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Evaluation of the expression level of miR-143 and CXCR4 receptor after silencing Snail-1 gene expression by siRNA in metastatic breast cancer cells

Masoumeh Sattarivand[1], Reza Mohammadzadeh[2]*

[1] *Department of Biology, Faculty of Basic Sciences, Ahar Branch, Islamic Azad University, Ahar, Iran*
[2] *Department of Biology, Maragheh University, Maragheh, Iran*

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Abstract: Many studies have shown that miRNAs can silent the genes involved in the growth and invasion of cancer cells. However, the exact effect of miRNAs on the expression of Snail-1 CXCR4 and miRNA-143 genes is not entirely clear in breast cancer cells. Therefore, the present study investigated the effects of siRNA on Snail-1, CXCR4 and miRNA-143 in breast cancer cells *in vitro*. In this experimental-laboratory study, MDA-MB-468 breast cancer cells were transfected by the specific siRNA of the Snail-1 gene. The expression levels of Snail-1 CXCR4 and miR-143 genes were evaluated by qRT-PCR. Cell proliferation was assessed by trypan blue test. Data were analyzed using student's t-test. Transfecting the MDA-MB-468 breast cancer cells by specific siRNA significantly decreased the expression level of Snail-1 gene CXCR4 and significantly increased the expression of miR-143 ($p < 0.05$). The results of this study showed that knocking down of diagnostic markers of breast cancer cells by specific siRNA can successfully reduce the proliferation and invasion of breast cancer cells.

Keywords: Snail-1, siRNA, cxcR-4, miR-143, Breast cancer

1 Introduction

Breast cancer is the most common cancer among women with a significant mortality rate (Aletaha et al., 2017). This cancer is mainly caused by a combination of heredity and environment factors and its physical symptoms include bulging and deformed breasts. Breast

*e-mail: rmohamadzadeh@maragheh.ac.ir

cancer has a high metastatic power and if not treated, it can metastasize to other tissues of the body and leave irreversible complications (Gumienny and Zavolan, 2015; Hattori et al., 2017). Small interfering RNA (siRNA) is a method used to study the function of genes and to treat diseases. In the treatment of cancer, the siRNA method is more effective, more specific, more efficient and less expensive, as well as more specific than other methods of cancer therapies including surgery and chemotherapy (Salguero-Aranda et al., 2019; Gumienny and Zavolan, 2015). Laboratory studies have shown that siRNA has significant inhibitory effects on Snail-1 . By transfecting siRNA, they were able to suppress the expression of the Snail-1 gene, and treatment with snRNA1 stops the cancer cell cycle. In addition, siRNA translocation affects breast adenocarcinoma cells and prevents migration, proliferation and induction of apoptosis. Snail-1 can be considered as a strong adjunct in the treatment of breast cancer (Aletaha et al., 2017). Laboratory studies have shown that siRNA has important effects on the transcription factor. It also suppresses tumor growth. From these findings, PLK1 and HSF1 may be considered for antitumor therapy purposes (Hattori et al., 2017). The results indicate that there is a significant relationship between siRNA and microRNA (miRNA). Currently, RNA interference (RNAi) - treated approaches to cancer screening have been scrutinized. In particular, siRNA and miRNAs are able to kill carcinogenic genes by targeting mRNA expression, which is the unique context of this therapeutic approach. In addition, it has been supported that co-administration of siRNA / miRNA with chemical drugs enhances their ability to overcome cancer resistance (Gandhi et al., 2014). Combination therapies consisting of several short-acting RNAs, such as siRNA and miRNA, have great potential in the treatment of cancer because they can precisely turn off a specific set of oncogenes and target multiple pathways of related disease. It has also been shown that siRNA can increase miR-143 expression. Laboratory studies have shown that miR-143 levels are negatively correlated with ERK5 (extracellular signal-regulated kinase 5) mRNA levels and with MAP3K7 levels. In addition, miR-143 upregulates the expression of ERK5, p-MAP3K7, MAP3K7 (mitogen-activated protein 3 kinase 7) and cyclin D1, as well as cell viability in MCF- cells. It has been reported that both ERK5 and MAP3K7 may be miR-143 target genes. Increased expression of miR-143 can inhibit cell growth, which may be associated with the expression of ERK5 and MAP3K7 in breast cancer cells (Zhou et al., 2017). Research shows that siRNAs can affect CXCR4 gene expression level (Liang et al., 2005). Studies show that use of antitumor drugs with siRNAs results in reduced CXCR4 gene expression which can be effective in the treatment of ovarian cancer (Yin and Qian, 2021). It has also been shown that the use of suppressors to reduce CXCR4 gene expression can be useful in predicting liver metastasis in colon cancer (Abedini et al., 2011). Inhibition of CXCR4 gene by siRNAs can be effective in the successful treatment of lung and breast cancer (Li et al., 2019; Guo et al., 2014). Inhibition of the CXCR4 gene by interfering RNAs also inhibits the growth of breast tumors *in vitro* (Lapteva et al., 2005). In an *in vitro* study of breast cancer cells, the role of the snail was identified as an invasive agent in breast cancer. It has been shown that snail and E-cadherin expression is inversely related. Increased miRNA-143 expression has been reported to prevent cancer progression. Research on CXCR4 has also shown that reduced siRNA expression in breast, ovarian, and laryngeal cancers can prevent cancer progression. Considering the effect of miR-143 and siRNA on breast cancer metastasis (Tokumaru et al., 2020; Skrzypek and Majka, 2020), the presents study aims to evaluate the expression level of

miR-143 and CXCR4 receptor after silencing the Snail-1 gene expression by siRNA in metastatic breast cancer cells.

2 Materials and Methods

In this experimental-laboratory study, metastatic breast cancer cell line (MDA-MB-468) was purchased from Pasteur Institute of Iran and in all stages, the principles of ethics in the research were observed according to international standards.

2. 1. Culture and counting of cancer cells

RPMI-1640 + 10% FBS medium was used for cell culture. The cells were transferred to a falcon tube containing 10 ml of RPMI-1640 medium and centrifuged at 1300 rpm for 5 minutes. The cell pellet was transferred to a 25 mm flask containing 7-10 ml of complete culture medium (RPMI1640 containing 10% FBS) and placed in an incubator at 37 °C containing 5% carbon dioxide. After culture, cell count was performed with Neubauer chamber.

2. 2. Cellular treatment

The Snail-1 specific siRNA and the negative control (both Santa Cruz Biotechnology, Inc), namely scrambled control siRNA, were prepared. MDA-MB-468 cells, at a concentration of 1×10^6 cells/well, were cultured in 6-well plates and transfected with different concentrations of transfection reagent (4-8 µl) and siRNA (40-80 ϱmol) at 70% confluence, according to the manuals provided by the company. The cells were harvested for RNA and protein isolation after 24, 48, and 72 h of transfecting.

2. 3. Micro RNA isolation and cDNA synthesis

Total RNA was extracted using miRCURY RNA Isolation Kit (Exiqon, Vedbaek, Denmark). Purity of isolated RNA was evaluated by determining the ratio of absorbance readings at 260 nm and 280 nm (A260/A280). cDNA was synthesized using cDNA synthesis kit. Total RNA was diluted and the reaction solution (enzymes and nuclease-free solution) was prepared according to the manufacturer's instructions. Diluted total RNA was added to the reaction solution and incubated at 45 °C for 60 minutes and at 95 °C for 5 minutes. Finally, it was cooled down at 4 °C quickly.

2. 4. Evaluation of gene expression

500 ng of cDNA was amplified by Real-time PCR utilizing SYBR Green-1 universal Master mix in a LightCycler® 96 system (Roche, Germany). The transcript level of β-actin, as the housekeeping gene, was also measured. The relative transcription levels were measured based on the comparative CT approach using $2^{-R\Delta CT}$ formula. All qRT-PCR tests were done in triplicate order. The primers applied in real-time PCR quantification are demonstrated in Table 1.

Table 1. The primers sequences.

Gene	FW/RV	Sequence 5'→ 3'	Size (bp)
Snail-1	FW	5′-GGTTCTTCTGCGCTACTGCTG-3′	161
	RV	5′-GTCGTAGGGCTGCTGGAAGG-3′	
CXCR4	FW	5′- ATCCGGCAAACTGGATCCCTC-3′	100
	RV	5′-AACTTCAGTTTGTTGGCTGC-3′	
β-actin	FW	5′-TCCCTGGAGAAGAGCTACG-3′	127
	RV	5′-GTAGTTTCGTGGATGCCACA-3′	

*** FW and RV represent forward and reverse, respectively.**

2. 5. Statistical analysis

All experiments were assayed at least three times. All statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Software, CA, USA). Data are expressed as mean ± Standard deviation (SD). Student's t-test was used to compare two groups (*P*-value < 0.05 was considered statistically significant).

3 Results and Discussions

To determine the effective time and dose, the amplification and melting curves of beta-actin gene and Snaill gene were obtained (Figures 1 and 2).

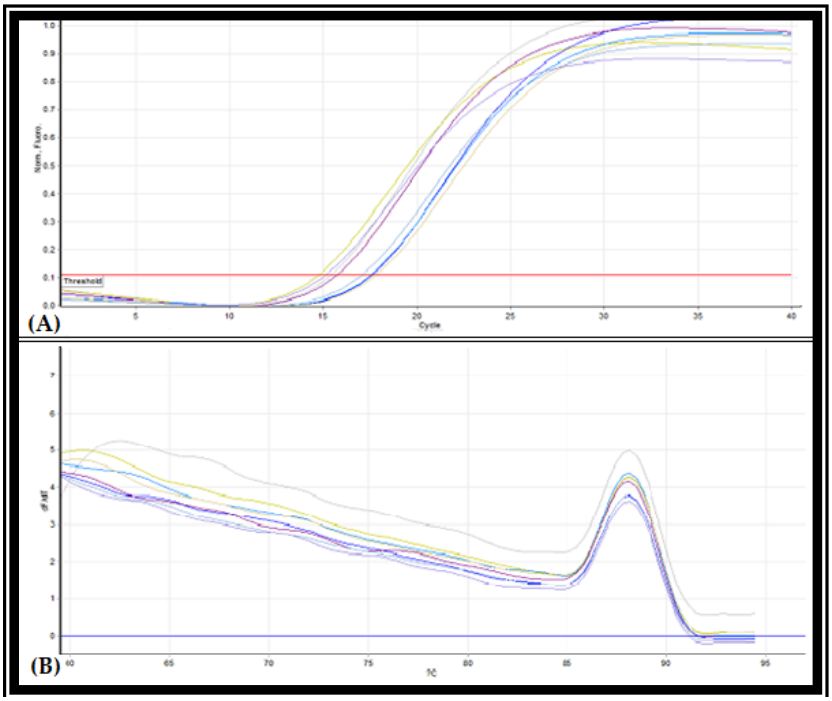

Figure 1: Amplification and melting curve of beta-actin gene. A) Amplification curve of beta-actin gene in and untreated cells and cells treated with specific siRNA of Snail-1 gene for 24, 48 and 72 hours and concentrations of 40, 60 and 80 picomoles. B) Melting curve of beta-actin gene in untreated cells and cells treated with specific siRNA of Snail-1 gene for 24, 48 and 72 hours and concentrations of 40, 60 and 80 picomoles.

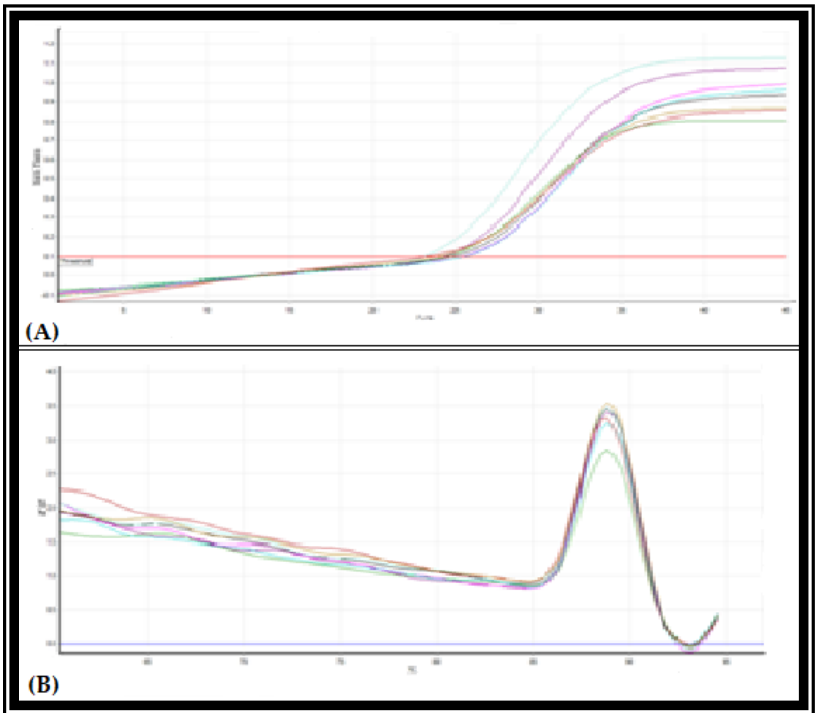

Figure 2: Amplification and melting curve of Snail-1 gene. A) The amplification curve of the Snail-1 gene in cells untreated cells and cells treated with the specific siRNA of the Snail-1 gene for 24, 48 and 72 hours and concentrations of 40, 60 and 80 picomoles. B) Melting curve of Snail-1 gene in untreated cells and cells treated with specific snail of Snail-1 gene for 24, 48 and 72 hours and concentrations of 40, 60 and 80 picomoles.

The results of specific siRNA study on Snail-1 gene expression showed that the relative level of Snail-1 gene expression in breast adenocarcinoma cells significantly reduced at an effective time of 48 hours and an effective dose of 60 picomol ($p < 0.05$). The relative expression of Snail-1 gene at 24, 48 and 72 hours was 92%, 23% and 113%, respectively. Also, the relative expression of mRNA in Snail-1 gene at concentrations of 40, 60 and 80 picomol was 85%, 24% and 61%, respectively. The expression level of beta-actin gene as internal control was the same in all groups (Figures 3 and 4).

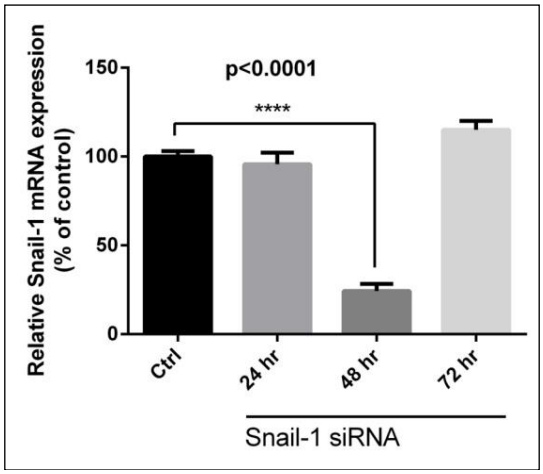

Figure 3: Expression level of Snail-1 gene in MDA-MB-468 cell line at 24, 48h and 72h after treatment. Data are represented as mean ± SD. *** indicates $p < 0.0001$ compared to the control group.

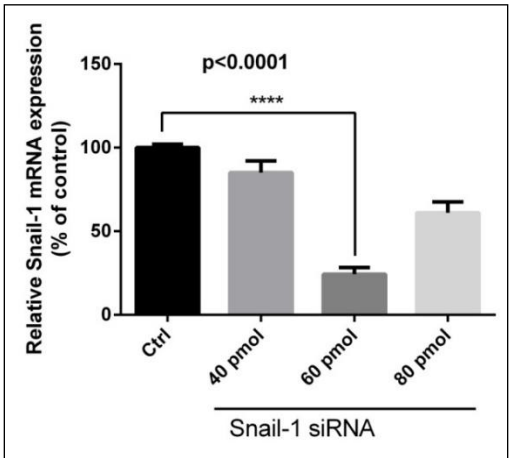

Figure 4: Expression level of Snail-1 gene in MDA-MB-468 cell line in response to 40, 60 and 80 picomoles of siRNA. Data are represented as mean ± SD. *** indicates $p < 0.0001$ compared to the control group.

To determine the effective time and dose, the amplification and melting curves of beta-actin gene and CXCR4 gene were obtained (Figure 5).

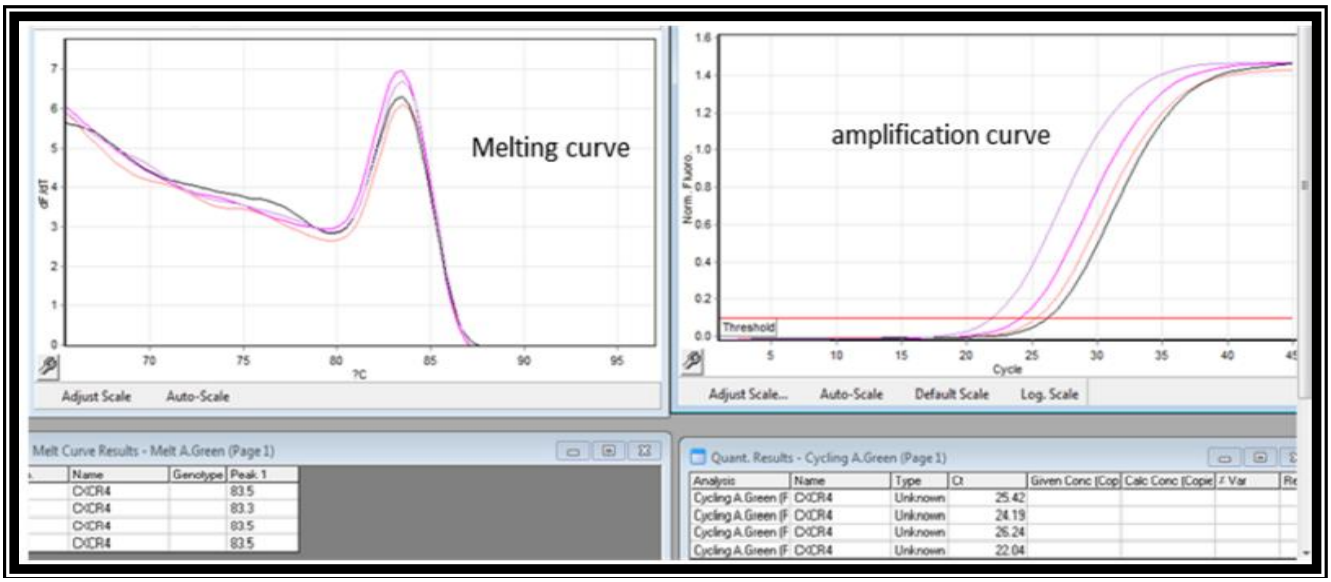

Figure 5: Amplification and melting curve of CXCR4 receptor gene in untreated cells and treated cells with Snail1 gene specific siRNA.

As shown in Figure 6, a significant decrease in CXCR4 receptor expression was observed after deletion of the Snail-1 gene.

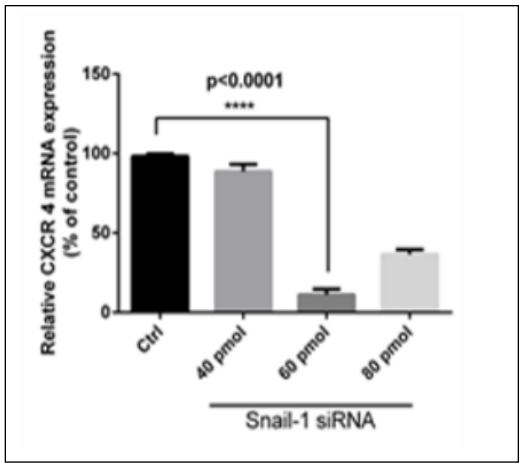

Figure 6: Expression of CXCR4 in cells treated with Snail-1 siRNA versus untreated cells.

The results of the study of the effect of specific siRNA of Snail-1 gene on the morphology of MDA-MB-468 cells showed that the growth rate of cancer cells decreases with knockdown of Snail-1 gene (Figure 7).

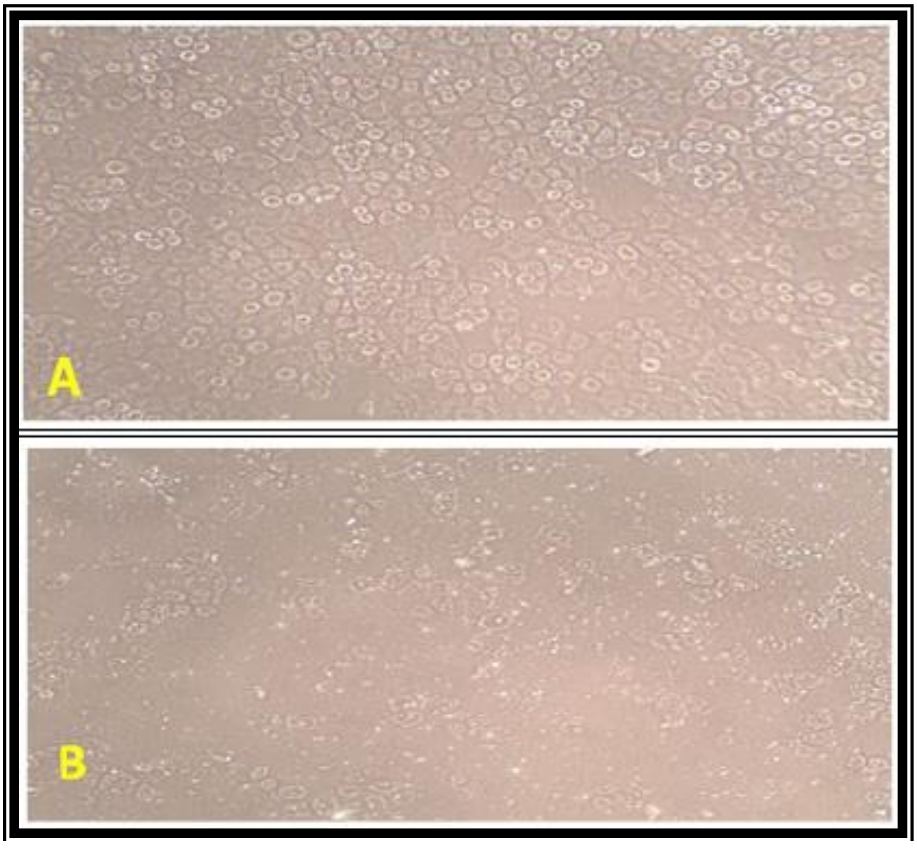

Figure 7: Effect of Snail-1 gene siRNA on cell morphology in MDA-MB-468 breast cancer cell line. A) Microscopic image of untreated cells (X40). B) Microscopic image of treated cells with specific siRNA of Snail-1 gene (40X).

The results of siRNA knockdown evaluation of Snail-1 gene showed that knockdown of this gene in cancer metastatic cells increases the relative expression of miR-143 at effective dose and

time compared to untreated cells (Figures 8 and 9).

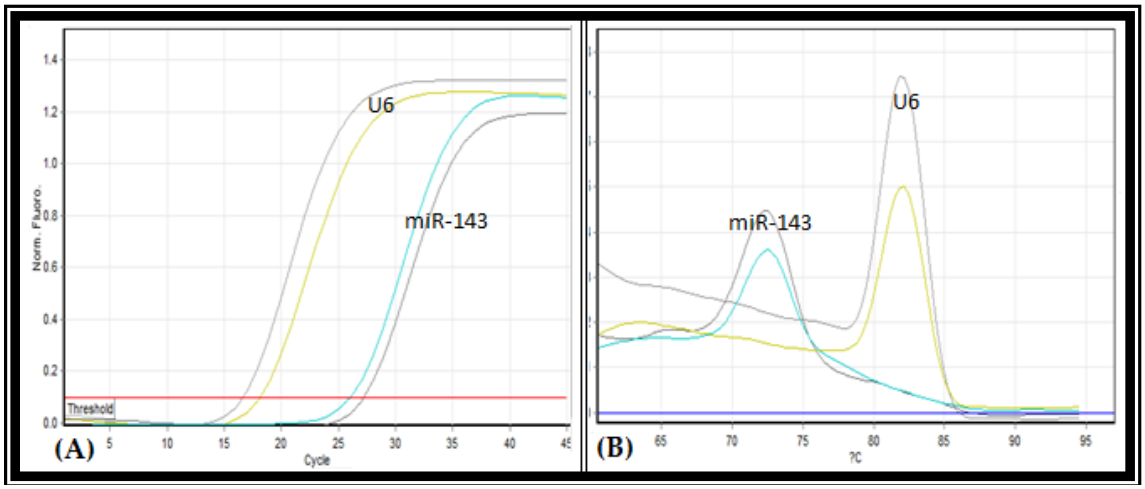

Figure 8: MiR-143 amplification and melting curve. A) The melting curve of MiR-143 in untreated cells and cells treated with the specific siRNA of the Snail-1 gene. B) The amplification curve of miR-143 in untreated cells and cells treated with the specific siRNA of the Snail-1 gene.

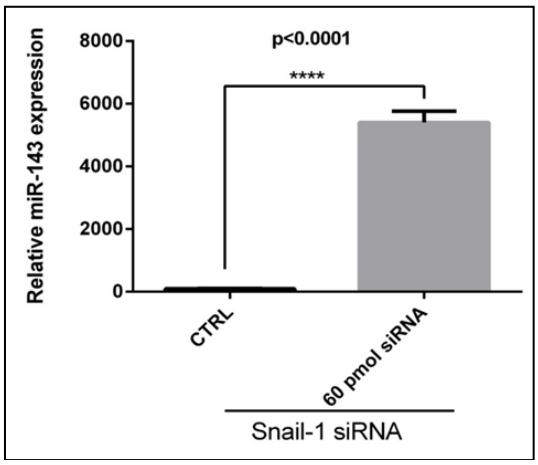

Figure 9: Effect of Snail-1 gene siRNA on miR-143 expression in MDA-MB-468 cells. The relative expression levels of miR-143 in cells treated with 60 picomol siRNA were compared with untreated cells and the data were shown as mean ± SD. $P < 0.0001$ compared to the control.

The results of this study showed that the Snail-1 gene is expressed in the metastatic cell line of breast cancer and silencing of this gene with specific siRNA decreases its expression level and increases the expression level of miR-143 resulting in reduced proliferation and invasion potential of the breast cancer cells. The results of this study show that the effects of microRNA function on the treatment of cancer cells, especially breast cancer cells, are of particular importance.

Breast cancer is the second most common cause of cancer death and according to statistics from the World Health Organization, one in 8 to 10 women develops breast cancer. Diagnostic markers of this cancer can play an important role in diagnosing and preventing it (Malthaner et al., 2004; Antoniou and Easton, 2006). Also, local recurrence and metastatic feature of this

cancer are its most prominent features. Accordingly, in the present study, metastatic cell line was used to investigate the effects of microRNA and Snail-1 gene on this aspect of breast cancer. In fact, clarifying the role of specific markers of breast cancer is one of the important issues for the diagnosis, prevention and treatment of breast cancer. Also these markers play a role in the process of cancer metabolism (Ng et al., 2014; Zaffaroni and Daidone, 2002).

Research has shown that siRNA has important effects on the transcription factor. In accordance with the findings of the present study, the research results have shown that siRNA in human breast cancer cell line (MDA-MB-231) and cervical cancer cells (HeLa) inhibit PLK1 and HSF1 mRNA expression, respectively (Hattori et al., 2017). Previous studies have shown that Snail-1 plays a key role in the development of various cancers in terms of metastasis, inhibition of apoptosis and cell cycle including breast and ovarian carcinoma (Martínez-Estrada et al., 2006), melanoma (Vila-Coro et al., 1999), oral squamous cell carcinoma (Müller et al., 2001).Increased Snail expression is associated with decreased cadmium E expression level (Yagi et al., 2011) in stomach, liver, colon, ovary, and breast cancer cells (Liu and Jessell, 1998; Peinado et al., 2004). The results of a study show that in breast cancer, excessive Snail expression suppresses the expression of Claudin-1, which is a membrane integral protein, and thus promotes tumor progression (Martínez-Estrada et al., 2006). It has been reported that transfecting cancer cells with siRNA suppresses Snail-1 gene expression and prevent cancer metastasis (Aletaha et al., 2017). In a study on the mechanism of action of siRNA on cancer cells, four STAT6-specific siRNA-specific sequences were tested *in vitro* using human colon adenocarcinoma cell lines and breast cancer cell lines, and the results showed that STAT6 silencing by siRNA significantly induces apoptotic events and reduces the number of cancer cells in a short time (Salguero-Aranda et al., 2019).

Studies on microRNAs have shown that MiR-143 inhibits the progression of cancers such as gastric and prostate cancer, and the expression level of MiR-143 is low in many tumors, indicating the inhibitory role of this microRNA in tumor progression (Gregersen et al., 2012; Wu et al., 2013). It appears that ERK5 and MAP3K7 may be the target genes for miR-143 (Zhou et al., 2017). Research has shown that siRNA/miRNA compounds with other anticancer agents can be used for cancer treatment (Gandhi et al., 2014). Recent studies have also shown that siRNAs play an important role in the expression of the CXCR4 gene so that they can inactivate it resulting in reduced invasion and metastatic potential of cancer cells (Liang et al., 2005). CXCR4 is known as the most important protected gene playing an important role in cancer cells metastasis so that reducing its expression can significantly reduce the potential for cancer cell metastasis (Bagheri et al., 2020). In line with this research data, our finding also showed that siRNA reduced CXCR4 expression in breast cancer cells. SiRNA combination therapies have a great potential in the treatment of cancer because they can precisely quench a specific set of oncogenes and target multiple pathways of related disease (Wang et al., 2020).

4 Conclusion

Overall, the results of this study showed that transfection of breast cancer cells by specific siRNA is associated with reduced relative expression level of Snail-1 gene, leading to reduced

expression level of CXCR4 receptor and increased relative expression level of miR-143 which in turn can successfully reduce the proliferation and invasion potential of breast cancer cells.

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Conflict of interests

The authors have no conflicts of interests to declare.

References

Abedini, F., Ismail, M., Hosseinkhani, H., Ibrahim, T. A., Omar, A. R., Chong, P. P., Bejo, M. H., & Domb, A. J. (2011). Effects of CXCR4 siRNA/dextran-spermine nanoparticles on CXCR4 expression and serum LDH levels in a mouse model of colorectal cancer metastasis to the liver. *Cancer Management and Research*, *3*, 301. doi: 10.2147/CMR.S11678

Aletaha, M., Mansoori, B., Mohammadi, A., Fazeli, M., & Baradaran, B. (2017). The effect of Snail-1 gene silencing by siRNA in metastatic breast cancer cell lines. *Iranian Journal of Public Health*, *46*(5), 659- 670. PMID: 28560197

Antoniou, A. C., & Easton, D. F. (2006). Models of genetic susceptibility to breast cancer. *Oncogene*, *25*(43), 5898-5905. https://doi.org/10.1038/sj.onc.1209879

Bagheri, M., Fazli, M., Saeednia, S., Kharanagh, M. G., & Ahmadiankia, N. (2020). Sulforaphane modulates cell migration and expression of β-catenin and epithelial mesenchymal transition markers in breast cancer cells. *Iranian Journal of Public Health*, *49*(1), 77. PMID: 32309226

Gandhi, N. S., Tekade, R. K., & Chougule, M. B. (2014). Nanocarrier mediated delivery of siRNA/miRNA in combination with chemotherapeutic agents for cancer therapy: current progress and advances. *Journal of Controlled Release*, *194*, 238-256. https://doi.org/10.1016/j.jconrel.2014.09.001

Gregersen, L. H., Jacobsen, A., Frankel, L. B., Wen, J., Krogh, A., & Lund, A. H. (2012). MicroRNA-143 down-regulates Hexokinase 2 in colon cancer cells. *BMC Cancer*, *12*(1), 1-10. https://doi.org/10.1186/1471-2407-12-232

Gumienny, R., & Zavolan, M. (2015). Accurate transcriptome-wide prediction of microRNA targets and small interfering RNA off-targets with MIRZA-G. *Nucleic Acids Research*, *43*(3), 1380-1391. https://doi.org/10.1093/nar/gkv050

Guo, P., You, J. O., Yang, J., Jia, D., Moses, M. A., & Auguste, D. T. (2014). Inhibiting metastatic breast cancer cell migration via the synergy of targeted, pH-triggered siRNA delivery and chemokine axis blockade. *Molecular Pharmaceutics*, *11*(3), 755-765. https://doi.org/10.1021/mp4004699

Hattori, Y., Kikuchi, T., Ozaki, K. I., & Onishi, H. (2017). Evaluation of in vitro and in vivo therapeutic antitumor efficacy of transduction of polo-like kinase 1 and heat shock transcription factor 1 small interfering RNA. *Experimental and Therapeutic Medicine*, *14*(5), 4300-4306. https://doi.org/10.3892/etm.2017.5060

Lapteva, N., Yang, A. G., Sanders, D. E., Strube, R. W., & Chen, S. Y. (2005). CXCR4 knockdown by small interfering RNA abrogates breast tumor growth in vivo. *Cancer Gene Therapy*, *12*(1), 84-89. https://doi.org/10.1038/sj.cgt.7700770

Li, Z., Chen, G., Ding, L., Wang, Y., Zhu, C., Wang, K., Li, J., Sun, M., & Oupicky, D. (2019). Increased survival by pulmonary treatment of established lung metastases with dual STAT3/CXCR4 inhibition by siRNA nanoemulsions. *Molecular Therapy*, *27*(12), 2100-2110. https://doi.org/10.1016/j.ymthe.2019.08.008

Liang, Z., Yoon, Y., Votaw, J., Goodman, M. M., Williams, L., & Shim, H. (2005). Silencing of CXCR4 blocks breast cancer metastasis. *Cancer Research*, *65*(3), 967-971. https://doi.org/10.1158/0008-5472.967.65.3

Liu, J. P., & Jessell, T. M. (1998). A role for rhoB in the delamination of neural crest cells from the dorsal neural tube. *Development*, *125*(24), 5055-5067. https://doi.org/10.1242/dev.125.24.5055

Malthaner, R. A., Wong, R. K., Rumble, R. B., & Zuraw, L. (2004). Neoadjuvant or adjuvant therapy for resectable esophageal cancer: a systematic review and meta-analysis. *BMC Medicine*, *2*(1), 1-17. https://doi.org/10.1186/1741-7015-2-35

Martínez-Estrada, O. M., Cullerés, A., Soriano, F. X., Peinado, H., Bolós, V., Martínez, F. O., Reina, M., Cano, A., Fabre, M., & Vilaró, S. (2006). The transcription factors Slug and Snail act as repressors of Claudin-1 expression in epithelial cells. *Biochemical Journal*, *394*(2), 449-457. https://doi.org/10.1042/BJ20050591

Müller, A., Homey, B., Soto, H., Ge, N., Catron, D., Buchanan, M. E., McClanahan, T., Murphy, E., Yuan, W., Wagner, S. N., Barrera, J. L., Mohar, A., Verástegui, E., & Zlotnik, A. (2001).

Involvement of chemokine receptors in breast cancer metastasis. *Nature*, *410*(6824), 50-56. https://doi.org/10.1038/35065016

Ng, E. K., Li, R., Shin, V. Y., Siu, J. M., Ma, E. S., & Kwong, A. (2014). MicroRNA-143 is downregulated in breast cancer and regulates DNA methyltransferases 3A in breast cancer cells. *Tumor Biology*, *35*(3), 2591-2598. https://doi.org/10.1007/s13277-013-1341-7

Peinado, H., Portillo, F., & Cano, A. (2004). Transcriptional regulation of cadherins during development and carcinogenesis. *International Journal of Developmental Biology*, *48*(5-6), 365-375. doi: 10.1387/ijdb.041794hp

Salguero-Aranda, C., Sancho-Mensat, D., Canals-Lorente, B., Sultan, S., Reginald, A., & Chapman, L. (2019). STAT6 knockdown using multiple siRNA sequences inhibits proliferation and induces apoptosis of human colorectal and breast cancer cell lines. *PLoS One*, *14*(5), e0207558. https://doi.org/10.1371/journal.pone.0207558

Skrzypek, K., & Majka, M. (2020). Interplay among SNAIL transcription factor, microRNAs, long non-coding RNAs, and circular RNAs in the regulation of tumor growth and metastasis. *Cancers*, *12*(1), 209. https://doi.org/10.3390/cancers12010209

Tokumaru, Y., Asaoka, M., Oshi, M., Katsuta, E., Yan, L., Narayanan, S., Sugito, N., Matsuhashi, N., Futamura, M., Akao, Y., Yoshida, K., & Takabe, K. (2020). High expression of microRNA-143 is associated with favorable tumor immune microenvironment and better survival in estrogen receptor positive breast cancer. *International Journal of Molecular Sciences*, *21*(9), 3213. https://doi.org/10.3390/ijms21093213

Vila-Coro, A. J., Rodríguez-Frade, J. M., De Ana, A. M., Moreno-Ortíz, M. C., Martínez-A, C., & Mellado, M. (1999). The chemokine SDF-lα triggers CXCR4 receptor dimerization and activates the JAK/STAT pathway. *The FASEB Journal*, *13*(13), 1699-1710. https://doi.org/10.1096/fasebj.13.13.1699

Wang, Y., Xie, Y., Kilchrist, K. V., Li, J., Duvall, C. L., & Oupický, D. (2020). Endosomolytic and tumor-penetrating mesoporous silica nanoparticles for siRNA/miRNA combination cancer therapy. *ACS Applied Materials & Interfaces*, *12*(4), 4308-4322. https://doi.org/10.1021/acsami.9b21214

Wu, X. L., Cheng, B., Li, P. Y., Huang, H. J., Zhao, Q., Dan, Z. L., Tian, D. A., & Zhang, P. (2013). MicroRNA-143 suppresses gastric cancer cell growth and induces apoptosis by targeting COX-2. *World Journal of Gastroenterology: WJG*, *19*(43), 7758. doi: 10.3748/wjg.v19.i43.7758

Yagi, H., Tan, W., Dillenburg-Pilla, P., Armando, S., Amornphimoltham, P., Simaan, M., Weigert, R., Molinolo, A. A., Bouvier, M., & Gutkind, J. S. (2011). A synthetic biology approach reveals a CXCR4-G13-Rho signaling axis driving transendothelial migration of

metastatic breast cancer cells. *Science Signaling*, *4*(191), ra60-ra60. doi: 10.1126/scisignal. 2002221

Yin, W., & Qian, S. (2021). CD44v6-O-MWNTS-loaded gemcitabine and CXCR4 siRNA improves the anti-tumor effectiveness of ovarian cancer. *Frontiers in Cell and Developmental Biology*, *9*, 1384. doi: 10.3389/fcell.2021.687322

Zaffaroni, N., & Daidone, M. G. (2002). Survivin expression and resistance to anticancer treatments: perspectives for new therapeutic interventions. *Drug Resistance Updates*, *5*(2), 65-72. https://doi.org/10.1016/S1368-7646(02)00049-3

Zhou, L. L., Dong, J. L., Huang, G., Sun, Z. L., & Wu, J. (2017). MicroRNA-143 inhibits cell growth by targeting ERK5 and MAP3K7 in breast cancer. *Brazilian Journal of Medical and Biological Research*, *50*(8). https://doi.org/10.1590/1414-431X20175891