MiR-424-5p and miR -142-3p Combination Synergistically Regulates the Biological Features of Breast Cancer Cells

Narges Dastmalchi

Tabriz University: University of Tabriz

Reza Safaralizadeh (✉ safaralizadeh@tabrizu.ac.ir)

University of Tabriz <https://orcid.org/0000-0002-6970-6998>

Seyed Mahdi Banan Khojasteh

Tabriz University: University of Tabriz

Behzad Baradaran

Tabriz Medical University: Tabriz University of Medical Sciences

Research

Keywords: miR-142-3p, miR-424-5p, breast cancer, cytotoxic effects, molecular function

Posted Date: March 30th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-339147/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: combination treatment was identified as a valuable procedure to decrease breast tumorigenesis. It has been confirmed that microRNAs (miRs) can be considered as a therapeutic agent in breast cancer (BC) combination treatment. Our aim was the examination of potential action and underlying molecular activity of miR-424-5p and miR-142-3p combination in the human BC cells.

Methods and results: MiR-424-5p and miR-142-3p mimics were transfected into MCF-7 cells, in a combination or separate manner. The proliferation of the cells was assessed through the MTT test. Also, apoptosis, autophagy, and cell cycle were analyzed by flow cytometry. Western blot and qRT-PCR determined the expression levels of downstream molecular factors of miR-424-5p and miR-142-3p. The results exhibited that miR-424-5p and miR-142-3p synergetically inhibited MCF-7 cell proliferation via apoptosis and autophagy induction and regulation of apoptotic factors, Bax and Bcl-2, and autophagy-associated gene, Beclin1 as well as proliferative factor c-Myc. Also, miR-424-5p and miR-142-3p combination treatment reduced MCF-7 cell colony numbers via modulating Oct-3 and resulted in G2 arrest through controlling STAT3 expression.

Conclusions: The simultaneous restoration of miR-424-5p and miR-142-3p showed more cytotoxic effects on BC cells as compared to mono-treatment. Thus, the combination of two miRs (miR-424 and miR-142) could be used as a new method for breast carcinoma treatment.

1. Introduction

An important threat to a woman's health is breast cancer (BC) because of its high frequency and mortality [1]. Developed countries showed more mortality rate of BC as compared with less developed countries [2]. A main reason for the poor survival in BC cases is therapy failure and rapid relapse of breast malignancy [3]. It has been indicated that a synergetic combination has been confirmed to show more safety and great responsiveness as compared with mono-treatment [4,5]. BC tumorigenesis occurred caused by the genetic and epigenetic variations [6]. In BC cells, microRNAs (miRs) expression pattern has altered which upregulate tumor cell proliferation and results in the BC progression [7,8]. MiRs have been currently determined as the therapeutic factors and modulators of breast malignancies [9]. It has been approved that modulation of aberrant miRs expression levels could be a valuable method in BC therapy [1]. Overall, miRs restoration was revealed as potentially useful method for breast carcinoma treatment. Besides, the progressive evidence has shown that a combined miRs replacement approach could be useful and valuable as compared to one miR restoration in the BC therapy [10]. MiR-424-5p and miR-142-3p have been confirmed as two critical tumor-suppressive miRs, and their aberrant expression profiles have been indicated in numerous cancers including human BC [11,12]. In previous examinations, miR-424 was identified to be more effective in a combined manner with miR-381 in renal cancer treatment [13]. In addition, miR-142 has been confirmed to be more valuable in combination treatments as compared to mono-therapies [14]. However, the participation of miR-424 and miR-142 combined manners or with other miRs, as well as their underlying molecular mechanisms have not been investigated in the BC therapy.

Thus, our project aimed at indicating the therapeutic usefulness of miR-424 and miR-142 combination and their underlying molecular mechanisms in BC cells. The data showed that miR-142 and miR-424 combination showed more efficiency on the regulation of apoptosis, proliferation, cell cycle, autophagy, and colony formation as compared to mono-treatment through modulating the mentioned biological pathways-related molecular factors.

2. Materials And Methods

2.1. Cell culture and miR transfection

We purchased a MCF-7 cell line from Pasteur Institute of Tehran, Iran. 10% culture media generating from RPMI-1640 (Gibco), 1% penicillin and streptomycin mixed, and fetal bovine serum (FBS) (Gibco) were used to culturing the cells. The culture condition was contained 95% humidity, 5% CO₂, and 37°C temperature. We detached and sub-cultured the cells with 80% confluency. MiR-424-5p and miR-142-3p nucleotide sequences were 5′-CAGCAGCAAUUCAUGUUUUGAA-3′ and 5′-UGUAGUGUUUCCUACUUUAUGGA-3′, respectively, which were obtained from “miRbase”. MiR control, miR-424-5p and miR-142-3p mimics were provided from GenePharma (Shanghai). Then, the electroporation strategy (BioRad instrument) was applied to mimic transfection into the cells. We assessed four cellular groups in the experiments, including miR-424-5p, miR-142-3p, miR-424 + miR-142, and negative controls (NCs). The mimics under study is endotoxin free.

2.2. RNA extraction and qRT-PCR

We used a Trizol extraction kit to extract RNAs from the BC cells, according to kit protocols. Extracted RNAs were assessed in terms of purification and concentration by measuring A260/A280 and A260/A230 absorbance using the NanoDrop (TermoFisher). Electrophoresis of the RNAs was done on 2% agarose gel to obtain the RNA quality. We synthesized the cDNAs from RNAs (1 µg) to measure the expression levels of miRs molecular targets via a BioFACT kit in a Thermal cycler (BioRad). Then, the SYBR green 2x-mediated qRT-PCR analyses was used to indicate the transcription levels of Bcl-2, Bax, c-Myc, Oct-3, Beclin-1, STAT-3 (Table 1) through the ABI Biosystems instrument.

Table 1
Primer sequences

Primers	Forward and Reverse	Sequences (5'-3')
β-Actin	F ^a	TCCCTGGAGAAGAGCTACG
	R ^b	GTAGTTTCGTGGATGCCACA
Bax	F	TTTGCTTCAGGGTTTCATCCA
	R	TCTGCAGCTCCATGTTACTGTC
Bcl-2	F	CTGTGGATGACTGAGTACCTG
	R	GAGACAGCCAGGAGAAATCA
Oct-3	F	GGGCTGAAGGTCCTGTCTTG
	R	GCCTGACATACATGAAATCAC
Beclin1	F	ATCATGTGGTGCCGAATGGTG
	R	GAAGTCTACGGCATCAACACC
STAT3	F	CACCTTTGACATGGAGTTGACC
	R	AGCAGATCACCCACATTCACT
c-Myc	F	AGGCTCTCCTTGCAGCTGCT
	R	AAGTTCTCCTCCTCGTCGCA
a: forward, b: reverse		

2.3. Western blot assay

We used radioimmunoprecipitation (RIPA) lysis buffer for the extraction of total proteins from the BC cells. Then, the electrophoresis of 50 µg total proteins was done on the gel. The polyvinylidene difluoride-based membranes were used to blotting the proteins through the semi-dry western blot system (BioRad). The mentioned membrane blocking was performed using 0.5% Tween-20. Then, the membranes were covered with the first monoclonal antibody (goat mA) (Santa Cruz Biotechnology) for Bcl-2, c-Myc, Bax, Oct-3, Beclin-1, STAT-3, and β-actin. The membranes were washed with PBS, and then they were incubated with the combination of secondary mA (rabbit anti-goat) and peroxidase for 1h at room temperature. To the end, the protein bands were observed and analyzed using the western blot imaging system and Image J program, respectively.

2.4. Cell viability assay

The viability of four cellular groups including miR-424-5p transfected, miR-142-3p transfected, miR-424 + miR-142 combination, and NC was assessed using the MTT test. After transfection of 12×10³ BC cells with mimics, they were cultured in the 96-well plates. Then, incubation of the cells was done at 37°C

incubator for 48 h. The wells were covered with 100 μ l of MTT with 2 mg/ml concentration. Then, the 100 μ l DMSO was added to the cells to dissolve the formed crystals. The incubation at 37°C for 30 min was occurred, and then the absorbance values were measured using the Eliza reader system (Sunrise) at 570 nm wavelength. Each test was repeated three times.

2.5. Apoptosis assay using Annexin V/PI

The BC cells were transfected with the mimics and 5×10^5 of them were cultured in the plates (6-well). 48 h after the transfection with miR-142-3p and miR-424-5p, in a combined status or separately, the cells were covered with annexin V/PI (exBio) at 25°C for 15 min. the apoptotic and necrotic rates of BC cells were examined and analyzed via MACSQuant flow cytometry and FlowJo program, respectively.

2.6. Apoptosis assay by DAPI

The apoptosis rate of the cells was assessed via DAPI staining. After transfecting the mimics into the cells, 5×10^5 of them were seeded in the 96-well plates. The cells were washed with PBS in a triplicate manner, following 48 h incubation at 37°C. The cells were fixed using paraformaldehyde.

Permeabilization of the cells was performed using Triton X-100. Then, the cells were covered with DAPI and visualized using citation 5 cell imaging system (Biotk).

2.7. Cell cycle

To assess the miR-424-5p and miR-142-3p impacts on cell-cycle, the restoration of the cells with mimics was occurred and 5×10^5 of them were cultured into 6-well plates. The cells were maintained at 37°C for 48 h, and then they were detached and covered with 70% ethanol. After incubation with ethanol, the cells were stained with 0.1% DAPI and visualized through flowcytometry (MACSQuant).

2.8. Colony formation

We transfected miR mimics into the BC cells and cultured 6×10^3 of them in each well of a 6-well plate. The cells were incubated in an incubator contained 5% CO₂ and 37°C temperature for 14 days, and the media of the cells was renewed every 4 days. Following 14 days, the cells were covered with crystal violet. To the end, the OPTICA inverted microscope was used to imaging the MCF-7 colonies.

2.9. Autophagy

The mimics were entered into the MCF-7 cell line and 5×10^5 of them were cultured into the 6-well plates. Then, following 48 h, the wells were washed with PBS and the medium of the wells was eliminated. Then, the cells were covered with 1 ml of monodansylcadaverine (MDC) and incubated at 37°C. After 15 min, the cells were detached and analyzed using flow cytometry and the FlowJo program.

2.10. Statistical analyses

Each experiment was repeated in a triplicate manner. The evaluation of data was done by GraphPad software (SanDiego, version 7) and represented as means $\pm$ standard deviation. The differences of two

cell groups as well as differences among three or more groups were evaluated using Student's *t*-test and ANOVA test, respectively. $P < 0.05$ was a significant result.

3. Results

3.1. The effects of the combination of miR-142-3p and miR-424-5p on the BC cellular viability

We identified a 40 pmol of either miR-142-3p or miR-424-5p as an optimal concentration in MCF-7 cells by MTT test. As compared to the NC cells, the MTT test indicated that BC cell viability was decreased by 34%, 29%, and 58% in miR-142-3p (40 pmol), miR-424-5p (40 pmol), and miR424 + miR142 groups, respectively (Fig. 1a). The miR424 + miR142 combined manner indicated decreased amounts of cell viability than the miR-142-3p-transfected or miR-424-5p-transfected cells alone ($p < 0.0001$). The results indicated that the combination treatment with miR-142-3p and miR-424-5p had more cytotoxic effects on the BC cells than the mono-treatment with either miR-424-5p or miR-142-3p. A crucial transcription modulator, c-Myc demonstrates a key action in modulating cell proliferation. C-Myc expression in both transcript and protein levels was evaluated to indicate the molecular mechanism of the reduced viability of MCF-7 cell line after the transfection with the mimics. Both transcript and protein levels of c-Myc were significantly downregulated in miR-424 and miR-142 combination groups as compared to the miR-142-3p-restored or miR-424-5p-transfected groups (Fig. 1b, c).

3.2. MiR-424-5p and miR-142-3p synergetically increased the apoptosis rate of BC cells

The annexin V/PI test showed that the amount of apoptosis was increased by 24%, 22%, and 41% in the miR-142-3p-transfected, miR-424-5p-restored, and miR142 + miR424 combination groups, respectively, as compared to the NC cells ($p < 0.0001$) (Fig. 2a). Both annexin V/PI and DAPI tests indicate that the combined manner of miR-142-3p and miR-424-5p, as compared to miR-142-3p or miR-424-5p alone, resulted in the maximum amount of the apoptosis upregulation ($p < 0.0001$) (Fig. 2a). Moreover, DAPI staining the seeded cells indicated the fragmented DNAs, as a specific characteristic of apoptotic cells (Fig. 2b), and revealed the highest amount of the fragmented DNAs in the dual transfected cells as compared to other cells. We assessed Bax and Bcl-2 transcripts and protein levels as the important effectors of apoptosis. A significant upregulation of Bax mRNA and protein levels was shown in miR142 + miR424 combined manner as compared to the separated manner of miR-424-5p and miR-142-3p (Fig. 2c). In contrast, the combination group showed the lowest levels of transcript and protein downregulation of Bcl-2 in comparison with mono-treatment groups (Fig. 2c).

3.3. The combination of miR-142/miR-424 synergetically increased the accumulation BC cell- cycle at the G2 phase

The findings indicated that miR-142-3p combined with miR-424-5p may enhance the arrest of BC cells in the G2 phase (Fig. 3a). Furthermore, miR-142-3p-transfected and miR-424-5p-transfected groups promoted the cellular arrest in the G2 phase, separately (16.9–44.5% for miR-142 and 16.9–40.8% for miR-424); however, the highest amount of BC cells in the G2 phase was demonstrated in the combination group (16.9–56%) (Fig. 3a). The aim was to assess the molecular action underlying the G2 phase arrest in the miR-142-restored, miR-424-transfected, and combination groups as compared to the NC cells by evaluating STAT-3 expression levels. STAT-3 was reduced at both transcript and protein levels in miR-142-restored, miR-424-transfected, and combined treated cells as compared to the miR control transfected cells. However, the lowest level of STAT-3 expression was obtained in the combination group in comparison with miR control restored cells ($p < 0.0001$) (Fig. 3b).

3.4. MiR-424-5p and miR-142-3p synergetically decreased the colony numbers in MCF-7 cell lime

The colony numbers were considerably downregulated after the transfection of BC cells with miR-142-3p or miR-424-5p in comparison to the miR control transfected group (Fig. 4a, b). As observed in Fig. 4a, the colony numbers were noticeably decreased in the combination group compared to the cells with mono-treatment. Thus, the observations showed the synergetic effects of the miR-142 + miR-424 combination on downregulating the colonies of MCF-7 cells. Also, the expression alteration of the key stemness factor, Oct-3 was evaluated in all investigated groups. A significant downregulation ($p < 0.0001$) in both transcript and protein expression of Oct-3 was found in all cellular groups in comparison with miR control transfected cells. However, the most downregulation ($p < 0.0001$) of Oct-3 was detected in the combination group as compared to separated miR-restored cells (Fig. 4c).

3.5. MiR-424-5p and miR-142-3p synergetically enhanced the autophagy in BC cells

To indicate the autophagy stimulation by miR-142-3p and miR-424-5p, the cells were labeled with MDC and cellular autophagic bodies were investigated via flow cytometry. The amounts of the cells in the MDC positive region in the NC, miR-424-transfected, miR-142-transfected, and combination groups were evaluated 0.12%, 2.9%, 0.74%, and 3.2%, respectively (Fig. 5a). Our observations indicated that the combination group showed more autophagy induction ($p < 0.0001$) as compared to mono-transfected cells (Fig. 5a). A well-identified factor participated in autophagy, Beclin1 was evaluated. As showed in Fig. 5b, Beclin1 in both mRNA and protein levels was upregulated in all cellular groups. However, the most upregulation ($p < 0.0001$) of Beclin1 was observed in the combination group (Fig. 5b).

4. Discussion

BC was identified as an incurable malignancy; though, the employment of various controlling strategies has led to the development in tumor treatment [15]. Breast malignancy recurrence after eliminating the

tumor is indicated as the important obstacle in BC treatment [16]. Thus, introducing the methods with enhanced cytotoxic effects on the tumor cells is important for the improvement of BC therapy [16]. It has been shown the combination of therapeutic agents can be more effective as compared to monotherapy [4,5]. The studies reveal that miRs control key signaling pathways participated in BC tumorigenesis, including cell growth, apoptosis, cell cycle, autophagy and so on [7]. Among the cancer-related miRs, miR-142-3p and miR-424-5p were identified to exert a tumor-suppressive activity as well as to suppress cell growth and cancer development in numerous malignancies. Malignancies in which miR-142-3p and miR-424-5p were demonstrated the mentioned features include colorectal carcinoma, ovarian malignancy, and BC [9,17–20]. Therefore, restoring the miR-424-5p and miR-142-3p transcription levels in tumorous cells can be potential as a useful strategy for breast carcinoma treatment. In the previous studies, we indicated the tumor-suppressive activities of miR-142-3p and miR-424-5p upregulation in MDA-231 and – 468 cells [9,21–23]. However, the effects of miR-142-3p and miR-424-5p alone or in a combined manner on cancer cells have not been completely evaluated in BC. Thus, in the present project, we evaluated a combinatory restoration therapy approach of miR-142-3p and miR-424-5p combination as well as mono-treatment with miR-142-3p or miR-424-5p on MCF-7 cells. We demonstrated that miR-142-3p combined with miR-424-5p decreased BC cell viability and growth more treatment of the MCF-7 cells with either miR-142-3p or miR-424-5p. To reveal the molecular effect of the anti-proliferative activity of miR-142-3p and miR-424-5p in BC cells, we evaluated the expression levels of c-Myc in miR-142-3p-transfected, miR-424-5p-restored, and miR-142 + miR-424 transfected cells. Here, both transcript and protein levels of c-Myc were more decreased in the combination group than those of miR-142-3p treated or miR-424-5p treated cells. It has been identified that c-Myc has oncogenic activity and regulates the transcription of cell-proliferation-related factors [24]. In our previous project, it was indicated that c-Myc sustained the growth activity and chemoresistant characteristics of MDA-MB-231 cells [21,22]. In another project, Si and co-workers revealed the c-Myc modulatory activity in breast tumorigenesis [25]. Thus, it might be proposed that miR-142-3p and miR-424-5p alone or in a combined manner could decrease BC cell viability through the control of the c-Myc transcription and translation. Here, we detected that miR-424-5p and miR-142-3p in a combination manner or separately enhanced the apoptosis rate of MCF-7 cells via modulating the apoptosis-associated proteins, including Bax, and Bcl-2 [26] in the MCF-7 cell line. Despite the downregulation of Bcl-2, Bax was found to be increased in transfected cells, particularly in the two miR combined manner. Therefore, it could be concluded that miR-142-3p and miR-424-5p can promote apoptosis of MCF-7 cells via overexpressing Bax and decreasing Bcl-2. The cytotoxicity of the miRs might be increased in a combination manner of miR-424-5p and miR-142-3p. This might be due to the synergetic effects of miR-424-5p and miR-142-3p on BC cells. Here, we observed that miR-142-3p and miR-424-5p in a combined or separate manner significantly increase the accumulation of MCF-7 cells in the G2 phase. It was identified that STAT3 promoted arrest in the G2 phase [27]. Regarding our results, STAT3 showed downregulation in mono-treated or combination-treated cells; however, more downregulation was recognized in the combination treated cells. This might describe the molecular mechanism of the increased G2 arrest rate in the miR424 + miR142 combined manner as compared with miR-142 treated or miR-424 restored cells alone. It has been demonstrated that inhibition of STAT3 upregulated the BC cell growth and development [28]. Thus, the effects of miR-424 and miR-142 in

suppressing BC could also be mediated via modulating STAT3. In addition, we indicated that miR-424-5p and miR-142-3p in a combined or separate manner could inhibit the colonogenic ability of MCF-7 cells, revealing the anti-colonization role of miR-142-3p and miR-424-5p. Researchers have revealed that Oct-3 is overexpressed in BC cells and could be a main biomarker for the prognosis of BC [29]. It has been confirmed that Oct-3 overexpression increased cell growth and improved colony-forming action in tumorous cells [30]. In this project, the decreased levels of Oct-3 were demonstrated in MCF-7 cells, following the transfection of the cells with either miR-424 or miR-142 compared with NC cells. However, the combination of miR-424-5p and miR-142-3p synergetically more decreases the Oct-3 expression compared with a separate manner of the mentioned miRs. Autophagy promotion may increase the autophagy rate of tumor cells that is valuable in BC treatment [31,32]. Researchers have described an important function of Bcl-2 in suppressing autophagy through modulating Beclin1 as a critical regulator of autophagy [21,33]. Here, the miR-424-restored and miR-142-transfected groups demonstrated reduced Bcl-2 and upregulated Beclin1 expression levels compared to NC cells. Taken together, miR-424-5p and miR-142-3p might involve in the autophagy induction in MCF-7 cells via modulating Bcl-2 and Beclin1. Upregulation of autophagy rate in miR424-miR424 combined manner might demonstrate the synergetic effects of miR-424-5p and miR-142-3p on BC cells. Therefore, the miR-142-3p and miR-424-5p could have therapeutic effects on inhibiting the growth and development of BC cells. Also, the combination of miR-142-3p and miR-424-5p synergetically enhanced the cytotoxic effects of either miR-142-3p or miR-424-5p on BC cells. However, it would be better to more assess the molecular actions of miR-142-3p and miR-424-5p in preventing BC development, especially through *in vivo* assays.

5. Conclusion

MiR-424-5p combined with miR-142-3p stimulated BC cells apoptosis. Moreover, miR-424-5p and miR-142-3p transfection synergistically decreased BC cell proliferation, colony-forming ability, autophagy, and cell cycle via controlling the expression of various biological factors. Our findings suggested the high efficiency of miR-424-5p and miR-142-5p combination as a potential method in developing BC treatment. More examinations assessing the downstream molecular targets of miR-424-5p and miR-142-3p would enhance the cytotoxic properties of these miRs in BC cells.

Declarations

Acknowledgment

We appreciated University of Tabriz for providing facilities. This project was supported by research grant (number: 3522) from University of Tabriz.

Funding

We received research grant from University of Tabriz (grant number: 3522).

Competing interests

None

Author Contributions

ND performed the tests and wrote the manuscript, RS supervised the project edited the manuscript, SMB edited the manuscript, BB edited provides facilities for conducting the project.

Ethics approval and consent to participate

Not applicable. This project has not used any human or animal samples.

Availability of data and materials

The data would be available if requested by Editor or reviewers.

Consent for publication

Not applicable

References

1. Dastmalchi N, Safaralizadeh R, Baradaran B, Hosseinpourfeizi M, Baghbanzadeh A (2020) An update review of deregulated tumor suppressive microRNAs and their contribution in various molecular subtypes of breast cancer. *Gene* 729: 144301.
2. Momenimovahed Z, Salehiniya H (2019) Epidemiological characteristics of and risk factors for breast cancer in the world. *Breast Cancer (Dove Med Press)* 11: 151-164.
3. Maeda H, Khatami M (2018) Analyses of repeated failures in cancer therapy for solid tumors: poor tumor-selective drug delivery, low therapeutic efficacy and unsustainable costs. *Clin Transl Med* 7: 11.
4. Bayat Mokhtari R, Homayouni TS, Baluch N, Morgatskaya E, Kumar S, et al. (2017) Combination therapy in combating cancer. *Oncotarget* 8: 38022-38043.
5. Ghasabi M, Majidi J, Mansoori B, Mohammadi A, Shomali N, et al. (2019) The effect of combined miR-200c replacement and cisplatin on apoptosis induction and inhibition of gastric cancer cell line migration. *J Cell Physiol* 234: 22581-22592.
6. Byler S, Goldgar S, Heerboth S, Leary M, Housman G, et al. (2014) Genetic and epigenetic aspects of breast cancer progression and therapy. *Anticancer Res* 34: 1071-1077.
7. Loh HY, Norman BP, Lai KS, Rahman N, Alitheen NBM, et al. (2019) The Regulatory Role of MicroRNAs in Breast Cancer. *Int J Mol Sci* 20.
8. Dastmalchi N, Safaralizadeh R, Banan Khojasteh SM (2019) The correlation between microRNAs and *Helicobacter pylori* in gastric cancer. *Pathog Dis* 77.

9. Mansoori B, Mohammadi A, Ghasabi M, Shirjang S, Dehghan R, et al. (2019) miR-142-3p as tumor suppressor miRNA in the regulation of tumorigenicity, invasion and migration of human breast cancer by targeting Bach-1 expression. *J Cell Physiol* 234: 9816-9825.
10. Chakraborty C, Sharma AR, Sharma G, Sarkar BK, Lee SS (2018) The novel strategies for next-generation cancer treatment: miRNA combined with chemotherapeutic agents for the treatment of cancer. *Oncotarget* 9: 10164-10174.
11. Wang J, Wang S, Zhou J, Qian Q (2018) miR-424-5p regulates cell proliferation, migration and invasion by targeting doublecortin-like kinase 1 in basal-like breast cancer. *Biomed Pharmacother* 102: 147-152.
12. Mansoori B, Mohammadi A, Gjerstorff MF, Shirjang S, Asadzadeh Z, et al. (2019) miR-142-3p is a tumor suppressor that inhibits estrogen receptor expression in ER-positive breast cancer. *J Cell Physiol*.
13. Chen B, Duan L, Yin G, Tan J, Jiang X (2013) Simultaneously expressed miR-424 and miR-381 synergistically suppress the proliferation and survival of renal cancer cells—Cdc2 activity is up-regulated by targeting WEE1. *Clinics (Sao Paulo)* 68: 825-833.
14. Liang L, Fu J, Wang S, Cen H, Zhang L, et al. (2020) MiR-142-3p enhances chemosensitivity of breast cancer cells and inhibits autophagy by targeting HMGB1. *Acta Pharm Sin B* 10: 1036-1046.
15. Redig AJ, McAllister SS (2013) Breast cancer as a systemic disease: a view of metastasis. *J Intern Med* 274: 113-126.
16. Oualla K, El-Zawahry HM, Arun B, Reuben JM, Woodward WA, et al. (2017) Novel therapeutic strategies in the treatment of triple-negative breast cancer. *Ther Adv Med Oncol* 9: 493-511.
17. Zhu X, Ma SP, Yang D, Liu Y, Wang YP, et al. (2018) miR-142-3p Suppresses Cell Growth by Targeting CDK4 in Colorectal Cancer. *Cell Physiol Biochem* 51: 1969-1981.
18. Fu Y, Sun LQ, Huang Y, Quan J, Hu X, et al. (2018) miR-142-3p Inhibits the Metastasis of Hepatocellular Carcinoma Cells by Regulating HMGB1 Gene Expression. *Curr Mol Med* 18: 135-141.
19. Xie D, Song H, Wu T, Li D, Hua K, et al. (2018) MicroRNA424 serves an antioncogenic role by targeting cyclindependent kinase 1 in breast cancer cells. *Oncol Rep* 40: 3416-3426.
20. Zhou Y, An Q, Guo RX, Qiao YH, Li LX, et al. (2017) miR424-5p functions as an anti-oncogene in cervical cancer cell growth by targeting KDM5B via the Notch signaling pathway. *Life Sci* 171: 9-15.
21. Dastmalchi N, Hosseinpourfeizi MA, Khojasteh SMB, Baradaran B, Safaralizadeh R (2020) Tumor suppressive activity of miR-424-5p in breast cancer cells through targeting PD-L1 and modulating PTEN/PI3K/AKT/mTOR signaling pathway. *Life Sci* 259: 118239.
22. Dastmalchi N, Safaralizadeh R, Hosseinpourfeizi MA, Baradaran B, Khojasteh SMB (2021) MicroRNA-424-5p enhances chemosensitivity of breast cancer cells to Taxol and regulates cell cycle, apoptosis, and proliferation. *Mol Biol Rep* 48: 1345-1357.
23. Dastmalchi N, Baradaran B, Banan Khojasteh SM, Hosseinpourfeizi M, Safaralizadeh R (2020) miR-424: A novel potential therapeutic target and prognostic factor in malignancies. *Cell Biol Int*.

24. Valovka T, Schonfeld M, Raffeiner P, Breuker K, Dunzendorfer-Matt T, et al. (2013) Transcriptional control of DNA replication licensing by Myc. *Sci Rep* 3: 3444.
25. Si W, Shen J, Du C, Chen D, Gu X, et al. (2018) A miR-20a/MAPK1/c-Myc regulatory feedback loop regulates breast carcinogenesis and chemoresistance. *Cell Death Differ* 25: 406-420.
26. Jan R, Chaudhry GE (2019) Understanding Apoptosis and Apoptotic Pathways Targeted Cancer Therapeutics. *Adv Pharm Bull* 9: 205-218.
27. Huang WW, Yang JS, Lin MW, Chen PY, Chiou SM, et al. (2012) Cucurbitacin E Induces G(2)/M Phase Arrest through STAT3/p53/p21 Signaling and Provokes Apoptosis via Fas/CD95 and Mitochondria-Dependent Pathways in Human Bladder Cancer T24 Cells. *Evid Based Complement Alternat Med* 2012: 952762.
28. Ma JH, Qin L, Li X (2020) Role of STAT3 signaling pathway in breast cancer. *Cell Commun Signal* 18: 33.
29. Zhao FQ, Misra Y, Li DB, Wadsworth MP, Krag D, et al. (2018) Differential expression of Oct3/4 in human breast cancer and normal tissues. *Int J Oncol* 52: 2069-2078.
30. Karmakar S, Seshacharyulu P, Lakshmanan I, Vaz AP, Chugh S, et al. (2017) hPaf1/PD2 interacts with OCT3/4 to promote self-renewal of ovarian cancer stem cells. *Oncotarget* 8: 14806-14820.
31. Zareba I, Huynh TYL, Kazberuk A, Teul J, Klupczynska A, et al. (2020) Overexpression of Prolidase Induces Autophagic Death in MCF-7 Breast Cancer Cells. *Cell Physiol Biochem* 54: 875-887.
32. Guo Y, Pei X (2019) Tetrandrine-Induced Autophagy in MDA-MB-231 Triple-Negative Breast Cancer Cell through the Inhibition of PI3K/AKT/mTOR Signaling. *Evid Based Complement Alternat Med* 2019: 7517431.
33. Marquez RT, Xu L (2012) Bcl-2:Beclin 1 complex: multiple, mechanisms regulating autophagy/apoptosis toggle switch. *Am J Cancer Res* 2: 214-221.

Figures

Figure 1

(a) The graph compares the cellular viability among miR424-restored, miR142-restored, and miR424+miR142 treated cells (b) c-Myc mRNA levels were analyzed using qRT-PCR in the cell groups (i.e., miR-424 restored, miR-142 restored, miR424-miR142 combination, and NC cells). (c) c-Myc protein expression levels were assessed via western blot in the mentioned cellular groups. ns: non significant, **p < 0.01, ***p < 0.001 and ****p < .0001

Figure 2

(a) The rate of apoptosis promotion was detected using annexinV/PI in miR-142 restored, miR-424 restored and miR142-miR424 treated cells. The graph shows the quantified manner of apoptotic cells. (b) Fragmented DNAs were detected using DAPI-based test. The apoptotic bodies were observed by citation 5. Apoptotic bodies were indicated via arrows in different cell groups. (d) Assessment of transcript and protein levels of Bcl-2 and Bax was done through qRT-PCR and western blot assays, respectively. *p < .05, ***p < 0.001 and ****p < .0001

Figure 3

(a) Flow cytometry showed the accumulation rate of MCF-7 cells in each phase of cell cycle. The graph shows the cell cycle distribution of MCF-7 cells in various cell groups (i.e., miR-142 restored, miR-424 restored, miR142-miR424 combination, and NC cells). (b) STAT3 mRNA and protein levels were compared among the mentioned cell groups. ns: non significant, ** $p < 0.01$, *** $p < 0.001$ and **** $p < .0001$

Figure 4

(a) Colony-forming ability of MCF-7 cell line was evaluated in different cell groups, including miR-142 restored, miR-424 restored, miR-142-miR-424 combination, and NC cells. Colony number was demonstrated as a graph. (b) Cell colonies were photographed through microscope. (c) Oct-3 mRNA and protein levels were assessed using western blot. * $p < .05$, *** $p < 0.001$ and **** $p < .0001$

Figure 5

(a) The percentages of autophagic cells were obtained via flow cytometry. Quantified manner of autophagic cells was observed in a graph. (b) Transcript and protein expression levels of Beclin1 were analyzed, then the results were represented as two graphs. **** $p < .0001$