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Original Article

Evaluation of the Effects of Sirna on Snail1 and miR-143 Gene Expression Levels in Metastatic Female Breast Cancer Cells During Mastectomy

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ABSTRACT

Introduction: The present study examines the siRNA effects on the expression level of Snail1 and miR-143 genes in metastatic breast cancer cells during mastectomy and the results of this study are in the field of etiology and cellular and molecular foundations.
Methodology: The synthesis of cDNA strand was done using hexamer primer, which binds non-specifically at different points of the RNA strand, and as a result, all RNA strands are used as synthesis templates. For this purpose, total RNA was initially extracted from the cell culture medium for cDNA synthesis.
Results: The results of knockdown evaluation of Snail1 gene by specific siRNA showed that the knockdown of this gene in metastatic cancer cells increases the relative expression of miR-143 in the effective dose and time compared to untreated cells.
Conclusion: Transfection of breast cancer cells by specific siRNA by reducing the relative level of snail1 gene expression and increasing the relative level of miR-143 expression can successfully reduce proliferation and invasion, become breast cancer cells, and this can play an important role in the use of cellular and molecular methods in the treatment of breast cancer.
Keywords: SIRNA, Snail1, MIR-143, Mastectomy.

1. Introduction

Breast cancer is basically caused by the interaction of heredity and environment, and its outward symptoms include swelling and deformation in breast cancer. This cancer has a high metastasis power and can metastasize to other tissues of the body if not diagnosed and treated on time and leave irreparable side effects. The Snail family is one of the zinc-dependent transcription factors, which has several types, including Snail1, Snail2, and Snail3, which play an effective role in the formation of the mesoderm layer and, therefore, are effective in the formation of the mammary gland structure. The role of Snail in the metastasis of metastatic cancer cells has been proven by its reducing effect on E-cadherin, which is one of the most important elements of cell junctions. On the other hand, today it is known that all types of microRNAs.

Among miRNAs, miR-143 can play an important role in the cancers pathogenesis, including breast cancer, by directly targeting several mRNAs. Likewise, studies have shown that Snail transcription factor is able to regulate the expression of some miRNA genes. These results prove that the Snail transcription factor plays a significant role in the invasion and metastasis of many tumors, especially in epithelial cancers, including breast cancer. In fact, miRNAs together with transcription factors in eukaryotic cells affect the expression of mRNAs and disrupt the biological pathways involved in carcinogenesis. Some recent studies have shown that miRNAs regulate the expression of a large number of genes involved in cancer progression. Laboratory research shows that Snails can significantly change the mRNA/miRNA interactions in the early stages of metastasis. Accordingly, have a significant effect on the process of metastasis and inhibition of gene expression, and thus the use of RNAs can be considered as a therapeutic tool to inhibit metastasis, especially in breast cancer. On the other hand, research results indicate that there is a significant relationship between siRNA and miRNAs. Recent studies show that siRNAs can increase the expression of miR-143 gene, can prevent the growth of cancer cells. Concerning the widespread incidence of breast cancer in women and the ability of this cancer to metastasize to different tissues of the body, which makes it one of the most dangerous cancers, and considering the extensive clinical and economic complications resulting from the disease to breast cancer in patients and given that previous studies regarding interactions between siRNAs and Snail family genes and miR-143 are very limited especially in breast cancer cells.

Therefore, the present study evaluates the effects of siRNA on Snail1 and miR-143 gene expression levels in metastatic female breast cancer cells during mastectomy, and the results of this study are in the field of etiology and cellular and molecular foundations. It can be used as diagnostic markers in the management of breast cancer prevention and treatment.

Method

1. Cultivation and counting of cancer cells: RPMI-1640+10% FBS culture medium was used for cell culture. The differentiated cells were transferred to Falcon containing 10 ml of RPMI-1640 medium and centrifuged at 1300 rpm for 5 minutes. The cell sediment from the bottom of the Falcon was transferred to a 25 mm square flask containing 7 to 10 ml of complete culture medium and transferred to a 37 °C incubator containing 5 carbon dioxide observations. After culture, cell counting was done with neo bar slides.

2. Cell treatment: In this research, the Snail1 gene kit (Santacruz Biotechnology, California, USA) was used to treat cancer cells with specific siRNA. After receiving the kit, the solutions were stored at -80 °C. RNase-free water was used to dilute the original stock according to the kit instructions. The dose considered for this step was a specific and fixed dose of 60 µg/µl for each volume, and the average dose of PM60 was used. For this purpose, control and treated cells were initially affected by the dose of 60 siRNA at three times of 24, 48, and 72, and after examining the gene expression, the effective time was obtained, and finally, at the effective time, the cells were the effect of three doses of 04, 06, and 08 were determined to obtain the effective dose and beta-actin gene was used for internal control. At the effective time and dose obtained, MDA-MB-468 metastatic cells were examined morphologically before and after transfection of the desired specific gene. Morphological changes resulting from the treatment in cancer cells were investigated using an optical

microscope. To measure the viability and growth of cells, trypan blue staining and observation with neobar slides were used.

3. RNA extraction: MDA-MB-468 cells were initially cultured in small flasks, and after reaching the desired number of cells, they were separated from the bottom of the flask, and after washing the cells, the supernatant liquid was removed and precipitated. The cell was opened. Next, the number of cells was counted using neobar slide.6 x 105 cells from each cell line were spread in 6-well plates. Cells were treated with specific siRNA in the next step. After the incubation time and washing twice with PBS and finally by 100 microliters of 0.25% Trypsin/EDTA solution, they were separated from the bottom of the plate and transferred to the Falcon after centrifugation at 1300 rpm for 10 minutes.

4. MicroRNA extraction and cDNA synthesis: To prepare the first strand of cDNA from the total RNA extracted in the previous step, the First Strand cDNA Synthesis Kit by Fermantase Company was used. This kit is prepared based on Moloney Murine cancer virus reverse transcriptase enzyme, which has mild RNase properties. Using this kit, cDNA can be synthesized from large patterns, even 13 kb. The synthesis of cDNA strand was done using hexamer primer, which binds non-specifically at different points of the RNA strand, and as a result, all RNA strands are used as synthesis templates. Total RNA was initially extracted from the cell culture medium for cDNA synthesis. Therefore, miRCURY RNA Isolation kit Vedbaek, Denmark was used. Total RNA extracted from Unisp RNA was used according to the instructions for quality control and purity. After total RNA extraction, cDNA synthesis was performed.

5. Evaluation of gene expression: After cDNA synthesis, gene expression was evaluated using qRT PCR. PCR panels for miRNA were used to perform qRT-PCR; Unisp6 RNA was used for internal control and calibrator inside the plate. To perform RT-PCR, first PCR SYBER Green master mix was combined with nuclease-free water, and then cDNA was added to it. The primers designed for miRNAs are stem loop type. It should be noted that all the ingredients should be on ice before and during the work.

Results

To determine the time and effective dose the Snail gene were obtained (Figures 1 and 2).

Treated cells not with Snail1 gene specific siRNA at 24, 48, and 72 hours and concentrations of 40, 60, and 80 pimol in Rotor-Gene 6000 device.

The results of the investigation of specific siRNA on Snail1 gene expression showed that the relative level of Snail1 gene expression in breast adenocarcinoma cells was significantly decreased at an effective time of 48 hours and an effective dose of 60 pmol. The relative percentage of Snail1 gene expression at 24, 48, and 72 hours was 29, 32, and 113%, respectively. In addition, the percentage of relative expression of mRNA in Snail1 gene at concentrations of 04, 06, and 08 pmol of beteratib was 58, 42 and 16%.

Results of knockdown evaluation of Snail1 gene by specific siRNA showed that the knockdown of this gene in metastatic cells increases the relative expression of miR-143 in the effective dose and time compared to untreated cells.

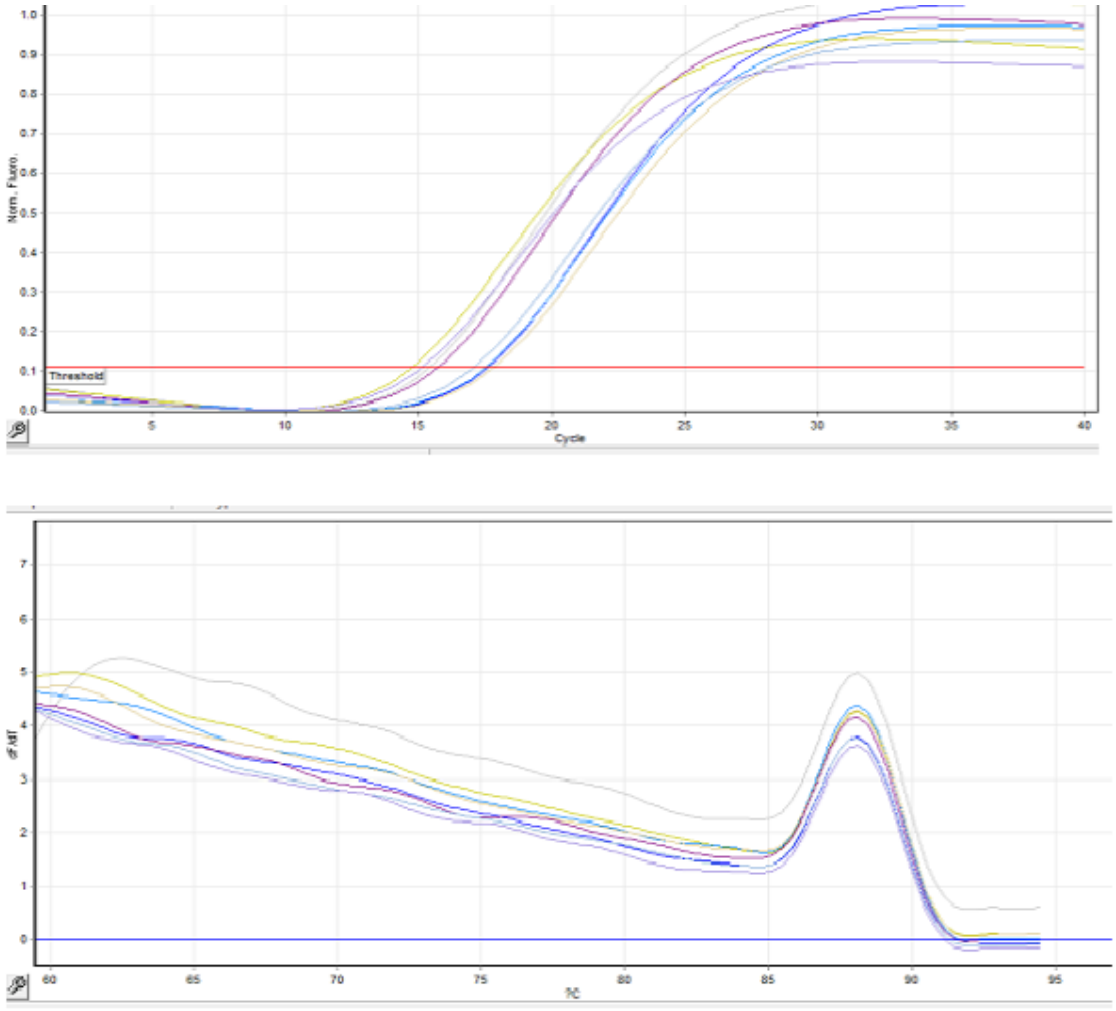

Figure 1. Amplification and fusion curve of beta-actin gene

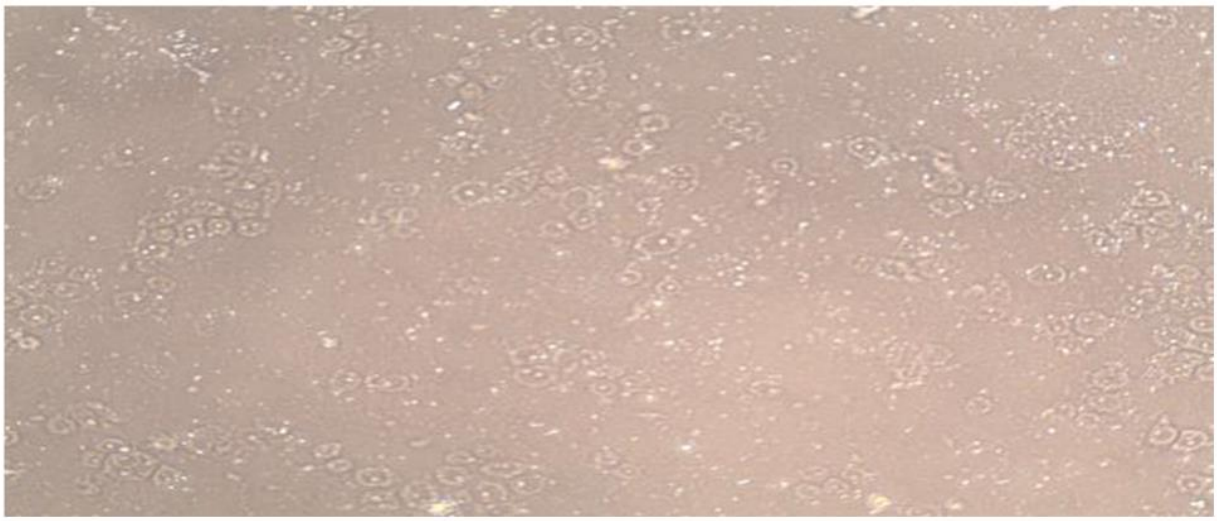

Figure 2. The effect of Snail1 gene siRNA

Discussion

In fact, breast cancer is the second most common cause of cancer-related death and according to the statistics of the world health organization, one out of every 8 to 10 women gets breast cancer, and the diagnostic markers of this cancer can make a significant contribution to its diagnosis and prevention to perform. In addition, the local recurrence and metastatic feature of this cancer is one of its most prominent features. Thus in this study, metastatic cell line was used to investigate the effects of microRNA and snail1 gene on this feature.

In fact, clarifying the role of breast cancer specific markers is one of the important issues for diagnosis, treatment, and prevention of breast cancer, and in this regard, microRNAs are considered as the most important regulatory protected genes related to physiological and pathological processes, which play an essential role in differentiation, proliferation, and angiogenesis as well as in the process of cancer metabolism. On this basis, miRNA-143 has been investigated in the present study. Research has shown that siRNA has important effects on transcription factors. In fact, and in agreement with the research findings of the present research, the research results have revealed that siRNA in human breast cancer cell line and cervical cancer cells inhibits cell growth by suppressing PLK1 and HSF1 mRNA expression, respectively. It becomes cancerous. Previous studies have shown that snail1 plays an essential role in the development of all types of cancers in terms of metastasis, inhibition of apoptosis, and cell cycle in breast, ovarian carcinoma, melanoma, and oral squamous carcinoma. In most cancers, the increase in the snail expression is accompanied by the decrease in expression of E-cadherin. Therefore, snail, as the cell lines of the stomach, liver, colon, ovary, and breast, inhibits E-cadherin and induces cell invasion. The results of a research show that in breast cancer, the high expression of snail suppresses the expression of claudin-1, which is an integral membrane protein, and as a result, it causes the tumor to progress, and of course, the reduction of its expression prevents the development. Tumor plays an important role. Another study showed that by transfecting breast cancer cells with siRNA, it suppresses snail1 gene expression and stops the cell cycle and inhibits cancer. In research regarding the mechanism of siRNA effect on cancer cells, four specific sequences of STAT6 specific to siRNA were tested *in vitro* using human colon adenocarcinoma cell lines and breast cancer cell lines and the results showed that silencing STAT6 by siRNA significantly induces apoptotic events and reduces the number of cancer cells in a short period. Therefore, the purpose of using siRNA in the present study was to inhibit and turn off the snail gene expression. On the other hand, various studies on micro RNAs have shown that miR-143 prevents the cancer progression such as stomach and prostate cancer, and the expression level of miR-143 is low in many tumors, which indicated the inhibitory role of this microRNA in tumor progression.

Conclusion

The transfection of breast cancer cells by specific siRNA by reducing the relative level of snail1 gene expression and increasing the relative level of miR-143 expression can successfully reduce the proliferation and invasion of cells.

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