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Knockdown of microRNA-29a Changes the Expression of Heat Shock Proteins in Breast Carcinoma MCF-7 Cells

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Breast cancer is the most commonly occurring cancer among women. MicroRNAs as noncoding small RNA molecules play pivotal roles in cancer-related biological processes. Increased levels of microRNA-29a in the serum of breast cancer patients have been reported. Since heat shock proteins (HSPs) play important roles in cell events, the quantitative fluctuations in their cellular levels could be deemed as key indicators of how the exerted treatment alters cell behavior. In this regard, using an antisense small RNA, we attempted to investigate the effects of miR-29a knockdown on the expression of HSPs genes in the MCF-7 breast cancer cell line. MCF-7 cells were cultured in high-glucose Dulbecco's modified Eagle's medium with 10% FBS. Studied cells were subdivided into five groups: treated with scramble, anti-miR-29a, anti-miR-29a+Taxol, Taxol, and control. Taxol was added 24 h post-anti-miR transfection and RNA extraction, and cDNA synthesis was done 48 h later. The changes in expression of HSP27, HSP40, HSP60, HSP70, and HSP90 were evaluated by real-time PCR. Our results revealed that inhibitors of microRNA-29a promote apoptosis through upregulation of HSP60 level and downregulation of HSP27, HSP40, HSP70, and HSP90 levels and could be contemplated as a compelling alternative for Taxol employment with similar effects and/or to sensitize cancer cells to chemotherapy with fewer side effects.

Key words: Breast cancer; miR-29a; Heat shock proteins (HSPs)

INTRODUCTION

Breast cancer, usually caused by genetic alterations, is one of the most common malignancies among women worldwide (1). It is a complex disease characterized by heterogeneity of genetic alterations and affected by several factors such as age, early menopause, familial history, and mutations (2). The current treatment for breast cancer is a combination of surgery with supplementary treatment including anticancer agents, hormonal therapy, radiotherapy, and biological treatment (3). It is estimated that half of patients with breast cancer will fail to respond to initial treatments or will rapidly acquire resistance to chemotherapeutic agents (4). As a result, finding new therapeutics attracts much attention (5). One of these therapeutic targets is microRNAs (miRNAs).

miRNAs are a family of naturally occurring small noncoding RNAs, with 18–25 nucleotides in length, that regulate gene expression by targeting mRNAs in a sequence of specific manner, inducing translational repression or mRNA degradation (6–8). miRNAs act as oncogenes or tumor suppressors (9). It has an influence on many biological processes, including cell proliferation, apoptosis, development, differentiation, cell migration, and survival (10). There is growing evidence showing the role of miRNAs in many human tumors, including ovarian, breast, pancreatic, thyroid, and lung cancers.

miRNAs dysregulation in human cancer came from molecular studies characterizing the loss of chromosomal region 13q14 in human chronic lymphocytic leukemia (CLL) (11), which revealed that two miRNAs, mir-15a and mir-16-1, were clustered within this chromosomal region (12). Following the first finding, miRNA dysregulation has been reported in various cancers. For instance, downregulation of miR-143 and miR-145 colon cancer

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(13,14), let-7 downregulation in lung carcinomas (15), and upregulated level of miR-155 in Burkitt's lymphoma has been reported (16).

It has been reported that miR-29a level was significantly elevated in breast cancer patient's sera (17). However, the phenomena of miR-29a downregulation have been shown to be the case in a broad spectrum of other solid tumors, including neuroblastoma, sarcoma, and brain tumor (18,19). The miR-29 family consists of miR-29a, miR-29b, and miR-29c, reported to be deregulated in various cancers and involved multiple pathways, indicating its vital role in tumor genesis, apoptosis, differentiation, metastasis, and epigenetic modulation (20). miR-29 family is involved in cell apoptosis through apoptosis-associated factors such as myeloid cell leukemia (Mcl), B cell lymphoma-2 (Bcl-2), T cell lymphoma breakpoint 1 (TCL-1), cell division control protein 42 (CDC42), and phosphatidylinositol 3-kinase regulatory subunit α (PIK3R1) (21).

It has been previously reported that there is a relation between breast cancer and heat shock proteins (HSPs), which led to proliferation and resistance to apoptosis. HSPs are a group of extracellular proteins. It has been suggested that extracellular proteins play a critical role in the regulation of cancer cell migration and invasion, and because of their availability, they provide good targets for drug development (22). HSPs as molecular chaperones are increased in many types of cancer. The elevated levels of HSPs in tumor cells play a pivotal role in protection from apoptosis associated with malignancy in addition to apoptosis following therapy (23).

Here we downregulated the expression of miR-29a and assessed the expression of HSP-27, -40, -60, -70, and -90 on MCF-7 human breast cancer cell line. We hypothesize that miR-29a downregulation leads to a change in the expression of HSPs. Here we report an alternative way of inducing apoptosis in breast cancer, which could be used as a treatment in combination with other routine procedures by targeting miRNAs and HSPs. Also, we aimed to compare these outcomes in cells treated with Taxol, as commonly used chemotherapy drug in breast cancer patients individually and in combination with anti-miR29a.

MATERIALS AND METHODS

Cell Culture

Human breast cancer cell line MCF-7 was obtained from the Stem Cell Technology Research Center (Iran) and cultured in the T25 flasks with appropriate culture media: Dulbecco's modified Eagle's medium (Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 U/ml penicillin G, and 100 μg/ml streptomycin (Invitrogen, USA). The cells were maintained at 37°C in incubator with 5% CO_2 atmosphere. After 48 h, the adherent cells were trypsinized and removed from the flasks.

Knocking Down miR-29a in MCF-7 Cells by Reverse Transfection

miR-29a was knocked down according to reverse transfection method by specific anti-miR-29a small RNA against 3'p miR-29a sequence. Cells were harvested in logarithmic growth phase and adjusted in desired number by culture medium. Transfection reagents for each group were prepared and added into 96-well plates in triplicate, and cells were added and shaken to enforce transfection efficiency. Transfection of treated cells was monitored in groups treated by FITC-conjugated scramble RNA under fluorescent microscopy and quantification of miR-29a in scramble and anti-miR-29a-treated cells. Preprepared master mix of all required reagents for scramble (final concentration of 5 nM in 200 μl culture medium) (Santa Cruz, USA) and anti-miR-29a (Invitrogen, USA) in a final concentration of 50 nM were vortexed and incubated along with the transfection reagent (Invitrogen, USA) for 20 min at room temperature. Then reagents were poured into the 96-well plate so that each of the three wells could be considered as a group, and finally, the cells (30×10^3 per well) were added (reverse transfection). Cell viability was assessed by trypan blue. Five groups were considered in this study. They were incubated for 24 h at 37°C and 5% CO_2. After that, Taxol, as an anticancer drug, was added to the fourth and fifth groups at the concentration of 10 ng/ml. The optimal concentration for Taxol in the cultured medium was determined after MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (data not shown). All five groups were brought to a final volume of 200 μl and placed in the incubator.

Cell Viability Assay

Measurement of cell viability was conducted on treated and nontreated cells of each group by MTT assay. Briefly, 72 h posttransfection, culture medium was disposed, and MTT solution (5 mg/ml) was added in equal volume and incubated in 5% CO_2 and 37°C. Overlay medium was replaced by DMSO, and absorbance was measured in 590-nm wavelength. Viability index was calculated by dividing the absorbance of each treated group to absorbance of nontreated group. All experiments were repeated three times.

RNA Extraction and cDNA Synthesis

Total RNA was extracted from seeded cells in different groups 72 h after transfection, using easy-BLUE™ Total RNA Extraction kit (iNtRON Biotechnology, Korea) according to the manufacturer's instructions. Then cDNA synthesis was performed for miR-29a (target gene) and

U6 snRNA (reference gene) by using specific primers (5′-GTCGTATCCAGTGCAGGGTCCGAGGT ATTCG CACTGGATCAGACTAACCGAT-3′ and 5′-AAAATAT GGAACGCTTCACGAATTTG-3′), respectively, in Stem loop RT-PCR with RevertAid H Minus First Strand cDNA Synthesis Kit according to the manufacturer's protocol. (Thermo Scientific, USA).

cDNA synthesis for HSP27, HSP40, HSP6, HSP70, HSP90 (target genes), and hypoxanthine phosphoribosyl transferase (HPRT) (reference gene) was conducted using general primers (Random and Oligo dT primers) by Maxime RT premix cDNA synthesis (iNtRON Biotechnology). The thermocycler temperature program was 25°C for 5 s, 45°C for 1 min, and finally at 95°C for 5 s.

Quantitative Real-Time PCR

To evaluate gene expression levels, quantitative real-time PCR performed on Corbett Rotor-gene 6000 instrument (Corbett Life Sciences) by using SYBR green Master Mix. miR-29a and U6 snRNA genes were evaluated with specific primers (miR-29; forward: 5′-TGCGC TAGCACCATCTGAAA-3′, reverse: 5′-CAGTGCAGG GTCCGAGGAT-3′ and U6 snRNA; forward: 5′-CTCG CTTCGGCAGCACATATAC-3′, reverse: 5′-ACGCTTCA CGAATTTGCGTGTC-3′) under the following condition: an initial step of 10 m at 95°C, followed by 95°C for 15 s, 60°C for 40 s, and 72°C for 20 s, for 45 cycles, followed by a melt cycle ramp from 55°C to 99°C. The PCR reactions were performed using cDNA samples (100 ng), 10 µl of the Master mix SYBER Green 2X in RealMOD Green PCR kit (iNtRON Biotechnology), and 10 pmol of each primer. The sterile deionized water was used to attain a total volume of 20 µl. To evaluate variations in levels of HSP expression, we quantified their fold change expression by specific primers as follows: HSP27 forward: 5′-AGGATGGCGTGGTGGAGAT-3′, reverse: 5′-GAT GTAGCCATGCTCGTCCTG-3′; HSP40 forward: 5′-CC AGTCACCCACGACCTTC-3′, reverse: 5′-CCCTTCTTC ACTTCGATGGTCA-3′; HSP60 forward: 5′-TGCCAAT GCTCACCGTAAG-3′, reverse: 5′-ACTGCCACAACCT GAAGAC-3′; HSP70 forward: 5′-CCATCATCAGCG GACTGTAC-3′, reverse: 5′-CCATCATCAGCGGACT GTAC-3′; HSP90 forward: 5′-CGCTCCTGTCTTCTGG CTTC-3′, reverse: 5′-TGGTATCATCAGCAGTAGGG TC-3′; hypoxanthine phosphoribosyl transferase (HPRT) forward: 5′-GGACAGGACTGAACGTCTTGC-3′, reverse: 5′-ATAGCCCCCCTTGAGCACAC-3′.

All reactions were performed in triplicate and used the average values for relative quantification. We used the Pfaffl method to determine the relative quantity of gene expression. Briefly, we normalized the cycle of the threshold (Ct) values of the target gene to the endogenous control gene and compared it with a calibrator (same gene in group without any treatment).

Statistical Analysis

The results were expressed as mean ± SD and analyzed using SPSS16 software. Difference between groups was evaluated by Kruskal Wallis, and comparison of studied groups for the expression of the desired genes was assessed by Mann/Whitney tests. A value of $p \leq 0.05$ was considered as significance level.

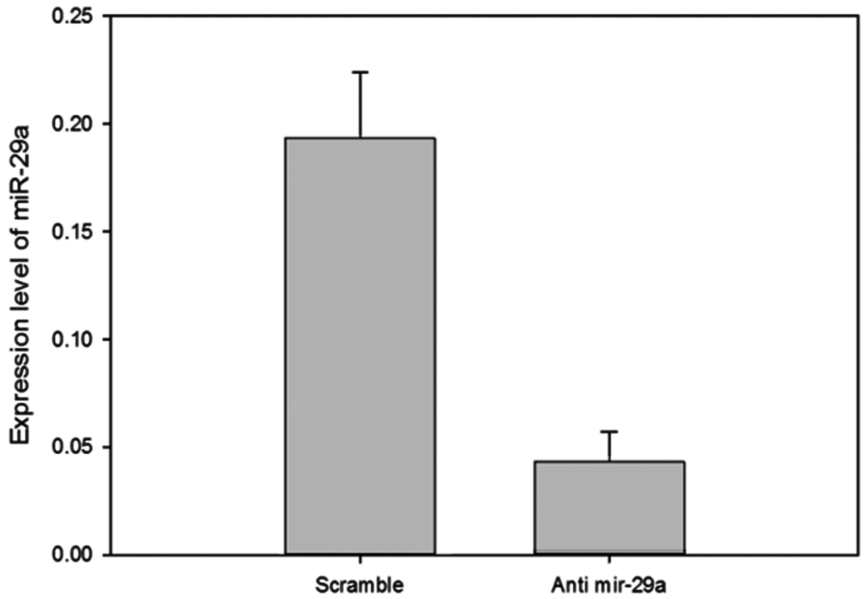

Figure 1. Level of miR-29a expression in scramble and anti-miR-29a transfected cells.

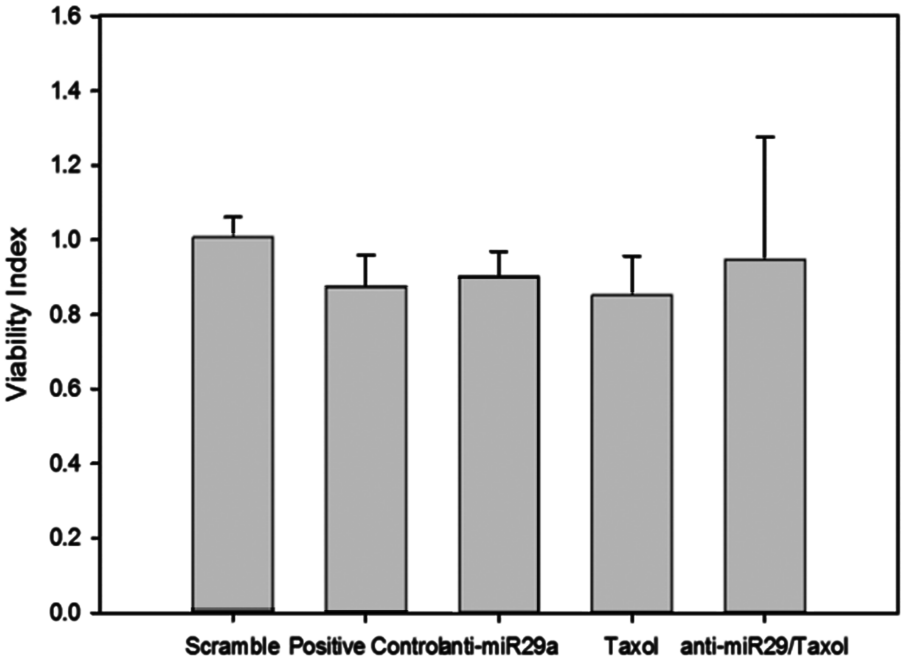

Figure 2. Cell viability index in MCF-7 line breast cancer cells after inhibiting the miR-29a. Data demonstrated as mean and standard deviation (SD) of ratio of obtained absorbance of formed formazan for treated groups to nontreated group.

RESULTS

Knockdown of miR-29a

To reveal knockdown of miR-29a in studied cells, we quantified the expression of anti-miR-29a-treated cells compared to nontreated and scramble transfected cells by real-time PCR. Melting curve was considered to determine specific amplification of target genes. Our result showed fold change ratios of 0.193 and 0.043 for scramble and anti-miR-29a transfected cells, respectively, compared to nontreated group. These finding suggested efficient downregulation ($p = 0.037$) of miR-29a in anti-miR-29a group (more than 99%) and 4.5 times more than the scramble-treated group (Fig. 1).

Effect of Knocking Down miR-29a on Cell Viability

The effects of inhibiting the miR-29a on viability of MCF-7 line breast cancer cells was evaluated through calculating the viability index in treated cells 72 h posttransfection of miR-29a antisense RNA. As demonstrated in Figure 2, our results showed maximum reduction in viability has been seen in Taxol-treated cells (15%). Likewise, viability has diminished in MCF-7 cells treated by anti-miR-29a, about 10% compared to the control group with lower standard deviation than the Taxol group. Combination of Taxol and anti-miR-29a led to less reduction in viability index (about 6%).

Downregulation of miR-29a

Relative change in miR-29a expression was analyzed by the Pfaffl method. The cycle of threshold (Ct) values of the target gene (miR-29a) was normalized by reference gene (U6 snRNA) and compared with the nontreated group as calibrator. Our results showed 0.2- and 0.045-fold changes in miR-29a expression, respectively, for the scramble and anti-miR-29a-treated cells. Therefore, anti-miR treatment caused a significant reduction (77.5%) in miR-29a expression compared with the scramble-treated group ($p \leq 0.05$).

Table 1. Fold Changes in HSP Gene Expression in Studied Groups

Category	HSP27	HSP40	HSP60	HSP70	HSP90
Scramble	1.68 ± 0.11	78.1 ± 0.53	2.45 ± 0.5	1.7 ± 0.1	14.77 ± 6.11
Anti-mir-29a	0.486 ± 0.42	59.9 ± 9.85	5.07 ± 0.51	0.17 ± 0.03	0.75 ± 0.05
Anti-mir-29a + Taxol	0.656 ± 0.52	53.63 ± 8.16	4 ± 0.92	0.5 ± 0.00	0.393 ± 0.04
Taxol	0.087 ± 0.007	25.63 ± 2.29	0.2 ± 0.01	0.25 ± 0.05	0.133 ± 0.03

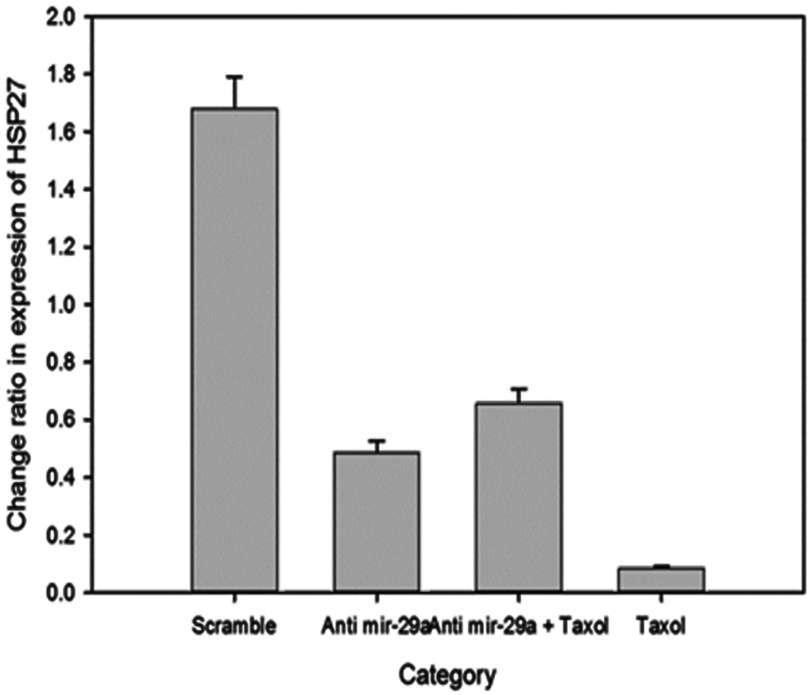

Figure 3. Chart of HSP27 gene expression changes in the groups treated with scramble, anti-miR-29a, anti-miR-29a+Taxol, and Taxol compared with calibrator.

Quantification of Heat Shock Protein Genes in the Studied Groups

The target genes including HSP27, HSP40, HSP60, HSP70, and HSP90 were normalized by HPRT as a reference gene. For each gene, we had five groups: nontreated (calibrator), treatment by scramble, anti-miR-29a, anti-miR-29a+Taxol and Taxol. Table 1 presents fold changes in the expression of HSPs in all the studied groups. Kruskal Wallis was used for analysis of the overall difference between the studied groups for the desired HSP genes.

miR-29a Inhibition Reduces HSP27 Expression. Our experiment showed that Taxol significantly reduced HSP27 expression by about 95% in treated cells ($p=0.023$), and anti-miR-29a addition also led to significant decrease in expression of HSP27 (more than 70%) compared to the scramble group ($p<0.05$). As demonstrated in Figure 3, the combination of Taxol and anti-miR-29a lowered the anti-HSP27 expression effect of Taxol in the treated group. There was no significant difference between the anti-miR-29a alone-treated group and the group treated with Taxol+anti-miR-29a (about 60% reductions).

HSP40 Reduced in Anti-miR-29a-Treated Cells but not as Much as Taxol-Treated Cells. We observed about 23% reduction in HSP40 expression in cells treated with anti-miR-29a in comparison with the scramble group ($p=0.05$). As illustrated in Figure 4, the most declines in HSP27 were seen in the Taxol-treated group (more than 67%). Decrease in HSP27 gene expression in combination of Taxol and anti-miR-29a-treated group was about 33%, which did not show significant difference between the anti-miR-29a alone group ($p=0.513$).

HSP60 Gene Upregulation in miR-29a Inhibited Cells. We found that inhibition of miR-29a leads to increased expression of HSP60 gene in MCF-7 cells by about 98% compared to the scramble group, while Taxol treatment alone reduced the expression to about 92%. As illustrated in Figure 5, contrary to Taxol that significantly decreased HSP60 ($p=0.046$), the combination of anti-miR-29a and Taxol increased the HSP60, which is less than the anti-miR-29a treatment alone. There is no significant difference between the anti-miR-29a-treated and combination groups ($p=0.127$).

Inhibition of miR-29a Alone can Efficiently Decrease HSP70 Expression. Our results demonstrated that the scramble treatment courses a 1.7-fold increase in HSP70 expression, while treatment with anti-miR-29a leads up to 90% decrease in HSP70 expression ($p=0.016$). The other treatments with anti-miR-29a in combination with Taxol and Taxol alone, respectively, results in 70% and 85% expression reduction compared to that of the scramble group. These results showed an efficient decline of HSP70 in the anti-miR-29a-treated cells ($p=0.05$). As presented in Figure 6, there is no apparent difference between HSP70 expression in the anti-miR-29a-treated

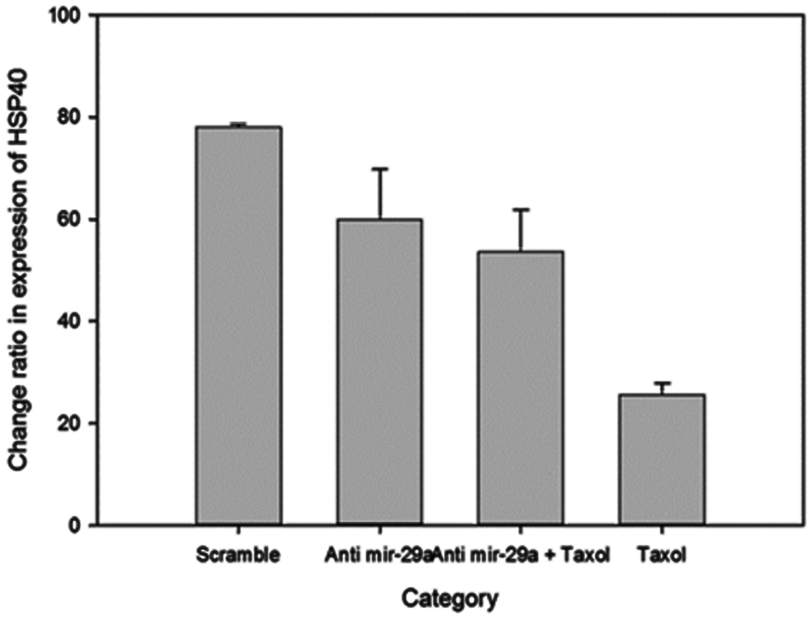

Figure 4. Chart of HSP40 gene expression changes in the groups treated with scramble, anti-miR-29a, anti-miR-29a + Taxol, and Taxol compared with calibrator.

and the Taxol-treated groups, but a marked reduction in HSP70 expression was seen in the anti-miR-29a group in comparison to two other mentioned groups ($p = 0.037$).

Decreased Expression of HSP90 Gene in miR-29a Inhibited Cells. In the scramble treatment, nearly 14.8-fold HSP90 expression was increased, while in the group treated with anti-miR-29a, HSP90 expression declined by 25% (Fig. 7). For the other studied groups, that is, treated with anti-miR-29a in combination with Taxol and Taxol alone, respectively, expression was reduced by about 60% and 97%. Thus, the treatment with anti-miR-29a could significantly reduce the expression of HSP90 by 95% lower than the scramble transfected group ($p < 0.05$). This study showed that Taxol

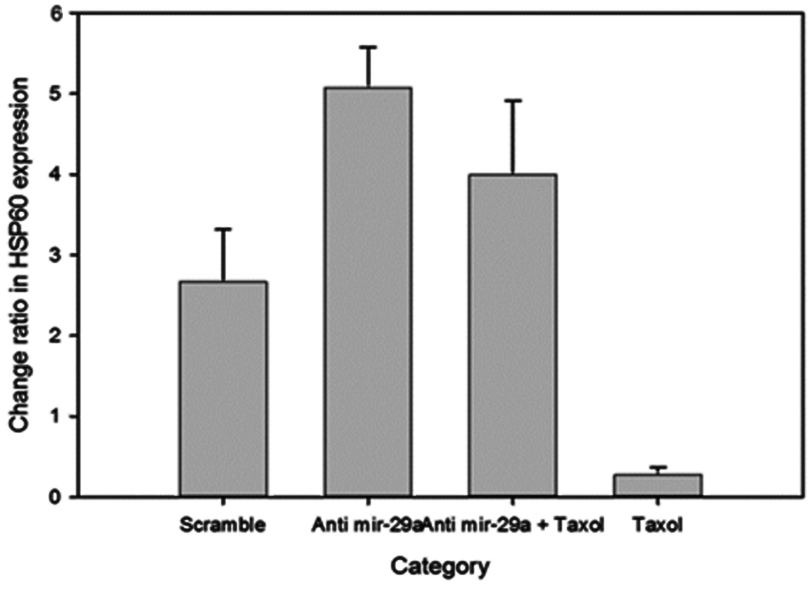

Figure 5. Chart of HSP60 gene expression changes in the groups treated with scramble, anti-miR-29a, anti-miR-29a + Taxol, and Taxol compared with calibrator.

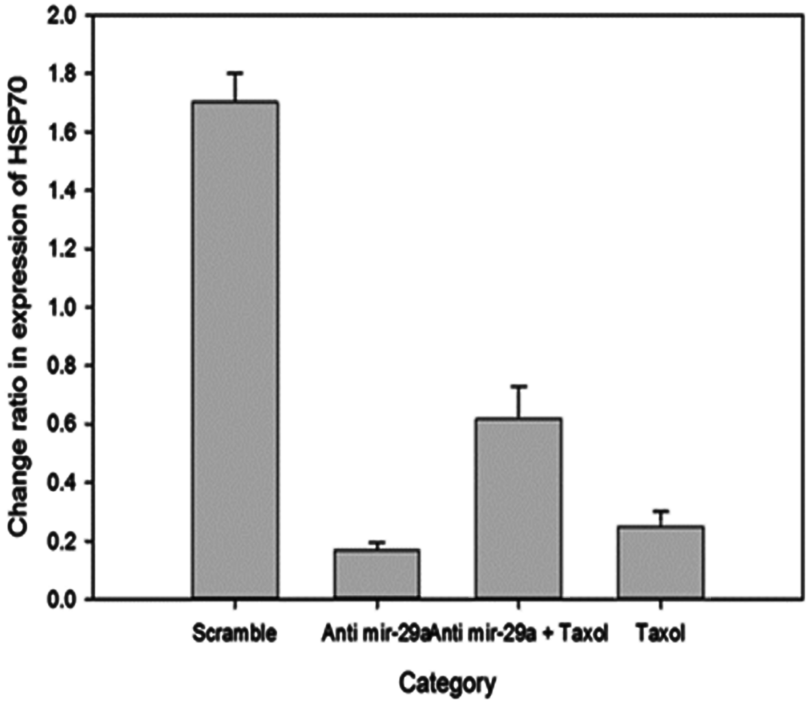

Figure 6. Chart of HSP70 gene expression changes in the groups treated with scramble, anti-miR-29a, anti-miR-29a+Taxol, and Taxol compared with calibrator.

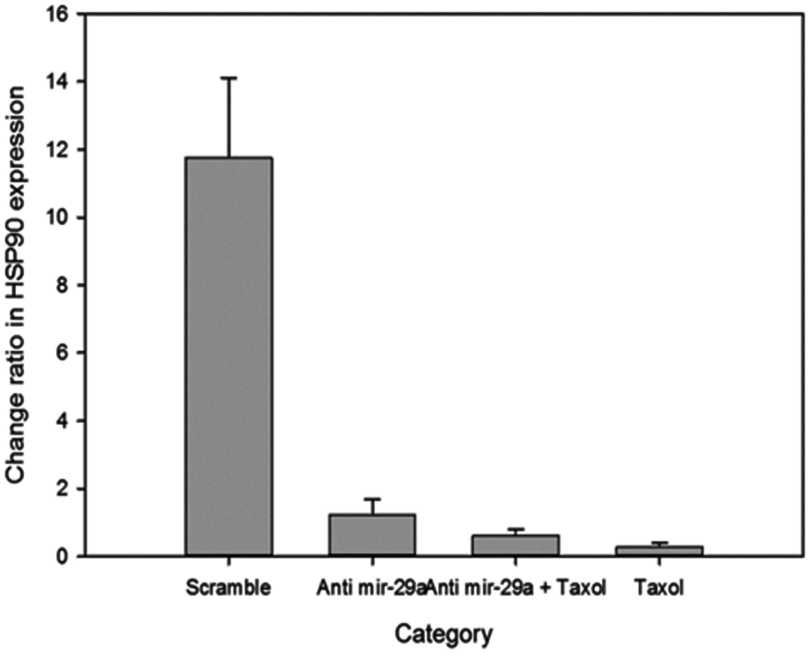

Figure 7. Chart of HSP90 gene expression changes in the groups treated with scramble, anti-miR-29a, anti-miR-29a+Taxol, and Taxol compared with calibrator.

can significantly reduce HSP90 expression in comparison with anti-miR-29a ($p < 0.05$).

DISCUSSION

In recent studies, it has been reported that miR-29a is highly expressed in the MCF-7 cell line, and some studies revealed a high level of this small molecule in the sera of patients with breast cancer (17). The aim of this study was to evaluate whether the inhibition of miR-29a could efficiently induce the expression of HSP genes that are involved in apoptosis or proliferation of the MCF-7 human adenocarcinoma breast cancer cell line.

miR-29a was downregulated by anti-miR-29a, and viability and the expression of HSP27, -40, -60, -70, and -90 were assessed in MCF-7 line breast cancer-treated cells. As presented in Table 1, although MCF-7 Taxol-treated cells showed the lowest viability among the studied groups (15%), there are no significance differences in the miR-29a-inhibited group with 10% reduction in viable cells ($p = 0.370$), suggesting comparable consequence of miR-29a downregulation on cancer cell apoptosis. Therefore, simultaneous use of Taxol and anti-miR-29a causes less reduction in viability, which is probably because of the negative interference of these two substances. These results agree with the expression pattern of HSPs in treated cells that have been previously described.

HSP27 is one of the most important HSPs in the growth of breast tumors owing to its prosurviving influence on breast cancer stem cells ($CD44^+$) (24) and inducing angiogenesis (25). Therefore, diminishing HSP27 can limit tumor growth and achieve a better response to chemotherapy (26). In this study, miR-29a downregulation significantly reduced HSP27 expression in anti-miR-treated MCF-7 cells. Although Taxol was more effective in reducing HSP27 (Fig. 2), we believe this effect is nonspecific and is a temporary and limited effect of anti-miR-29a. Statistical analysis showed no significant difference between the Taxol and anti-miR-29a groups.

HSP40, as a chaperone, plays an important role in protein folding, transportation, and degradation in coordination with HSP70 (27). Regarding its relation to HSP70/HSP90 complex, HSP40 can be involved in preventing cell death and the growth of cancer. Previous studies have shown a high level of JDP1, a member of the DnaJ/Hsp40 family, in estrogen receptor-positive breast cancer cells (28). As demonstrated in Figure 3, inhibiting miR-29a expression reduced the HSP40 mRNA level in MCF-7 cells, almost one third of the cells treated with Taxol. There was no significant effect in concomitant use of anti-miR-29a and Taxol compared to anti-miR-29a alone treatment, suggesting the modifying role of anti-miR-29a on Taxol-treated cells.

HSP60 is a mitochondrial chaperone and is present in cytosol, cell surface, and extracellular regions of normal and tumor cells (29). HSP60 interacts with $\alpha_3\beta_1$ integrin and plays a role in adhesion of metastatic breast cancer cells to lymph nodes and osteoblasts. This interaction was inhibited by mizoribin, which was attached to HSP60.

HSP60 extracellular molecular interactions and intracellular signaling play important roles in tumor cells. Through different mechanisms, such as the inhibition of intracellular antiapoptotic pathways and the activation of proapoptotic mechanisms, HSP60 shows its therapeutic properties. The suppression of HSP60 through small interfering RNA (siRNA) caused apoptosis in colon and breast adenocarcinoma via mitochondrial dysfunction and impairment of P53–HSP60 complex. In our experiment, we found that miR-29a inhibition led to a reduction in P53 (data not presented). According to these mechanisms, siRNA against HSP60 could be utilized as therapeutic agents in cancers such as colon and breast. In our experiments, we observed that anti-miR-29a caused an approximately 5.5-fold increase in the expression of HSP60. There are many reports regarding prosurvival and proapoptosis induction of HSP60. Some studies reported that accumulation of HSP60 can trigger apoptosis-independent mitochondrial release by activating caspase 3 (30). Moreover, HSP60 can be the most common immunologic response to tumor cells through activation of T cells and releasing IFN-γ and stimulating antigen-presenting cells (31–35). In contrast, Taxol leads to a significant decrease in the level of HSP60 (approximately 90%). The combination of Taxol and anti-miR-29a leads to a 4.8-fold increase in the level of HSP60. This finding demonstrated that the inhibition of miR-29a has a negative role in apoptosis augmentation in the breast cancer cell line through HSP60, while Taxol results in a decline in the level of HSP60. The incompatible effects of anti-miR-29a and Taxol could be explained by the nonspecific mechanism of the Taxol function. While anti-miR-29a as a smart drug candidate targets specific molecules and, therefore, specific cellular pathways in tumor cells, Taxol interacts via a nonspecific mechanism involving a variety of targets in tumor and nontumor cells. The unwanted side effects and the unwanted effects of Taxol, among which is the reduction of HSP60, could be rationalized by this fact.

HSP70 plays an important role in tumor genesis through the apoptosis inhibition, induction of chemotherapy resistance, and regulation of oncoprotein chemoresistance (29,36). Although the HSP70 expression level is low in normal cells, it has been elevated according to the poor prognosis and properties of malignancy. Thus, the inhibition of HSP70 attracts much attention as an anticancer treatment. Here we demonstrated that miR-29a inhibition significantly decreased (about 90%) the expression of HSP70. Taxol, a common agent in cancer chemotherapy, reduced its expression around 85%. Using the

combination of anti-miR-29a + Taxol caused a decrease in the level of HSP70, which is less than that of each of the treatments (around 70%). According to these results, anti-miR-29a could be applied as a potent agent in the treatment of breast cancer by increasing drug sensitization in cancer cells.

The previous reports revealed that HSP90 is one of the most important factors in the survival and growth of tumor cells. So the inhibition of this HSP could be potentially useful in treatment. 17-N-Allylamino-17-demethoxygeldanamycin (17AAG), known as a HSP90 inhibitor, had antitumor influence on colon, prostate, and breast cancer. It acts as a tyrosine kinase inhibitor.

Due to its interaction with a variety of proteins that play key roles in breast neoplasia, such as estrogen receptors (ER), tumor-suppressor p53 protein, angiogenesis transcription factor HIF-1α, antiapoptotic kinase Akt, Raf-1, mitogen-activated protein kinase, and a variety of receptor tyrosine kinases of the erbB family, the pharmacological inhibition of HSP90 could bring about new hope in the field of breast cancer treatment. Multiple oncogenic signaling pathways could be simultaneously suppressed using various inhibitors of HSP90, which in turn would reduce the possibility of molecular feedback loops and mutations leading to tumor resistance (15,27,34). Herein, we demonstrated that anti-miR-29a caused a reduction in the expression of HSP90, which is less than the Taxol effect. When anti-miR-29a was used with Taxol, we observed less reduction. One of the explanations for this finding can be that anti-miR-29a and Taxol were added to the test at different time points. Anti-miR-29a was added to the test group because anti-miR-29a was demolished by endonucleases in a short time. But Taxol was added 24 h following the addition of anti-miR-29a. It is important to mention that, although Taxol and anti-miR-29a separately reduced the HSP90 expression, they had no synergistic effect according to results.

CONCLUSIONS

Taxol, known as a chemotherapeutic agent with a long life span and high permeability, is infiltrated all over the body through the blood and had side effects on nontumor cells. Due to the adverse influences of this drug on normal cells, its application has been limited. Unlike Taxol, miR-29a inhibitors could be potentially utilized as efficient smart drugs, which directly influence breast-derived tumor cells that express miR-29a at a high level. Also, downregulating the miR-29a could potentially target susceptible cancer cells with a lower dose of chemotherapy. However, to reveal the in vivo therapeutic effect of miR-29a inhibition strategy, it should be chemically modified to improve its shelf life. However, we believe that more investigation is required at the cell and animal levels to verify these claims.

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