



Short Communication

miRNAs Authoritarians in Calibrating the MAP Kinases and Serine Threonine Protein Kinases in the Breast Cancer Microenvironment

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ABSTRACT

Breast cancer is the second most common cancer that is diagnosed worldwide as it affects one in eight women approximately during their lifetime and also accounts for 23% of cases among all cancer cases. Deregulation of miRNAs in cell proliferative and cell cycle regulatory pathways is also attributed to resistance towards breast cancer treatments aimed at suppressing cell growth and proliferation. The present study aims to identify relative expression of miRNA221, 222, 146b, 181b in the breast cancer tissue and parallel change in the gene expression of MAPK1, MAP3K9, AKT, ER- α , p53 and EGFR. Breast cancer tissue samples of the patients undergoing mastectomy and lumpectomy were collected along with the patients' demographic and clinical data. Relative gene expression profiling of miRNA221, 222, 146b, 181b, MAPK1, MAP3K9, AKT, ER- α , p53 and EGFR was carried by RT-PCR and the fold change in gene expression was determined by delta-delta Ct method. Disease associated clinical parameters revealed the complications associated with the patients. Majority of the patients (62.50%) were in pre-menopause age. All the patients belong to stage I and stage II of the breast cancer and seventy percent of the cases reported metastasis. It was observed that the increase in the expression of miR-221 causes the increase in expression of MAPK1 (30%), MAP3K9 (80%) and AKT (70%). While the increase in expression of miR-222 causes the increase in expression of MAPK1 (0%), MAP3K9 (80%) and AKT (60%). Whereas the expression of ER- α increases among 40% cases with increase in the expression of miR-221 and the expression of ER- α changes irrespective of the expression of miR-222. Furthermore, the results revealed that 70% of the disease occurrence was reported on the left side among the patients as compared to the right side (30%). As far as the location of the disease is concerned, higher incidence of disease was reported in outer and upper quadrant of the gland. The current study concludes that the miR-221 and miR-222 regulate the expression of oncogenes MAPK1, MAP3K9, and AKT among the breast cancer patients.

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Authors' Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by SAA, MH, QM and RMB. SAA, MH and RK and SA, RM and QM. The first draft of the manuscript was written by SAA and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Key words

Breast cancer, Expressional deregulation, miR-221, MAP3K9, ER- α , MAPK1

Breast cancer is the most frequently diagnosed cancer among women in developing and developed countries and the second leading cause of cancer-related death after lung cancer in women (Momenimovahed and Salehiniya, 2019). According to the ranking in 2020, breast cancer is categorized first in the majority of world countries in both incidences as well as in mortality. Among all the cancer cases, breast cancer comprised about 24.50% while it is

responsible for 15.50% of cancer-related deaths in women (Lei *et al.*, 2021). Based on demographic changes, 2.4 million new cases of breast cancer were reported globally, accounting for almost 1 in 4 cancer cases among women in 2018 (Zaheer *et al.*, 2019). miRNAs form a distinct subtype of non-coding RNA of 18-25 nucleotides that regulate the gene expression in mammalian cells at post-transcriptional level. These miRNAs have been associated with numerous human cancers, including breast cancer. The loss of several tumor suppressor miRNAs and the overexpression of certain oncogenic miRNAs have been observed in many breast cancers. Breast cancer is the most common cancer among the women worldwide and evidence support that miRNAs are playing key roles in breast cancer tumorigenesis and development (O'Day and Lal, 2010; Sidorova *et al.*, 2023). Regulation of gene expression by miRNAs is of great importance as miRNAs act as a control unit for the work of the proteins which

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are responsible for the DNA repair; therefore, miRNAs can function as oncogenes or tumor growth suppressors (Sidorova *et al.*, 2023). Many studies have highlighted the role of miRNAs in the regulation of activity and specificity of ERK/MAPK cascade. miRNAs are involved in the regulation of expression of several ERK/MAPK-related targets while, this pathway has been shown to regulate the miRNA apparatus as well. In-vitro studies have demonstrated that a number of miRNAs enhance the activity of signaling pathway (Safa *et al.*, 2020). The purpose of the current study was to access the relative gene expression profiling of miRNA221, 222, 146b, 181b, and MAPK1, MAP3K9, AKT, ER- α , p53 and EGFR gene by using RT-PCR in order to highlight the interplay of miRNAs with MAP kinases and serine threonine protein kinases in breast cancer patients.

Materials and methods

Clinically diagnosed breast cancer patients were selected for the study. Only female patients with invasive ductal carcinoma were selected in this study. Stages I and II of breast cancer, along with, metastasis and non-metastasis cases were included. Breast cancer cases having any other disease were not included in the study also the patients not willing to participate or sign the consent form were not targeted in the study. Executing ethical consideration for the study, pregnant females and cases with any psychological illness were not included.

Demographic data including age and age of menarche, marital status, pre and postmenopausal status were recorded. The clinical hallmark i.e., metastasis, type of carcinoma, grade, location, nipple discharge, and type of surgery were also recorded.

Fifty fresh tissue samples of breast cancer, along with adjacent healthy tissue as a control were included in the study. These patients were clinically confirmed for breast cancer at tertiary care hospitals. Samples were collected in 5ml RNA later at the time of surgery and stored at -18°C for further processing.

Total RNA extraction was carried out by using the Trizol method (Masood *et al.*, 2011). RNA quantification was done and first-strand cDNA was synthesized from the total RNA extracted following the kit instructions (Revert Aid First Strand cDNA Synthesis Kit, K1622, Thermo Fisher Scientific, USA).

Expression profiling was carried out for miRNAs; miR-221, miR-222, miR-146b and miR-181b along with the target genes i.e., MAPK1, MAP3K9, AKT, ER- α , p53, EGFR through RT-PCR. Expression of GAPDH gene was performed for all samples for relative quantification of all the genes under investigation. The RT-PCR was performed in a final volume of 25 μ l using gene specific primers 0.5 μ l

of 100pmol/ μ l of each forward and reverse primer, 12.5 μ l of Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, Lithuania), 2 μ l cDNA and 9.5 μ l PCR water in standard cycling program for SYBR Green on SLAN96 RT-PCR Machine (Sansure, China). A fold change in the expression of the genes was calculated by delta delta Ct method (Apostolou *et al.*, 2019).

Results

Table I shows the demographic data of the breast cancer patients with higher incidence of disease at mean age of 49.55 $\pm$ 15 years and 15.33 $\pm$ 2 years age of menarche. A high prevalence of the disease (62.50%) occurred in women at pre-menopause stage. More than 70% cases were metastatic and all the cases were equally distributed in grade I and II of breast cancer. The left sided breast was pertinently affected in 70% of the cases. Nipple discharge was observed among 60% of the patients. While, 60% of

Table I. Demographic data of the patients.

Description	Patients (n=50)
Age	
Mean age	49.55 $\pm$ 15years
Age at menarche	15.33 $\pm$ 2years
Grade	
I	50%
II	50%
Metastasis	
Yes	70%
No	30%
Location	
Right breast	30%
Left breast	70%
Menopausal status	
Premenopausal	62.50%
Postmenopausal	37.50%
Type	
Invasive ductal carcinoma	100%
Lactation history	
Positive	100%
Oral contraceptive pills	
Positive	50%
Negative	50%
Nipple discharge/deformities	
Yes	60%
No	40%
Type of surgery	
Mastectomy	60%
Lumpectomy	10%
No history for surgery	30%

the cases has undergone mastectomy and 10% undergone lumpectomy at some stage of their life. All the patients were married and suffering from invasive ductal carcinoma.

Figure 1 shows relative gene expression in comparison with the housekeeping gene GAPDH. The fold change in the expression for mir-146b, EGFR, MAPK1, and mir-181b showed a two to four fold (2.07, 2.14, 2.36, and 3.92) increase. While an increase of fifteen and twenty-one fold (15.00, 21.21) was observed for p53 and ER- α , respectively. A much higher increase in the expression of mir-221; 27.96 folds and AKT expression up to 29.34 folds was seen. Moreover, the highest increase in expression was observed for MAP3K9 and mir-222 i.e., forty-one to forty-eight fold (41.77-48.38).

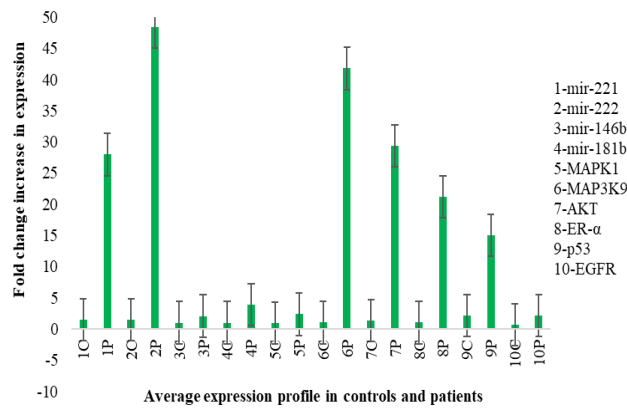


Fig. 1. Average expression profile in cases and controls.

Discussion

The breast cancer patients presented in the study are in the borderline age of menopause. More than two third (70%) of the disease occurrence was reported on the left side among the patients as compared to the right-side breast (30%). As far as the location of the disease is concerned, higher incidence of disease was reported in outer and upper quadrant of the gland. The gene expression profile revealed that an increase in the expression of miR-221 causes the increase in expression of MAPK1 (30%), MAP3K9 (80%) and AKT (70%). While the increase in expression of miR-222 causes the increase in expression of MAPK1 (0%), MAP3K9 (80%) and AKT (60%). Whereas the expression of ER- α increases among 40% cases with increase in the expression of miR-221 and the expression of ER- α changes irrespective of the expression of miR-222 (Fig. 2). Existing evidence highlights the activation of AKT/mTOR pathway in miR-221-expressing cancer cells. As miR-221 negatively regulates PTEN thus pro-survival signaling is activated in breast cancer cells (Yin *et al.*, 2020). Overexpression of miRNA-221 potentiated

the activation of PI3K/AKT pathway which promotes cell transformation. Moreover, an increase in expression of miRNA-221/222 has been associated with the altered response to chemotherapy (Di Martino *et al.*, 2022). miR-221 and miR-222 are downstream effectors of EGFR-RAS-RAF-MEK pathway and fuel the progression of metastatic transformation of breast cancer tumors (Shah and Calin, 2011).

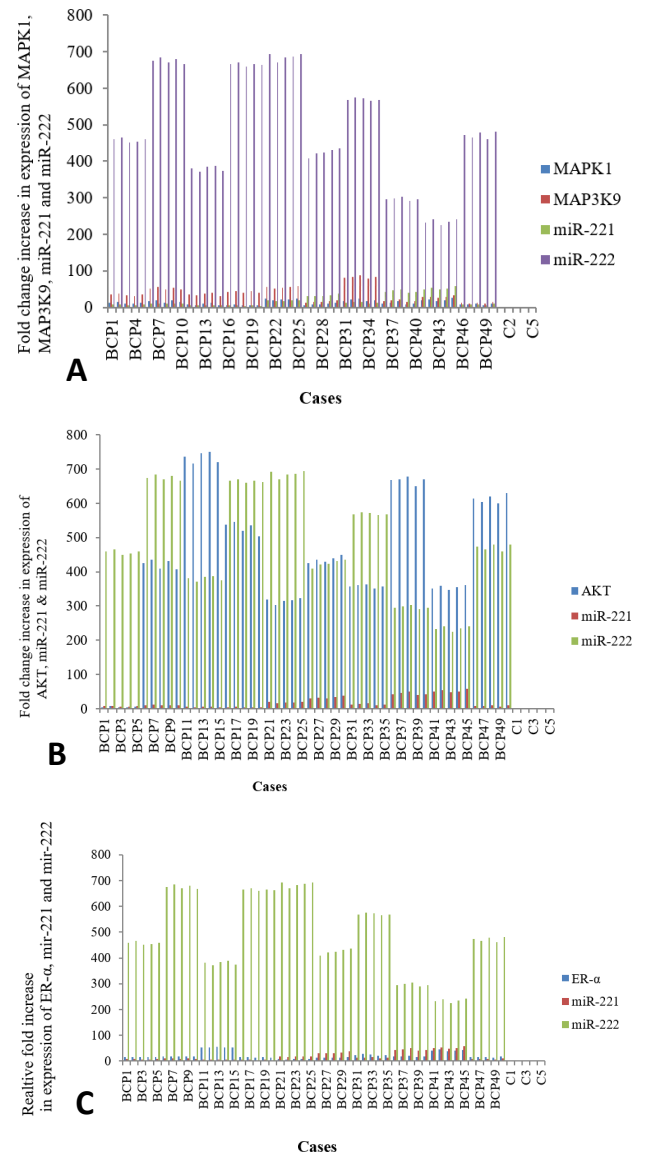


Fig. 2. Fold increase in the expression of MAPK1, MAP3K9, miR-221 and miR-222 (A), AKT, miR-221, miR-222 (B), and ER- α , miR-221 and miR-222 (C) among cases and controls.

Conclusion

Overall, our findings suggest that miR-221/miR-222 are oncogenic miRNAs having central role in breast carcinogenesis. Inactivation of PTEN mainly because of post-transcriptional processing causes the progression of breast cancer. Future studies should be focused on targeted inhibition of miR-221/miR-222 to mechanistically analyze metastatic dissemination in breast cancer models. Likewise, patients derived xenografts will enable us to develop a better understanding of the complex interplay among miR-221/miR-222, PTEN and AKT/mTOR pathways.

DECLARATIONS

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IRB approval

The study was approved by the Ethical Review Committee, PMAS-Arid AAU, Rawalpindi, Pakistan.

Ethical statement

The study was approved by the ethical committee of PMAS-Arid Agriculture University Rawalpindi, Pakistan, as well as by the ethical committees of hospitals. All the protocols, methods, and experiments used in the study were as per guidelines and approved by IBGE, Islamabad, Pakistan. The patients were requested to participate in this study after informed consent.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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