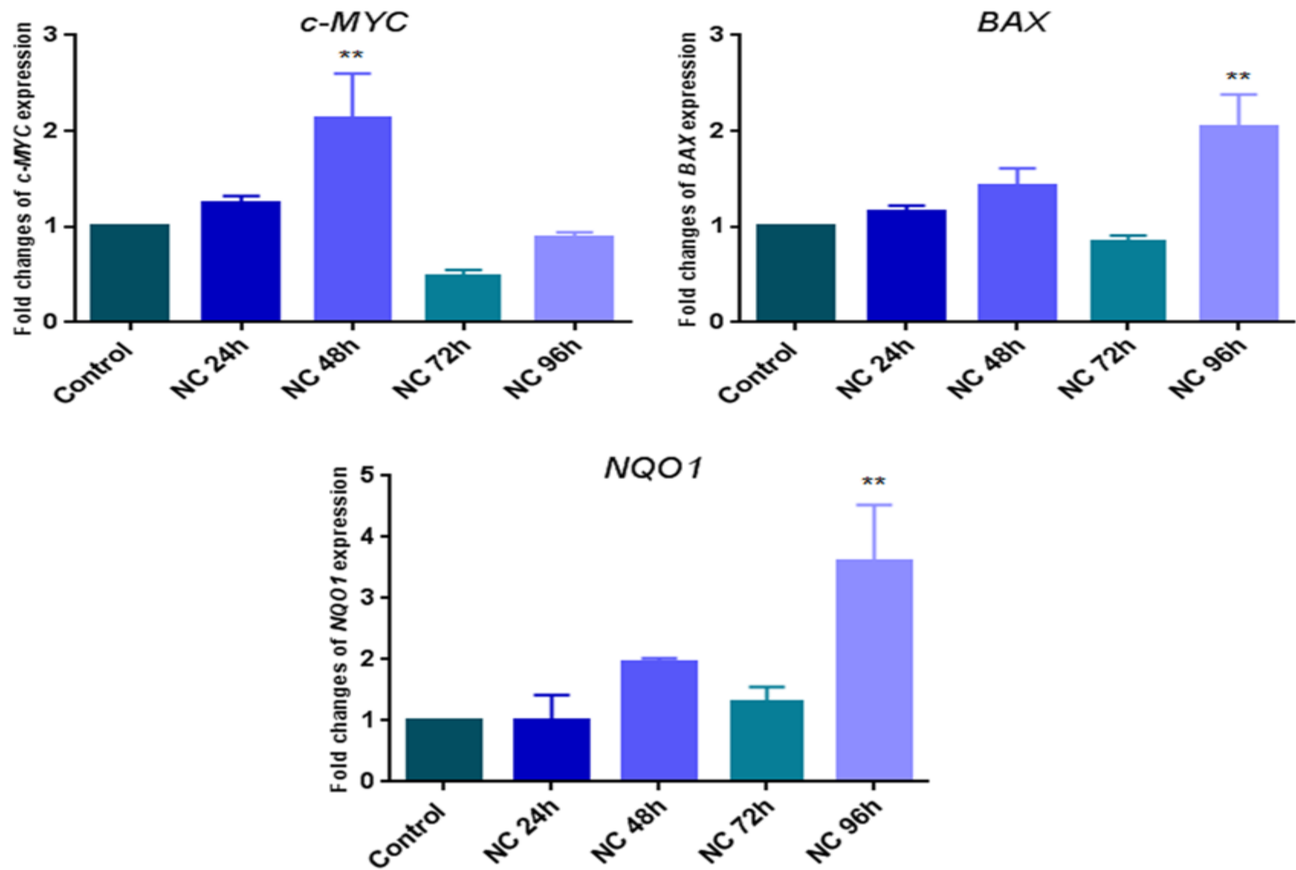


Figure 4. Gene expression analysis of cMYC, BAX and NQO1 factors. Fold change expression of genes in control and treated K562 cells with nanocurcumin (15μM) at different times were measured by real-time RT-PCR. The data was analyzed using the 2 -ΔΔCT method. (Sample size (n) = 4, *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001, NC: nanocurcumin).

DISCUSSION

Megakaryopoiesis is a phenomenon responsible for producing megakaryocytes and platelets in our body. This process is triggered via thrombopoietin [24]. Various factors, including cytokines and growth factors regulate megakaryopoiesis in physiological conditions. Scientists demonstrated that many chemical components such as Phorbol-12 myristate-13 (PMA) and nanocurcumin can induce megakaryocyte differentiation from megakaryocyte progenitors or K562 leukemia cell lines.

PMA is a small molecule that induces differentiation of hematopoietic stem cells and K562 cells towards megakaryocytes[25]. Nanocurcumin contain curcumin and nanocarrier. Void curcumin has many limitations for therapeutic application because of its low bioavailability, water insolubility, and low cellular uptake. In previous experiments, nanocurcumin was synthesized in our laboratory to overcome these limitations [22].

NFκB is a transcription factor that regulates the expression of thousands of genes involved in main cellular processes. NFκB signaling has been known as a central mediator of inflammation and is involved in the thrombotic processes, platelet production, and platelet activity[26]. NFκB activity induces megakaryocyte progenitor cells to convert systemic or local inflammatory conditions to a transcriptional response, which may have consequences on the phenotype of megakaryocytes and released

platelets[27,28]. Available studies showed that activation of thrombopoietin receptor (MPL) by interaction with its ligand leads to immediate induction of IκB kinase (IKK) activity during megakaryocyte differentiation in physiological conditions. However, over time leading to a significant reduction in IKK and NFκB activities[27,29]. IKK factor is responsible for the phosphorylation of IκBα inhibitory protein, dissociation of IκBα from NFκB, and formation of active NFκB. It has also shown that the NFκB pathway regulates TPO production through the stimulation of transferrin[30].

The results of the current study demonstrated the induction of MK differentiation by nanocurcumin. In this experiment, nanocurcumin increased the expression of CD41 and CD61 markers more than other megakaryopoiesis inducers such as phytosphingosine, 3-Hydrogenkwadaphnin[31], Lapatinib[32], and many small molecules [33].

The outcome of NFκB activity experiments supports that nanocurcumin can increase transiently nuclear localization of NFκB (P65 subunit) during megakaryopoiesis induction. This finding is consistent with the process that takes place in normal physiological condition[17], so activation of NFκB after 48 hours of nanocurcumin treatment possibly participates in megakaryocyte differentiation triggering.

Curcumin is widely used in diet and nutritional supplements and can regulate various cellular pathways [34]. The inhibitory effect of curcumin on canonical NFκB signaling pathway is well-described previously [35, 36]. Scientists indicated that among 84 target genes of NFκB, 29 mRNAs were significantly down-regulated in Curcumin-treated K562 cells [37].

In accordance with the current study, the ability of some anti-inflammatory agents such as 15-deoxy-Δ12,14- Prostaglandin J2 and parthenolide (a feverfew plant-derived compound) to induce megakaryocyte differentiation and platelet production through NFκB modulation have been indicated [38,39]. Differentiation of K562 cells is also induced by 8-Hydroxydaidzein, an Isoflavone from Fermented Soybean by activation of NFκB signaling pathway [40].

The expression of NFκB target genes including BAX, NQO1, and c-MYC have altered at different times during nanocurcumin treatment in the current investigation. C-MYC gene expression was enhanced earlier than NQO1 and BAX genes. Previous studies have demonstrated that NFκB target genes have distinct kinetic patterns. Early and late-activated P65 target genes have different chromatin configurations. Early target genes express directly by P65 activity and late target genes respond indirectly to other P65-activated transcription factors[41]. Hence, it seems that the c-MYC gene is a member of the early-activated target genes of NFκB whereas NQO1 and BAX are late-activated genes.

CONCLUSION

According to the results of the present experiment, the NFκB pathway can be a new mechanism in the megakaryopoiesis induction by nanocurcumin. Transiently nuclear localization of NFκB (P65) may be related to the upregulation of NFκB target genes during this process. Therefore, the NFκB pathway could be considered for future studies about small molecules that induce megakaryopoiesis. Further experiments are also needed to determine whether inhibition of NFκB causes megakaryopoiesis inhibition in the cells treated with nanocurcumin or other MK differentiation inducers.

ACKNOWLEDGMENTS

The research was supported by the Royan Stem cell Technology Institute of Tehran (grant number: F/96-14890) and the Tarbiat Modares University of Tehran. The authors would like to thank Alborz Nanomed Technology Company (www.Nanomed.ws).

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

1. Chang Y, Bluteau D, Debili N, Vainchenker W. From hematopoietic stem cells to platelets. J Thromb Haemost. 2007. 5(Suppl.1):318-27.

2. Noetzli LJ, French SL, Machlus KR. New insights into the differentiation of megakaryocytes from hematopoietic progenitors. Arterioscler Thromb Vasc Biol. 2019;39(7):1288-1300.

3. Severin S, Ghevaert C, Mazharian A. The mitogen-activated protein kinase signaling pathways: role in megakaryocyte differentiation. J Thromb Haemost. 2010; 8(1):17-26.
4. Rojnuckarin P, Miyakawa Y, Fox NE, et al. The roles of phosphatidylinositol 3-kinase and protein kinase Cζ for thrombopoietin-induced mitogen-activated protein kinase activation in primary murine megakaryocytes. J Biol Chem. 2001;276(44):41014-22.
5. Hirao A. TPO signal for stem cell genomic integrity. Blood. 2014; 123(4):459-460.
6. Fox N, Lim J, Chen R, et al. F104S c-Mpl responds to a transmembrane domain-binding thrombopoietin receptor agonist: proof of concept that selected receptor mutations in congenital amegakaryocytic thrombocytopenia can be stimulated with alternative thrombopoietic agents. Exp Hematol. 2010; 35(5):384-391.
7. Alcaina PS. Platelet Transfusion: And Update on Challenges and Outcomes. J Blood Med. 2020; 11:19-26.
8. Soltani B, Ghaemi N, Sadeghizadeh M, et al. Redox maintenance and concerted modulation of gene expression and signaling pathways by a nanoformulation of curcumin protects peripheral blood mononuclear cells against gamma radiation. Chem Biol Interact. 2016; 257:81-93.
9. Goel A, A.B. K, Aggarwal BB. Curcumin as “curecumin”: From kitchen to clinic. Biochem Pharmacol. 2008; 75(4):787–809.
10. Javidi MA, Kaeidi A, Mortazavi Farsani SS, et al. Investigating curcumin potential for diabetes cell therapy, in vitro and in vivo study. Life Sci. 2019;239:116908.
11. Babaei E, Sadeghizadeh M, Hassan ZM, et al. Dendrosomal curcumin significantly suppresses cancer cell proliferation in vitro and in vivo. Int Immunopharmacol. 2012;12(1):226-34.
12. Farsani SSM, Sadeghizadeh M, Gholampour MA, et al. Nanocurcumin as a novel stimulator of megakaryopoiesis that ameliorates chemotherapy-induced thrombocytopenia in mice. Life Sci. 2020; 256:117840.
13. Bharti AC, Donato N, Singh S, et al. Curcumin (diferuloylmethane) down-regulates the constitutive activation of nuclear factor-kappa B and IkappaBalpha kinase in human multiple myeloma cells, leading to suppression of proliferation and induction of apoptosis. Blood. 2003; 101(3):1053–62.
14. Carrà G, Torti D, Crivellaro S, et al. The BCR-ABL/NF-κB signal transduction network: a long lasting relationship in Philadelphia positive Leukemias. Oncotarget. 2016; 7(40):66287-66298.
15. Yang Y, Wu J, Wang J. A database and functional annotation of NF-κB target genes. Int J Clin Exp Med. 2016; 9(5):7986-7995.
16. Kim KW, Kim SH, Lee EY, , et al. Extracellular signal-regulated kinase/90-KDA ribosomal S6 kinase/nuclear factor-κB pathway mediates phorbol 12-myristate 13-acetate-induced megakaryocytic differentiation of K562 cells. J Biol Chem. 2001;276(16):13186-91.
17. Zhang Y, Sun S, Wang Z, et al. Signaling by the Mpl Receptor Involves IKK and NF-kB. J Cell Biochem. 2002;85(3):523-35.
18. Cui L, Moraga I, Lerbs T, et al. Tuning MPL signaling to influence hematopoietic stem cell differentiation and inhibit essential thrombocythemia progenitors. Proc Natl Acad Sci U S A. 2021;118(2):e2017849118.
19. Leger DY, Liagre B, Beneytout JL. Role of MAPKs and NF-κB in diosgenin-induced megakaryocytic differentiation and subsequent apoptosis in HEL cells. Intl J Oncol.2006; 28(1):201-207.
20. Jacquel A, Herrant M, Defamie V, et al. A survey of the signaling pathways involved in megakaryocytic differentiation of the human K562 leukemia cell line by molecular and c-DNA array analysis. Oncogene. 2006; 25(5):781-94.
21. Kaur J, Rawat Y, Sood V, et al. Megakaryopoiesis in Dengue virus infected K562 cell promotes viral replication which inhibits endomitosis and accumulation of ROS associated with differentiation. bioRxiv. 2020.
22. Mirgani MT, Isacchi B, Sadeghizadeh M, et al. Dendrosomal curcumin nanoformulation downregulates pluripotency genes via miR-145 activation in U87MG glioblastoma cells. Int J Nanomedicine.2014; 9:403-17.
23. Kruger NJ. The Bradford method for protein quantitation. Methods Mol Biol. 1994;32:9-15.
24. Geddis AE. Megakaryopoiesis. In: Seminars in hematology: Elsevier; 2010. pp. 212-219.
25. Li C, Peart N, Xuan Z, et al. PMA induces SnoN proteolysis and CD61 expression through an autocrine mechanism. Cell signal. 2014; 26(7):1369-78.
26. Kojok K, El-Kadiry AE-H, Merhi Y. Role of NF-κB in platelet function.
Int J Mol Sci. 2019;20(17):4185.
27. Mussbacher M, Salzmann M, Brostjan C, et al. Cell type-specific roles of NF-κB linking inflammation and thrombosis. Front Immunol. 2019; 10:85.
28. Vallance TM, Sheard JJ, Meng Y, et al. Development and characterization of a novel, megakaryocyte NF-κB reporter cell line for investigating inflammatory responses. J Thromb Haemost. 2021;19(1):107-120.
29. Zhang Y, Sun S, Wang Z, et al. Signaling by the Mpl receptor involves IKK and NF-κB. J Cell Biochem. 2002;85(3):523-35

30. Ono-Uruga Y, Tozawa K, Matsuoka S, et al. A Novel Mechanism of Megakaryopoiesis from Pre-Adipocytes: Involvement of Transferrin/CD71/TPO Pathways. In: American Society of Hematology Washington, DC; 2014.
31. Meshkini A, Yazdanparast R. Induction of megakaryocytic differentiation in chronic myelogenous leukemia cell K562 by 3-hydrogenkwadaphnin. J Biochem Mol Biol. 2007;40(6):944-51.
32. Huang HL, Chen YC, Huang YC, et al. Lapatinib induces autophagy, apoptosis and megakaryocytic differentiation in chronic myelogenous leukemia K562 cells. PloS one. 2011; 6(12):e29014.
33. Zou X, Qu M, Fang F, et al. Small molecule supplements improve cultured megakaryocyte polyploidization by modulating multiple cell cycle regulators. Biomed Res Int. 2017;2017:2320519.
34. Shehzad A, Lee YS. Molecular mechanisms of curcumin action: signal transduction. Biofactors. 2013; 39(1):27-36.
35. Ghasemi F, Shafiee M, Banikazemi Z, et al. Curcumin inhibits NF-kB and Wnt/β-catenin pathways in cervical cancer cells. Pathol Res Pract. 2019;215(10):152556.
36. Marquardt JU, Gomez-Quiroz L, Camacho LOA, et al. Curcumin effectively inhibits oncogenic NF-κB signaling and restrains stemness features in liver cancer. J Hepatol. 2015;63(3):661-9.
37. Reuter S, Charlet J, Juncker T, et al. Effect of curcumin on nuclear factor κB signaling pathways in human chronic myelogenous K562 leukemia cells. Ann N Y Acad Sci. 2009;1171:436-47.
38. Sahler J, Bernard JJ, Spinelli SL, et al. The Feverfew plant-derived compound, parthenolide enhances platelet production and attenuates platelet activation through NF-κB inhibition. Thromb Res. 2011;127(5):426-34
39. Straus DS, Pascual G, Li M, et al. 15-Deoxy-Δ12, 14-prostaglandin J2 inhibits multiple steps in the NF-κB signaling pathway. Proc Natl Acad Sci U S A. 2000; 97(9): 4844–4849.
40. Wu PS, Yen JH, Wang CY, et al. 8-Hydroxydaidzein, an Isoflavone from Fermented Soybean, Induces Autophagy, Apoptosis, Differentiation, and Degradation of Oncoprotein BCR-ABL in K562 Cells. Biomedicines. 2020; 8(11):506.
41. Zhao M, Joy J, Zhou W, et al. Transcriptional outcomes and kinetic patterning of gene expression in response to NF-κB activation. PLoS Biol. 2018; 16(9):e2006347.