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ASSOCIATION OF MICRO RNA 21 (MIRNA21) EXPRESSION AND METASTATIC BREAST CANCER: A STUDY PROTOCOL

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Abstract

Objective of the study is to compare the miRNA21 expression in plasma of metastatic and non-metastatic carcinoma of breast patients. We also aim to find out whether there is an association between metastasis and miRNA21 levels in plasma in breast cancer as well as to assess whether plasma miRNA21 can be a potential biomarker to predict metastasis in Ca breast.

Fifty metastatic breast cancer patients (stage IV), fifty Ca breast patients without metastasis (Stage 0 to IIIC) and fifty healthy, age matched women will be included in the study.Their demographic profile, stage of cancer,dose and treatment history will be recorded. Five ml of venous blood will be collected, RNA will be isolated, cDNA will be synthesized by reverse transcriptase kit and miRNA quantification will be done by using real time PCR.Using suitable statistical tests, association between miRNA and metastasis will be analyzed.

The study may be useful if miRNA 21 can be used as a potential biomarker to predict metastasis in carcinoma of breast.Early prediction may be useful in personalizing the therapy ,hence may improve patient outcome as well as quality of life in breast cancer patients

Key words: *miRNA, metastasis, breast cancer*

Introduction

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 centers 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]. miRNA-21 is upregulated and promotes metastasis in several cancers [11,12]. A Chinese study by Kunita et al proved that plasma levels of miRNA-21 were up-regulated in large B-cell lymphoma patients [13]. The epithelial–mesenchymal transition (EMT) is a process that epithelial cells lose their cell polarity and cell adhesion ability, which will lead to cancer metastasis [14,15].

Although miRNA-21 is indicated to play a crucial role in the metastasis of lung cancer, ovarian cancer and head and neck cancer though several signalling pathways, the molecular mechanism of how miRNA-21 regulates the epithelial–mesenchymal transition process in breast cancer is not clear [16,17].

The study aimed to explore the association of miRNA-21 expression with the metastatic breast cancer.

Breast cancer is the most common cancer type in female, and many patients are suffered from recurrences and metastasis. MicroRNAs (miRNAs) are non-coding, single-stranded RNA molecules that regulate target gene expression via posttranscriptional processing.

Recently, several studies indicated the promising role of miRNA in the diagnosis and outcome prediction in several cancers [18-24]. Studies suggest that miR-21 is up regulated and promotes metastasis in several cancers[25-32]. Study by Wang et al also proved that plasma levels of miR-21 were up regulated in large B-cell lymphoma patients in China [33]. The epithelial–mesenchymal transition (EMT) is a process that epithelial cells lose their cell polarity and cell adhesion ability, which will lead to cancer metastasis [34,35]. Epithelial cells exhibit the property of regular cell-cell contacts,adhesion to the surrounding cellular fabric ,preventing the detachment of individual cells.Whereas mesenchymal cells donot form intercellular contacts.

Although miR-21 was indicated to play a crucial role in the metastasis of lung cancer, ovarian cancer and head and neck cancer though several signaling pathways, the molecular mechanism of how miR-21 regulates the EMT process in breast cancer is not clear [24-31].

Novelty/Innovation:

There is no established biomarker for the prediction of metastasis in carcinoma of breast so far.If miRNA21 emerges to be a potential biomarker for predicting metastasis, early prediction of metastasis 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.

Objectives of the Study:

i) to compare miRNA21 expression in metastatic breast cancer patients and non metastatic breast cancer patients

ii) find the association between miRNA21 levels and metastasis of malignancy of breast

Methods

i.Study design: Prospective cross sectional

ii.Sample size:

Sample size = $4pq/d^2$

Considering the incidence of metastatic breast cancer to be 5%,

Sample size = $\frac{4x5x95}{5^2}$

$$= \frac{4x5x95}{25} = 76$$

p = prevalence rate

q= 100-p

d =Precision rate

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: Fifty metastatic breast cancer patients (stage IV)

Group II: Fifty Ca breast cancer patients without metastasis (Stage 0 to IIIC)

Group III: Fifty healthy, age matched women

Exclusion Criteria: Patient having any one of the following criteria will be excluded:
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 parents/care takers and assent will be taken from children

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)

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 (PreAnalytiX, Hombrechtikon, Switzerlan) and will be stored at –80°C until RNA isolation. RNA will be isolated from blood, using the PAXgene system and following the manufacturer's instructions. Briefly, PAXgene Blood RNA tubes will be centrifuged, and the pellets will be washed and resuspended in buffer. Using the PAXgene Blood RNA Kit (Qiagen, Valencia, California, USA), 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 (Thermo Scientific, United States). 1 ug of RNA will be converted into cDNA by using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermofischer scientific, USA). The gene-specific suitable oligonucleotide primers (Integrated DNA Technologies) 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 tumour, lymph node involvement and distant metastasis will be assessed and their association with miRNA expression will be evaluated.

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 analysed using statistical package for social sciences (SPSS), version 19.0 software. Chi-square test will be used to find the association of miRNA expression and metastatic breast cancer.Kruskal Wallis test will be used to compare miRNA levels of different groups.

Significance of proposed study

miR-21 expression could be a promising biomarker in the diagnosis and outcome prediction of breast cancer.

Conclusion and Expected Outcome

- This study may reveal the miRNA expression pattern prevailing in Coastal Karnataka and North Kerala region.
- This study might reveal that miRNA 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 triple negative Ca breast cases

References

1. Malvia S, Bagadi SA, Dubey US, et al. Epidemiology of breast cancer in Indian women. Asia Pac J Clin Oncol. 2017;13:289–295.

2. Agarwal G, Ramakant P. Breast cancer care in India: The current scenario and the challenges for the future. Breast Care (Basel) 2008;3:21–27.

3. Gogia A, Raina V, Deo SVS, et al. Triple-negative breast cancer: An institutional analysis. Indian J Cancer. 2014;51:163–166.

4. Gennari A, Conte P, Rosso R, et al. Survival of metastatic breast carcinoma patients over a 20-year period: A retrospective analysis based on individual patient data from six consecutive studies. Cancer. 2005;104:1742–1750.

5. Dafni U, Grimani I, Xyrafas A, et al. Fifteen-year trends in metastatic breast cancer survival in Greece. Breast Cancer Res Treat. 2010;119:621–631.

6. Chopra R. The Indian scene. J Clin Oncol. 2001;19(18):106S–111S.

7. Mohr AM, Mott JL. Overview of microRNA biology. Semin Liver Dis. 2015;35(1):3–11.

8. Lai EC. Micro RNAs are complementary to 3′ UTR sequence motifs that mediate negative post-transcriptional regulation. Nat Genet. 2002;30(4):363–4.

9. Mirzaei H, Masoudifar A, Sahebkar A, Zare N, Sadri Nahand J, Rashidi B, et al. MicroRNA: A novel target of curcumin in cancer therapy. J Cell Physiol.2018;233(4):3004–15.

10. Liu SY, Li XY, Chen WQ, Hu H, Luo B, Shi YX, et al. Demethylation of the MIR145 promoter suppresses migration and invasion in breast cancer.Oncotarget. 2017;8(37):61731–41.

11. Kunita A, Morita S, Irisa TU, Goto A, Niki T, Takai D, et al. MicroRNA-21 in cancer-associated fibroblasts supports lung adenocarcinoma progression. Sci Rep. 2018;8(1):8838.

12. Liu Z, Jin ZY, Liu CH, Xie F, Lin XS, Huang Q. MicroRNA-21 regulates biological behavior by inducing EMT in human cholangiocarcinoma. Int J Clin Exp Pathol. 2015;8(5):4684–94.

13. Chen WQ, Wang H, Chen H, Liu S, Lu H, Kong D, et al. Clinical significance and detection of microRNA-21 in serum of patients with diffuse large B-cell lymphoma in Chinese population. Eur J Haematol. 2014;92(5):407–12.

14. Brabletz T, Kalluri R, Nieto MA, Weinberg RA. EMT in cancer. Nat Rev Cancer. 2018;18(2):128–34.

15. Ye X, Brabletz T, Kang Y, Longmore GD, Nieto MA, Stanger BZ, et al. Upholding a role for EMT in breast cancer metastasis. Nature. 2017;547(7661):E1–3.

16. Su C, Cheng X, Li Y, Han Y, Song X, Yu D, et al. MiR-21 improves invasion and migration of drug-resistant lung adenocarcinoma cancer cell and transformation of EMT through targeting HBP1. Cancer Med. 2018;7(6):2485–503.

17. Liu CH, Huang Q, Jin ZY, Zhu CL, Liu Z, Wang C. miR-21 and KLF4 jointly augment epithelial-mesenchymal transition via the Akt/ERK1/2 pathway. Int J Oncol. 2017;50(4):1109–15.

18. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov. 2017;16(3):203–22.

19. Mirzaei H, Masoudifar A, Sahebkar A, Zare N, Sadri Nahand J, Rashidi B, et al. MicroRNA: A novel target of curcumin in cancer therapy. J Cell Physiol. 2018;233(4):3004–15.

20. Sethi S, Sethi S, Bluth MH. Clinical Implication of MicroRNAs in Molecular Pathology: An Update for 2018. Clin Lab Med. 2018;38(2):237–51.

21. Lee SH, Jung YD, Choi YS, Lee YM. Targeting of RUNX3 by miR-130a and miR-495 cooperatively increases cell proliferation and tumor angiogenesis in gastric cancer cells. Oncotarget. 2015;6(32):33269–78.

22. Yu L, Zhou L, Cheng Y, Sun L, Fan J, Liang J, et al. MicroRNA-543 acts as an oncogene by targeting PAQR3 in hepatocellular carcinoma. Am J Cancer Res. 2014;4(6):897–906.

23. Li PL, Zhang X, Wang LL, Du LT, Yang YM, Li J, et al. MicroRNA-218 is a prognostic indicator in colorectal cancer and enhances 5-fluorouracil induced apoptosis by targeting BIRC5. Carcinogenesis. 2015;36(12):1484–93.

24. Liu SY, Li XY, Chen WQ, Hu H, Luo B, Shi YX, et al. Demethylation of the MIR145 promoter suppresses migration and invasion in breast cancer. Oncotarget. 2017;8(37):61731–41.

25. Kunita A, Morita S, Irisa TU, Goto A, Niki T, Takai D, et al. MicroRNA-21 in cancer-associated fibroblasts supports lung adenocarcinoma progression. Sci Rep. 2018;8(1):8838.

26. Lopez-Santillan M, Larrabeiti-Etxebarria A, Arzuaga-Mendez J, Lopez-Lopez E, Garcia-Orad A. Circulating miRNAs as biomarkers in diffuse large B-cell lymphoma: a systematic review. Oncotarget. 2018;9(32):22850–61.

27. Niu J, Shi Y, Tan G, Yang CH, Fan M, Pfeffer LM, et al. DNA damage induces NF-kB-dependent microRNA-21 up-regulation and promotes breast cancer cell invasion. J Biol Chem. 2012;287(26):21783–95.

28. Panagal M, SK SR, P S, M B, M K, Gopinathe V, et al. MicroRNA21 and the various types of myeloid leukemia. Cancer Gene Therapy. 2018;25(78):161–6.

29. Zhou B, Wang D, Sun G, Mei F, Cui Y, Xu H. Effect of miR-21 on Apoptosis in Lung Cancer Cell Through Inhibiting the PI3K/ Akt/NF-κB Signaling Pathway in Vitro and in Vivo. Cell Physiol Biochem. 2018;46(3):999–1008.

30. Liu Z, Jin ZY, Liu CH, Xie F, Lin XS, Huang Q. MicroRNA-21 regulates biological behavior by inducing EMT in human cholangio carcinoma. Int J Clin Exp Pathol. 2015;8(5):4684–94.

31. Yan LX, Huang XF, Shao Q, Huang MY, Deng L, Wu QL, et al. MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. RNA. 2008;14(11):2348–60.

32. Liu ZL, Wang H, Liu J, Wang ZX. MicroRNA-21 (miR-21) expression promotes growth, metastasis, and chemo-or radio resistance in non-small cell lung cancer cells by targeting PTEN. Mol Cell Biochem. 2012;372(1–2):35–45.

33. Chen WQ, Wang H, Chen H, Liu S, Lu H, Kong D, et al. Clinical significance and detection of

microRNA-21 in serum of patients with diffuse large B-cell lymphoma in Chinese population. Eur J Haematol. 2014;92(5):407–12.

34. Brabletz T, Kalluri R, Nieto MA, Weinberg RA. EMT in cancer. Nat Rev Cancer. 2018;18(2):128–34.

35. Ye X, Brabletz T, Kang Y, Longmore GD, Nieto MA, Stanger BZ, et al. Upholding a role for EMT in breast cancer metastasis. Nature. 2017;547(7661):E1–3.