European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 09, Issue 07, 2022

Original Research Article

Micro Rna 22 (Mirna22) And Breast Cancer:A Review

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ABSTRACT

WHO has reported that, 2.3 million women were diagnosed with breast cancer in 2021 and 685000 deaths occur globally due to breast cancer. As estimated at the end of 2020, there were 7.8 million women diagnosed with breast cancer in the past 5 years were alive, making it the world's most prevalent cancer. Breast cancer is the most common cancer type in Indian female, and many patients suffer from recurrences and metastasis. There is no marker to predict the severity and invasiveness of breast cancer. Single-stranded, non-coding RNA molecules called microRNAs (miRNAs) control the expression of target genes through post-transcriptional alterations . Numerous studies suggested that miRNA has a promising role in the diagnosis and prognosis of various malignancies. In a number of malignancies, including gastric cancer, esophageal squamous cell carcinoma, breast cancer, and prostatic cancer, miR-22 is aberrantly expressed either up or down. miRNA-22 may be used a diagnostic as well as prognostic marker for breast cancer.

Keywords: miRNA22, breast cancer,pathogenesis

BACKGROUND:

Breast cancer is the most common cancer in Indian females [1]. Incidence of metastatic breast cancer (MBC) has been reported to be approximately 5% to 25% from various centres in India [2,3]. MBC is unlikely to be cured; meaningful improvements in survival have been seen, coincident with the introduction of newer systemic therapies in Western literature [4,5]. MBC carries a poor prognosis in the Indian subcontinent, 5-year and 10-year overall survival have been reported to be 22% and 5% [6].

MicroRNAs (miRNAs) are non-coding, single-stranded RNA molecules that regulate target gene expression via post-transcriptional modifications [7,8]. Several studies indicated the promising role of miRNA in the diagnosis and outcome prediction in several cancers [9,10]. The miR-22 aberrantly expressed upregulation or downregulation in various cancers, such as prostatic cancer, esophageal squamous cell carcinoma, breast cancer, and gastric cancers [11–14]. Besides, reports also showed that miR-22 may prominently influence cancer biological behaviours, such as proliferation, invasion and metastasis, and it genetically alters expression of numerous related genes, which unveils the intrinsic mechanisms of miR-22 in regulating cancer formation by means of multi-approaches and multi-layers, indicating the central roles of miR-22 in manipulating the occurrence and development of different cancers[15-17].

miR-22 has been reported to biologically impinge on the processes of senescence, energy supply, angiogenesis, EMT (epithelial-mesenchymal transition), proliferation, migration, invasion,

ISSN 2515-8260 Volume 09, Issue 07, 2022

metastasis and apoptosis, but also it genetically or epigenetically exerts dual (inhibitory/promoting cancer) effects in various cancers via CNAs (copy number alterations), SNPs (single nucleotide polymorphisms), methylation, acetylation and even more momentously hydroxymethylation. Additionally, miR-22 expression may fluctuate with cancer progression in the body fluids of cancer patients and miR‐22 could amplify its inhibitory or promoting effects through partaking in positive or negative feedback loops and interplaying with many other related miRNAs in the cascade of events, making it possible for miR-22 to be a promising and complementary or even independent cancer biomarker in some cancers and engendering profound influences on the early diagnosis, therapeutics, supervising curative effects and prognosis.

Recently, several studies indicated the promising role of miRNA in the diagnosis and outcome prediction in several cancers [9,18-22]. MicroRNAs (miRNAs) are a family of small non-coding RNAs that regulate a wide array of biological processes including carcinogenesis. In cancer cells, miRNAs have been found to be heavily dysregulated. miRNAs regulate target gene expression via post-transcriptional processing.

Hanahan and Weinberg have proposed the abnormal miRNA expression in tumors, it is believed that the dysregulated miRNAs could affect one or several of the cancer hallmarks for tumor initiation and progression[23]. Depending on their target genes, miRNA could function as either oncogene (oncomiRNAs) or tumor suppressor(tsmiRs) under certain circumstances.

The role of miRNA dysregulation in breast cancer was first reported in 2005. Various studies have shown altered expression of miRNAs in breast cancer and their role in breast cancer occurrence and development[24].OncomiRNAs are usually upregulated in breast cancer, suppressing the expression of potential tumor suppressor genes and leading to breast malignancy [25]. Conversely, tsmiRNAs can inhibit the expression of oncogenes that promote breast tumorigenesis [26]. Therefore, their downregulation can lead to breast malignancy.

The miR-22 has been aberrantly expressed,either upregulated or downregulated in various cancers, such as prostatic cancer, oesophageal squamous cell carcinoma, breast cancer, and gastric cancers [11–14]. Recently various studies have indicated the role of miRNA-22 3p in breast cancer. It has been demonstrated that miR-22 is down regulated in estrogen receptor alpha-positive human breast cancer tissues and cell lines, and overexpression of miR-22 could inhibit the growth of breast cancer cells via directly targeting estrogen receptor alpha [27, 28]. It was also found that miRNA-22 inhibits the growth and metastasis of breast cancer cells by targeting GLUT1, EVI-1, PHF8, and CD147, and downregulation of miR-22 was significantly correlated with the TNM stage, local relapse, distant metastasis, and survival time of patients with breast cancer [29- 33]. In addition, miR-22 can also inhibit lipid and folate metabolism in breast cancer cells. The suppressed expressions of miR-22 target genes are associated with poorer outcomes in breast cancer patients, suggesting a beneficial effect of miR-22 on clinical outcomes in breast cancer [34]. On the contrary, Damavandi reported that miR-22 exhibited a significant upregulation in breast invasive ductal carcinoma tissues compared with their matched non-tumor tissues [13]. Pandey *et al* reported that miR-22 was upregulated in breast cancer, which is associated with poor overall survival [35].Furthermore, miR-22 is a promising prognostic biomarker for breast cancer, and ectopic expression of miR-22 inhibits the proliferation and invasion of breast cancer cells by targeting GLUT1 [29].Many targets of miR-22, have been identified in the last 10 years, confirming the importance of miR-22 in tumorigenesis[36].

A study demonstrated that miR-22 expression was downregulated and sirt1 was upregulated in breast cancer cells. Further studies revealed that exogenous expression of miR-22 suppressed tumorigenesis and improved adiosensitivity of breast cancer cells by targeting sirt1. Therefore, miR-22 may be a promising therapeutic target for the treatment of breast cancer [37].

Song et al suggested that miR-22 have decreased expression in breast cancer tissues relative to healthy, non-tumor tissues[38]. Forced expression of both miR-365 and miR-22 through miRNA

ISSN 2515-8260 Volume 09, Issue 07, 2022

mimics gave rise to decreased breast cancer cell growth and increased sensitivity to fluorouracil and paclitaxel, respectively. miR-365 and miR-22 gain their functions in overcoming chemoresistance through respectively targeting GALNT4 and NRAS. GALNT4 is responsible for glycosylation-based post-transcriptional protein modification, whereas NRAS is an oncogenic activator of PI3K/Akt-, MAPK/ERK-, and NF-κB kinase-associated pathways; both are therefore important for cell proliferation and tumor progression [38,39].However, whether other targets of miR-22 exist in breast cancer still needs to be studied. Hence the study is intended to investigate the clinical significance of miR-22 expression in breast cancer patients as compared to healthy individuals. Moreover there are no studies have evaluated the expression of miRNA-22 in blood serum/plasma. The study also aims to assess whether miR 22-3p be used as a biomarker for predicting breast cancer.

Objectives of the Study:

Primary Objectives of our study are to

a) compare the miRNA-22 expression in serum of breast cancer patients as compared to healthy individuals
b) find the association of miRNA-22 expression and breast cancer
c) find the association of miRNA-22 expression with the clinical staging of breast cancer so as to find its association with the disease progression

Secondary objectives are to

a) Evaluate the quality of life of the breast cancer patients
b) Find the association between the miRNA-22 expression and QOL

Methodology:

i.Study design: Observational cross sectional

ii.Sample size:

Sample size = $(2(S)^2[Z_{(1-\alpha/2)} + Z_{(1-\beta)}]^2)/\mu_{d^2}$

$$S^2 = \frac{S_1^2 + S_2^2}{2} = \frac{(1.18)^2 + (0.29)^2}{2} = 0.74$$

$$Z_{\left(1-\frac{\alpha}{2}\right)} = 1.96$$

$$Z_{1-\beta} = 0.84$$

$$\mu_d = 0.4$$

$$n = 2(0.74)\,[1.96+0.84]^2 / (0.4)^2$$

$$= 73$$

Total sample size will be 73 cases and 73 controls[40]

iii. Project implementation Plan

Study site: Molecular division of Central research laboratory,KSHEMA in collaboration with Department of Oncology, Justice K S Hegde charitable Hospital, Mangalore.

Inclusion Criteria: Patients fulfilling all below mentioned criteria will be included in the study;

Group I: Seventy three diagnosed breast cancer patients in any stage of the disease,irrespective of ER/PR/HER status

Group II: seventy three healthy, age matched women

Exclusion Criteria: Patient having any one of the following criteria will be excluded:

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Associated illnesses like DM, congestive heart disease, MI and autoimmune disorders where miRNA may have a role

Ethics Review

- NITTE University Central Ethics Committee approval will be obtained prior to the study
- Written Informed Consent will be taken from the patients.

Sample collection and analysis

Sampling:

- 5mL of whole blood (EDTA) will be collected at the time of diagnosis
- Sample will be stored at stored at -80°C
- Clinical documentation of patient demographics, ER/PR & HER2 status,
- treatments, responses and survival will be done
- Clinical documentation of pattern of metastases (bone only vs. visceral) if any

Gene (mRNA) Expression by Real time PCR(qPCR) Analysis

a. RNA extraction

Five millilitres of whole blood will be collected in to two 2.5 mL PAX gene blood RNA tubes and will be stored at −80°C until RNA isolation. RNA will be isolated from blood, using the PAX gene system and following the manufacturer's instructions. Briefly, PAX gene Blood RNA tubes will be centrifuged, and the pellets will be washed and resuspended in buffer. Using the PAXgene Blood RNA Kit, lysis buffer will be applied to the resuspended pellets, and RNA purification and extraction will be performed using the columns. The extracted RNA will be stored at –80˚C until further analysis.

b. cDNA Synthesis

Purity and RNA concentration will be assessed by measuring the absorbance at 260 and 280nm using Nano drop 2000. 1 ug of RNA will be converted into cDNA by using High Capacity cDNA Reverse Transcription Kit. The gene-specific suitable oligonucleotide primers will be used.

c. Real time PCR(qPCR)

CFX96 Real-Time PCR Detection System will be used for evaluating the gene expression levels by using SYBR green and probe master mix (Roche, Indianapolis, IN).

In order to confirm the presence of a single PCR product in PCR reaction, melting curve analysis will be performed. Relative fold change will be calculated by using 2−ΔCt method (Livak& Schmittgen, 2001).

Clinical Evaluation

Size of the tumor, lymph node involvement and distant metastasis will be assessed and their association with miRNA expression will be evaluated.

QOL of the patients will be measured and screening for psychologic morbidity will be assessed usingtwo instruments: the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire–Core 30, version 2.0 (EORTC QLQ-C30), and the Hospital Anxiety and Depression Scale (HADS)[41,42].

Functional Assessment of Cancer Therapy-Breast cancer

Health-related QOL was measured with the Functional Assessment of Cancer Therapy-Breast cancer (FACT-B) version 4 which comprises of 36-items to measure both the 27-item general QOL associated with cancer (FACT-G) and the additional 9-item breast cancer related QOL, breast

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cancer subscale (BCS). The subscales of FACT-G are physical well-being (PWB), functional well-being (FWB), emotional well-being (EWB), and social/family well-being (SWB). The psychometric properties of the FACT-B are well documented [43] and validated in Korean population [44]. The test was conducted and scored in accordance with the instructions of the version 4 provided by the Functional Assessment of Chronic Illness Therapy (FACIT) Measurement System (www.facit.org). A total FACT-B score is calculated by summing the subscales. The instrument asks respondents to rate how true each statement is for the last 7 days with a response scale ranging from 0 (not at all) to 4 (very much).

Global QOL

The overall QOL of the patients was assessed using the Ladder of Life, a single-item measure. The respondents are asked to circle the number that represents how they feel at the present time. One represents the worst possible life and 10 represents the best possible life, Ladder of Life is widely used scale and provides a good summary of QOL [45].

Statistical analysis

The values will be expressed as mean ± S.D/SEM for parametric data and median (interquartile range) for nonparametric data and will be analyzed using statistical package for social sciences (SPSS), version 23.0 software. Chi-square test will be used to find the association of miRNA expression and breast cancer as well as severity of the disease (staging).Mann Whitney U test will be used to compare miRNA levels of different groups.Chisquare test will be used to find the association of QOL of Ca breast patients on adjutant therapy and miRNA 22 expressions.

Significance of proposed study

miR-22 expression could be a promising biomarker in the diagnosis and outcome prediction of breast cancer.There is no established biomarker for the prediction of metastasis in carcinoma of breast so far. If miRNA-22 emerges to be a potential biomarker for detection of cancer or in predicting metastasis, it may help in personalizing the therapy which may lead to better prognosis.Early detection and treatment will definitely improve quality of life of breast cancer patients.

Expected Outcome

- This study may reveal the miRNA 22 expression pattern prevailing in Coastal Karnataka and North Kerala region.
- This study might reveal that miRNA 22 expression in plasma may be a potential biomarker to predict metastasis in breast cancer patients
- This study may also guide or help the oncologists to personalize the treatment in breast cancer.
- It may help to overcome the challenges faced in treating Ca breast casesby giving an insight in to chemo sensitivity interms of patient well being.

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ISSN 2515-8260 Volume 09, Issue 07, 2022

13. Timeline
Timeline: 2 years
Table: – Work plan

First 6 months:	•Standardization of all procedures, commencement of patient enrolment(20%) collection of samples
Second 6 months:	•Further enrolment of patients(40%) and analysis of samples
Third 6 months:	•Further enrolment of patients (40%) and analysis of samples
Fourth 6 months	•statistical analysis, report writing and publication

Budget

Item	**1st Year (INR)**	**2nd Year (INR)**	**Total (INR)**
Consumables for mRNA Analysis: 2.5ml PAXgene blood RNA tubes, PAX gene RNA kit, High Capacity cDNA Reverse Transcriptase Kit, Gene specific oligonucleotide primer, SYBR green probe master mix.	**150000**	**50000**	**200000**