# Effect of Resveratrol on Apoptosis and MDM2, RUNX3, RB Gene Expressions in Human Acute Myeloid Leukemia Cells by Transfection of MATRA-Mediated miR-150

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## ABSTRACT

Acute myeloid leukemia (AML) is a heterogeneous group of hematopoietic malignancies. Magnet assisted transfection (MATRA) is one of the most effective non-viral transfection methods. We aimed to evaluate the apoptotic effect of resveratrol (RES), MDM2, RUNX3, RB gene expression exchanges and MicroRNA- 150 (miR-150) transfected with MATRA to HL-60 cells. MATRA was used as non-viral vector carrier for miR-150 transfection. $IC_{50}$ dose of RES was determineted by Fahri et al. Resveratrol and miR-150 transfected cells were performed apoptosis and MDM2, RUNX3, RB gene expression assays in Human promyelocytic leukemia (HL-60) cells. $IC_{50}$ dose of RES was used as 5 µM. In HL-60 cells, it was found that miR-150, miR150-Resveratrol combination, resveratrol alone induces apoptosis by 6.48, 6.93 and 4.54 fold, respectively, compared to the control cells. Compared to control cells, MDM2, RUNX3, RB, gene expressions decreased (miR-150) 1.7, 1.3 and 1.4 fold, (miR-150-resveratrol) MDM2 expression increased 2.73 fold, RUNX3 and RB expression decreased 6.1, 1.07 fold, respectively. Combination with resveratrol, MDM2, RB decreased 2.9 and 1.4 fold, respectively. Non-viral miR-150 transfection may be effective in leukemia cells, induction of apoptotic effects and gene expression changes, following treatment with resveratrol and miR-150-resveratrol combinations.

**Keywords:** miR-150, Acute myeloid leukemia, MATRA, Resveratrol, gene expressions

## ÖZET

**MATRA Aracılı miR-150'nin Transfeksiyonu ile İnsan Akut Myeloid Lösemi Hücrelerinde Resveratrol'ün MDM2,RUNX3,RB Gen Ekspresyonları ve Apoptoz Üzerine Etkisi**

Akut myeloid lösemi (AML), hematopoetik malignitelerin heterojen bir grubudur. "Magnet assisted transfection" (MATRA), non-viral transfeksiyon metodlarında oldukça etkilidir. Çalışmamızda; İnsan promiyelositik lösemi (HL-60) hücrelerine MATRA ile MicroRNA-150 (miR-150) transfekte edilip; MDM2,RUNX3,RB gen ekspresyon değişimleri ve resveratrolün apoptotik etkisini değerlendirmeyi amaçladık. MATRA, miR-150 transfeksiyonu için non-viral taşıyıcı olarak kullanılmıştır. HI-60 hücrelerinde Resveratrolün $IC_{50}$ dozu, Fahri et al tarafından saptanmıştır. Resveratrol ve miR-150 ile transfekte edilmiş HL-60 hücrelerinde apoptoz ve MDM2, RUNX3, RB gen ekspresyon analizleri yapıldı. HL-60 hücrelerinde Resveratrolün $IC_{50}$ dozu, 5 µM olarak saptandı. kontrol hücreleriyle karşılaştırıldığında; HL-60 hücrelerinde sırasıyla; miR-150, miR150-Resveratrol kombinasyonu, resveratrolün tek başına apoptozu 6.48, 6.93 ve 4.54 kat indüklediği tespit edilmiştir. Kontrol hücrelerine göre miR-150 tranfeksiyonu yapılanlarda MDM2, RUNX3, RB gen expresyonlarının sırasıyla 1.7, 1.3 ve 1.4 kat azaldığı; miR-150-resveratrol kombinasyonlarında ise MDM2 ekspresyonu 2.73 kat artmış; RUNX3 ve RB ekspresyonları sırasıyla; 6.1, 1.07 kat azalmıştır. Resveratrol ile kombinasyonda MDM2, RB sırasıyla 2.9 ve 1.4 kat azalmıştır. miR-150-resveratrol ve resveratrol kombinasyonları ile tedaviyi takiben gen ekspresyon değişimleri ve apoptotik etkiyi indüklemeleri; non-viral miR-150 transfeksiyonunun lösemi hücrelerinde etkili olabileceğini göstermektedir.

**Anahtar Kelimeler:** miR-150, Akut myeloid lösemi, MATRA, Resveratrol, Gen ekspresyonları

## INTRODUCTION

Acute myeloid leukemia is a heterogeneous group of malignant hematopoietic diseases characterized by uncontrolled proliferation of clonal neoplastic cells and accumulation of blasts in the bone marrow.[1] Acute promyelocytic leukemia is a subset of acute myeloid leukemia characterized by the expression of PML-RARα fusion proteins and specific chromosomal translocations t(15;17)(q24;q21).[2-4]

Natural products have been attention in the treatment of cancer, recently like resveratrol [trans-3,5,4'- trihydroxystilbene, (RES)] which a is natural product found in grapes, red wine, cranberries, blueberries, and peanuts plant.[5] Significant cytotoxic and apoptotic effects are demonstrated in various types of cancer cells by the absence of damage to normal healthy cells.[6] Resveratrol inhibits the proliferation of HL-60 cells depending on time and dose. It also causes cell cycle arrest in G1/S phase.[7-9]

miRNAs are important regulators of normal hematopoietic differentiation.[10-15] miRNAs, which play an important role in the regulation of gene expression, are single chain unencoded RNA molecules and 18-25 nucleotides in length. Deregulation of miRNA expressions is important in the pathogenesis of many genetic and multifactorial diseases. miRNAs may provide a promising strategy for cancer therapy, targeting specific proteins that play a role in the mechanism of proliferation, invasion, anti-apoptosis, drug resistance, and metastasis.[16]

In the case of primary leukemia, various miRNAs were identified that expressed blast crisis chronic myeloid leukemia or acute myeloid leukemia, compared to normal hematopoietic progenitor cells.[17,18] Recently, miR-150 roles (MLL-rearranged) have been defined in AML. Expression of miR-150 maturation by MLL-fusion genes was accelerated in leukemogenesis in the MLL-AF9 mouse model and miR-150 expression inhibited leukemia cell proliferation.[19]

MATRA is an easy, fast and very effective technology for transfection of cells in culture.[20] For this purpose; transfection of miR-150, which was downregulated in leukemic cell lines and control cell lines, was performed via MATRA. In this study, we aimed to evaluate the effect of resveratrol on apoptosis in human HL-60 acute promyelocytic leukemia cells after miR-150 transfection via MATRA.

## MATERIALS AND METHODS

### Chemicals and Cell Lines

RES was purchased from Sigma-Aldrich, a stock solution of RES was prepared in dimethyl sulfoxide (DMSO) at a concentration of 100 mmol/L and then diluted in cell culture medium. The DMSO concentration did not exceed 0.1% in any experiment.[21] Cell culture medium and fetal bovine serum (FBS) were purchased from Biological Industries (Kibbutz Beit-Haemek, Israel) and Sigma–Aldrich (St Louis, MO, USA).

HL-60 cell line was obtained from ATCC (USA). Human promyelocytic leukemia cell line was cultured and maintained in RPMI-1640 medium; containing 2 mM L-glutamine supplemented with 10% inactivated FBS and 1% penicillin/streptomycin. Cell lines were maintained in a humidified incubator at 37°C with 5% $CO_2$. Analysis of Cell Viability was carried with the Cedex XS Analyzer (Roche).

### Transfection of miR-150 by MATRA

Before the transfection miR-150 with MATRA, cells were seeded in 6 well-plate ($5x10^5$-$1x10^6$/ ml). Magnet plate was used for the adhesion of cells.[20] Plate was put in a humidified incubator at $37^0$C with 5% $CO_2$. After the transfection miR-150 with MATRA to HL-60 cells, efficiency of transcription was checked via fluorescence-labeled control oligo (siGLO Red Transfection Indicator) after 6 hours, cells were observed under the fluorescence microscope (Olympus, Japan), and images were recorded. Transfection efficiency of miR-150 and miR-150 with RES was examined, respectively. As a control group, HL-60 (not transfection) was used.

### Analysis of Apoptosis

The apoptosis was determined by Annexin V Apoptosis Detection Kit (BD Pharmingen).Cells in each group were seeded in six-well plate at a den-

sity of1x $10^6$ /well. After 96 h of incubation in 5% $CO_2$ incubator and RPMI-1640 medium, the cells were collected by centrifugation at 1,200 x g for 5 min. FITC Annexin V Cells that stain positive for FITC Annexin V and negative for PI are evaluated as cells undergoing apoptosis. Apoptotic, necrotic and alive cells were determined in the end stage of this Annexin method. Cells were subjected to the process of apoptosis and re-suspended in 500 ml PBS before being analyzed by BD Accuri C6 Flow Cytometry (FL1-A and FL3-A channels), with the data being analyzed using CFlow Software (Accuri Cytometers, Inc.). All studies were carried out in triplicate.

### MDM2, RUNX3, RB Expression Profiling

After transfection for 96 h, total RNA isolations and cDNA synthesis were done. The RNA concentration was determined with a spectrophotometer (NanoDrop™, ND-8000, Thermo Scientific, MA, USA) ( 260/280 nm), RNA was extracted using miRNeasy Kit. qRT-PCR was carried out with a miRCURY LNA Universal RT microRNA system kit. qPCR for MDM2,RUNX3,RB and the reference gene were used in SYBR Green PCR Master Mix and primers (Thermo Scientific, USA). Twenty microlitre of resulting cDNA were subjected to PCR reactions using specific hsa-miR-150-5p LNA PCR primers, and SYBR Green. As a housekeeping gene, GAPDH, ACTB and RN18S1 were used. A negative control lacking cDNA was used to detect possible contamination, and a Universal cDNA synthesis kit was used for reverse transcription. Twenty nanogram RNA and RNA "spike-in" for expression normalization were used.

### Statistical Analysis

Possible variations of results of apoptosis and expression changes have been compared with the one-way ANOVA test. Post-hoc comparison of the groups involved the Tukey test (significance taken as $p< 0.05$). Data are expressed as mean standard deviation (SD). Statistical analyses used SPSS 15.0 software.

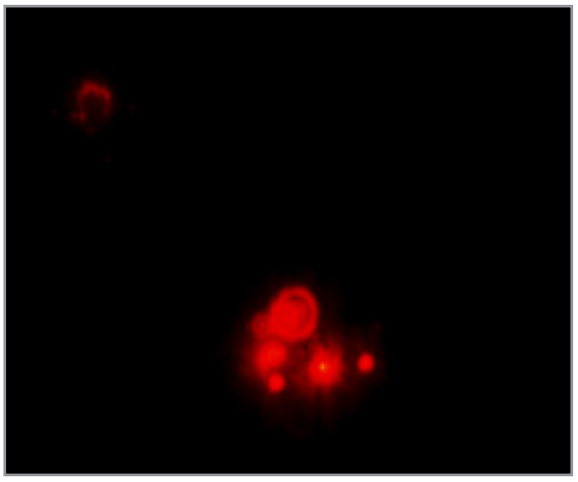

**Figure 1.** The activity of MATRA mediated transfection

## RESULTS

### Analysis of Transfection

Transfection efficiency of mir-150 via MATRA was determined as effective by fluorescence microscopic imaging. At the same time, siGLO Red Transfection Indicator was showed by fluorescence microscope (Figure 1).

### Results of Apoptosis

$IC_{50}$ dose of RES was used as 5 $\mu$M by Fahri et al. determined.[21] Apoptosis analysis was performed using Annexin V method. MATRA was used as non-viral vector carrier for miR-150 transfection. Then, given resveratrol to cells, respectively. After 96 h; Compared to control cells; 6.48 (miR-150) and 6.93 (miR150-Resveratrol), 4.54 (resveratrol) fold have been determined to induce apoptosis in HL-60 cells. (As a FITC Annexin V method) (Figure 2).

### Expression Exchanges

MATRA was used as non-viral vector carrier for miR-150 transfection. Resveratrol was applied to the cells. In three different combinations (miR-150), (miR-150-resveratrol), (Resveratrol) analyzes were performed. Compared to control cells, MDM2, RUNX3, RB, gene expressions decreased

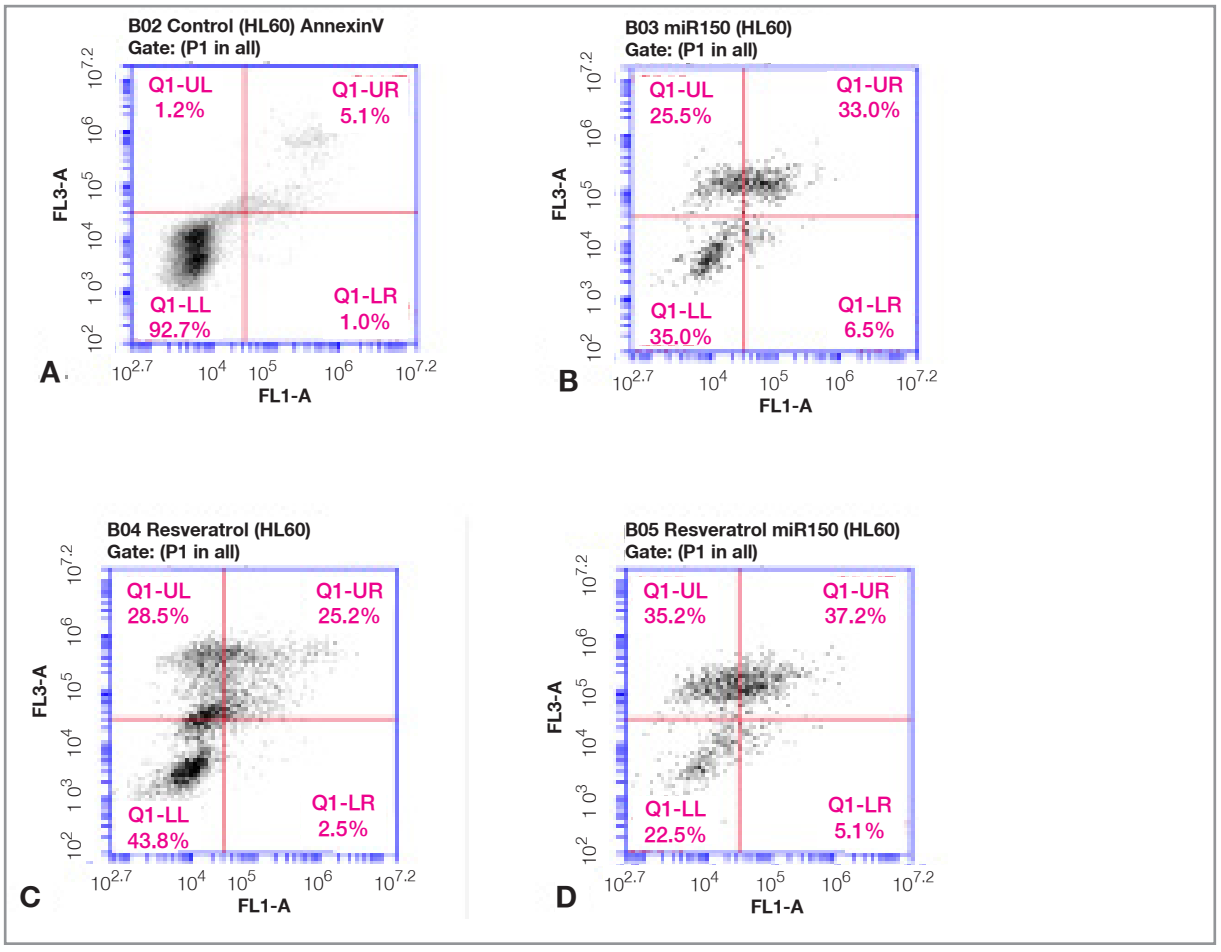


**Figure 2.** Analysis of apoptosis after 96h in HL-60 cell line **(A)** Control (HL-60 cell line) **(B)** Transfection of miR-150 **(C)** Resveratrol **(D)** Resveratrol and miR-150 combination

(miR-150) 1.7, 1.3 and 1.4 fold, (miR-150-resveratrol) MDM2 expression increased 2.73 fold, RUNX3 and RB expression decreased 6.1, 1.07 fold, respectively. Combination with resveratrol, MDM2, RB decreased 2.9 fold and 1.4 fold, respectively (Figure 3).

## DISCUSSION

There are different strategies for the treatment of patients with acute myeloid leukemia. For this reason, basic scientific researches that natural products may have strong anticancer efficacy are always attentive to the clinicians.[22] At the present time, the fact that many of the natural products are not harmful to normal healthy cells shows significant cytotoxic and apoptotic effects in various types of cancer cells.[6] Various studies have identified the anti-proliferative effect of resveratrol in leukemic cell lines.[23] Resveratrol is found to inhibit the proliferation of HL-60 cells by time and dose-dependent. It also causes cell cycle arrest in G1 / S phase.[7-9]

Clement et al. have shown that resveratrol induces Fas signal-mediated apoptosis in HL-60 human leukemia cells.[24] Resveratrol, which causes cell death, is associated with intracellular caspase. Estrov Z et al. investigated the effect of resveratrol on acute myeloid leukemia cells.[25] Aggarwal al. found that S-phase cell arrest inhibited the proliferation of AML cells. Interleukin-1 (IL) -1 also plays a key role in the proliferation of AML cells.[26] Dörrie et al, looked at the effects of Resveratrol on ALL patients and cell lines. Resveratrol has shown that

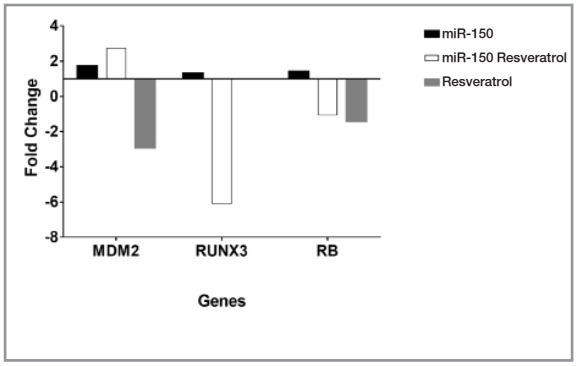


**Figure 3.** Expression Exchanges (miR-150, miR-150- Resveratrol, Resveratrol) in HL-60 cell line

it can induce apoptosis in B-lineage and T-lineage ALL cells. It has been suggested that resveratrol may be used as a chemotherapeutic agent in the treatment of ALL. Also; It is suggested that it may be important in patients with B-lineage ALL, including the t (4;11) subgroup, which is resistant to CD95.[27] In our work; when resveratrol was given to HL-60 cells, we found that apoptosis increased 4.5 times compared to control. The findings indicate that resveratrol may exert an anti-cancer effect on cell proliferation.

In our study, HL-60 cells interacting with resveratrol showed 2.9-fold decreased in MDM2 gene and 1.4-fold decreased in RB gene, indicating that the genes behave as tumor suppressor. It suggests that resveratrol may have an effect on anti- cancer cell proliferation.

Deregulation of miRNA expressions is important in the pathogenesis of many genetic and multifactorial diseases.[16] miRNAs are important regulators of normal hematopoietic differentiation.[10-15] miRNA expression profile studies ranked in normal hematopoietic cell populations were detected in normal stem / progenitor cells that were expressed of miR-150.[12] Recent studies focused on cell proliferation with decreased expression of miR-150. In the study of Morris et al. showed that loss of miR-150 expression inhibited myeloid differentiation. They found that increased miR-150 expression by terminal myeloid differentiation of miR-150 expression and found support that increased miR-150 expression promoted granulocytic and monocytic differentiation of CD34+ progenitor cells in healthy individuals. These observations suggest that miR-150 plays a role in normal myelopoiesis.[28] Currently, mir-150 transfection is performed with non-viral delivery. MATRA is an easy, fast and very effective technology for transfection of cells in culture. Different nanaoparticle formulations can be applied in the clinic. Manthawornsiri et al. used PCR and Magnetic Nanoparticles (MNP) in enzyme-substrate technique to detect BCR / ABL abnormal gene in KML patients. It has been suggested that CML-positive cell lines and leukemic patients are the appropriate technique. It is thought that CML patients may be different method in the diagnosis and treatment process.[29] El-Boubbou et al. has advanced a chemotherapeutic nanoformulation loaded with the anticancer drug, Doxironide, as a drug carrier in various types of AML cancer cells. As a result of the studies, it has been concluded that Doxinoride magnetic nanoparticles may be positive as anti-cancer agents in AML treatment.[30] In our study, approximately 6.48 fold increase in apoptosis was observed when miR-150 transfection was performed via MATRA to the cells. In addition, approximatively 7-fold increase in apoptosis was observed when resveratrol was administered to the miR-150 transfected cells. The findings indicate that miR-150 and the combination of resveratrol-miR-150 can inhibit proliferation in leukemia cells.

In our study, after miR-150 transfection, a 1.7-fold decrease in MDM2 gene, 1.3-fold decreased in RUNX3 gene, and a 1.4-fold decrease in RB gene, indicate that these genes may be tumor suppressors because they inhibit cell proliferation.

In the combination of MiR-150 and resveratrol, MDM2 gene increased 2.73-fold, but RUNX3 gene decrease 6.1-fold and RB decreased 1.07-fold. These genes (RUNX3, RB) may be anti-cancer effect in leukemia cells.

Substitution of tumor suppressor miR-150, resveratrol and mir150-resveratrol combination to leukemia cells with non-viral transfection yielded promising results for the treatment of leukemia. Supporting the data of our study with in vivo animal experiments, an additional treatment protocol will be achieved in the treatment of leukemia.

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