MiR-574-5p dysregulation in metastatic breast cancer through cell migration

Ozge Doğan, Şermin Linton, Ceren Öztürk, Oruç Murat [1*]

Abstract

Breast cancer is the most frequently diagnosed malignancy among women worldwide. The occurrence rate of metastasis is also increased. It's reported that 20–30% of breast cancer patients may develop metastases after diagnosis and primary tumor treatment, and approximately 90% of cancer-related deaths are attributed to metastasis. Recently, a growing number of studies have highlighted the significant role of miR-574-5p, a member of the miRNA family, in multiple human diseases that induce apoptosis in cancer cell lines completely understood yet. the majority of studies indicated that miR-574-5p was a promoter of NSCLC development. indicated that miR-574-5p was involved in the progression and metastasis of NSCLC. The purpose of this study is to investigate the effect of miR-574-5p migration in breast cancer. Seventy tumor and adjacent non-tumor tissues were examined in the study. The effects on cell proliferation and migration were investigated with MTT assay and scratch test, respectively.

The effects on cell proliferation and migration were investigated with MTT assay and scratch test, respectively. Bioinformatics analysis was performed through enrichment and hub gene finding for miRNA targets. Metastatic breast cancer, proliferation, and migration-related to overexpression of miR-574-5p. In conclusion; miR-574-5p increased in breast cancer cell lines with a role in the growth, metastasis, and migration of breast cancer.

Keywords: miR-574-5p; Breast cancer; Proliferation

Introduction

Breast cancer is the most frequently diagnosed malignancy among women worldwide with greater than 268,000 new diagnoses expected per year in the USA, representing approximately 30% of all new cancer cases in women. It is the second cause of cancer-related death in women and responsible for more than 41,000 deaths per year in the USA, accounting for an estimated 15% of all cancer-related deaths in women [1]. Due to advances in early diagnosis and comprehensive treatment strategies, the prognosis of breast cancer patients has improved. The occurrence rate of metastasis is also increased. It's reported that 20–30% of breast cancer patients may develop metastases after diagnosis and primary tumor treatment, and approximately 90% of cancer-related deaths are attributed

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to metastasis [2]. The 5-year overall survival rate of breast cancer patients without metastasis is greater than 80%; however, distant metastasis can lead to a dramatic reduction of this rate to only approximately 25%. Breast cancer exhibits a metastatic propensity to distinct organs, including bone, lung, liver, and brain, which is termed metastatic heterogeneity and leads to varied responses to treatment and patient prognosis. Bone metastasis accounts for approximately 75% of metastatic cases [3], and the 5-year overall survival rate is 22.8% [4]. The most widely accepted model of metastasis is the "seed and soil" hypothesis, which was put forward by Stephen Paget. It preliminarily revealed that a successful second organ-colonization required the intrinsic properties of tumor cells and a compatible and supportive microenvironment [5]. However, the answer to the question of when and how metastases spread is diverse, and two classical models of tumor metastasis are widely acknowledged. In the linear progression model, heterogeneous clones within the primary tumor undergo a successive mutation and selection, and a subset of clones accumulates mutational changes that are necessary for survival and growth in target organs [6]. In contrast, the parallel progression model postulates that tumor cells acquire metastatic potential very early, even when the primary lesion is small or undetectable, and different tumor clones can be seeded in parallel to multiple secondary sites [7].

Overall, these two models indicated a similar but not identical perspective that genetic and non-genetic alterations as well as selection pressures from the microenvironment give rise to heterogeneous cellular populations and metastatic potential. MicroRNAs (miRNAs) are a group of small, single-stranded, non-coding RNAs approximately 22 nucleotides in length [8]. Since first discovered in the nematode C. elegans. in 1993 [9], thousands of miRNA genes have been annotated in diverse species, including humans. Either residing in the intergenic or intragenic regions of the genome, miRNAs account for approximately 1-5% of the human genome and regulate over 30% of protein-coding, survival-related genes based on predictions [10]. In recent years, a large body of literature has accrued regarding their critical role in post-transcriptionally regulating gene expression, mainly acting through binding to the 3'-untranslated regions (UTRs) of messenger RNAs (mRNAs). According to the different degree and nature of complementarity between miRNA sequences and the target mRNA 3'UTRs, the latter translation could be completely degraded or partially inhibited, resulting in delicate modulations of a variety of fundamental cellular and physiological activities, such as cell proliferation, embryogenesis, and development [11]. With advances in experimental and computational methodologies in the miRNA research field, the dysregulation of miRNAs has been widely investigated in various pathological processes, including tumorigenesis, rendering them a promising diagnostic and therapeutical target in the future [12]. Recently, a growing number of studies have highlighted the significant role of miR-574-5p, a member of the miRNA family, in multiple human diseases. Human miR-574-5p (has-miR-574-5p) is encoded by mir574, which is located on the human chromosome 4p14 and is generated in a multi-step process. Transcription begins with RNA polymerase II, which produces an initial long transcription chain called pri-miRNA. Unlike the canonical miR biogenesis pathway, pri-miRNAs can be spliced and debranched into pre-miRNA hairpins that are suitable for Dicer cleavage, thus bypassing the microprocessor [13]. Upon being exported to the

cytoplasm by exportin-5 (XPO5), pre-miRNAs are cleaved by two RNases and turned into an RNA duplex of approximately 22 nucleotides [14]. In the case of miR-574-5p, the strand originating from the 5' side of the pre-miRNA (thus annotated with the suffix -5p) is selectively loaded on the argonaute protein (AGO) to form the miRNA-induced silencing complex (RISC) [15]. Unlike the usually degraded undominated strand, the 3' strand of miR-574 still gives rise to the functional miR-574-3p counterpart, and the 5p/3p ratio reportedly contributes to some pathologies [16].

Statistical analysis

In this cross-sectional, descriptive study, the mean age and incidence of cytogenetic abnormalities, using the SPSS software package (version 18). Moreover, we performed comparisons in terms of cytogenetic subclasses and age groups using the Pearson chi-square test with MED CALC software.

Materials and Methods

Seventy tumor and adjacent non-tumor tissues were examined in the study. miR-574-5p levels were evaluated by qPCR in breast cancer cell lines and tumor tissues. Pre- miR-574-5p was cloned in the pEGFPN1 vector. Next, human breast cancer MCF7 cells were cultured, and pre- miR-574-5p vector was transfected to the breast cancer line. The effects on cell proliferation and migration were investigated with MTT assay and scratch test, respectively. Bioinformatics analysis was performed through enrichment and hub gene finding for miRNA targets.

pre- Mir Assimilation-574-5p

To create the vector structure - miR-574-5p fragment containing miRNA precursor sequence by primersF: TCGAAAGCTTAGGGTGCAGCAGGTCAGC R:TCGAGGTACCGGGGTGGGGAGTCAGG and GA was amplified by PCR method. In the next step, the vector pEGFP using the plasmid extraction kit-N1 (Gene Favor-Taiwan) was purified. Vector and cut pre in separate reactions by–Mir Reproduced 574-5p Cutting enzymes KpnI and HindIII were cut to Prepare for the next step, which is connecting the part to the vector. At this stage, using 4T ligase enzyme, for 3 hours at 22°C, the binding reaction was carried out. Then the experiments with the transformation of the Neuter cube vector are created 5DH continued. After time to cells prone to α, about 18 hours on the colonies grown in the plate Agar, the PCR colony process was performed. Cloning PCR using direct CMV and reverse GFPN primers was taken to determine the colonies containing the recombinant plasmid. After that, for the final confirmation of the determination of assimilation The recombinant vector was sequenced. including miRDIP, miRMap, TargetScane miRTarbase, PicTar, MirWalk, Miranda miRDB, RNAHybrid, MicroT4, RNA22 were miRBridge and miRTar2GO, PITA. The listed databases are based on different algorithms There are various influencing factors such as: the type of positions available in mRNA, the number of sites, the distance between sites, Game composition in UTR',3 percent AU, length of UTR',3 Number of desired miRNA targets in other mRNAs and as a result of its availability, duplex energy 9 and target genes of different miRNAs 10 binding energy they predict and score.

Results

Investigation of miRNA target genes to examine miRNA target genes from 14 databases used

Genes carried by at least 70% of databases were validated as miRNA targets were chosen. Databases used in this step. Examining expression in examples tumor grade 3 tumor compared to non-tumor samples also increased miRNA expression showed significant (To evaluate specificity and p >0.007 (miR to distinguish between expression sensitivity - miR-574-5p tumor and non-tumor samples, operating curve analysis (ROC) was drawn. The area under the curve (AUC) 12 receiver p>0.0005 miR is equal to 66 percent) for miR-574-5p is and as a result, the possibility that miRNA can
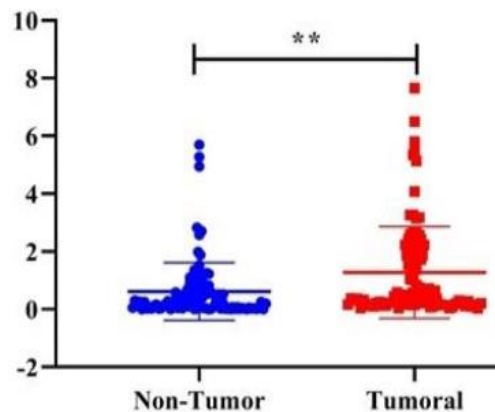

Figure 1.

The graph of expression level miR-574-5p adjacent healthy tissue compared to breast cancer cell lines.
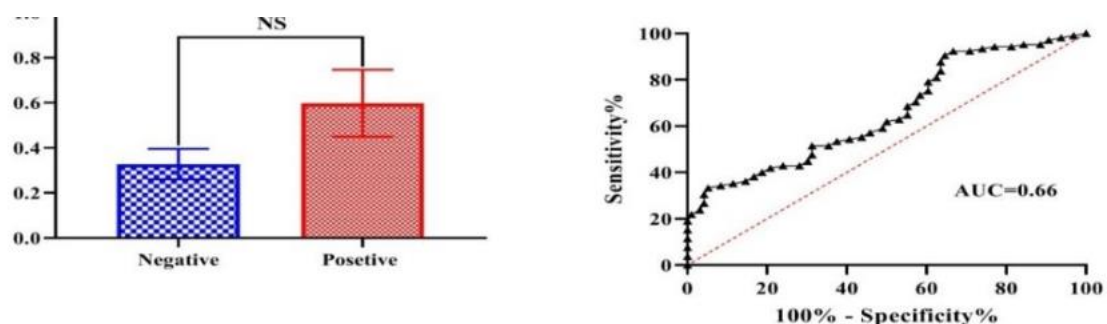

Figure 2.
ROC curve displays the area under the curve of 66%. Comparative expression of miR -574-5p in negative and positive metastasis samples.

According to the analyzes performed, the expression of miR-574-5p increased It has been shown in metastasis samples compared to non-metastasis but this increase in expression was not statistically significant. The correlation of gene expression level p =0.11, tumor grades 1 to 3 were also measured.

Tumor In this study, the rate of increase in grade 3. In investigating the relationship between gene expression and)p =0.05 was observed) Tumor size, considering that the average size of tumors in / 4 cm was calculated, the samples were divided into two groups About 1 cm and larger and tumor sizes smaller than 4 They were divided equally to 4 cm. Comparison of gene expression in each (and a significant difference in – d Two groups were done (Figure 2). miR observed in these two groups Expression level miR-574-5p.) p < 0.05 didn't work) miR to cell line, first expression in the study of induction miR-574-5p mature miRNA in structures designed by the method.

Discussion

Despite available therapies for metastatic breast cancer, such as cytotoxic chemotherapies, endocrine therapies, and targeted therapies, the 5-year overall survival rate of metastatic breast cancer remains less than 30% [17]. Moreover, the extent of response and potential resistance to treatments varies among different metastatic types and patients. Therefore, more effective and targeted treatments that build on the molecular mechanism of metastatic breast cancer are needed. Regarding treatment targeting bone metastasis of breast cancer, the above-mentioned molecules have promising clinical applications. Denosumab, a monoclonal antibody against RANKL, is one of the most effective approaches in the prevention of skeletal-related events [18]. In addition, the small-molecule RANKL inhibitor AS2676293 markedly inhibits bone metastasis of breast cancer cells and tumor-induced osteolysis [18], indicating its potential for clinical application. Moreover, using independent clinical settings, the expression of Jagged1 is associated with more aggressive breast carcinoma and reduced disease-free survival (DFS) and metastasis-free survival, and the presence of Jagged1 in circulating tumor cells (CTCs) is strongly related to reduced progression-free survival in patients treated with bisphosphonates [19]. Dysregulated miR-574-5p has been found in all pathological processes of tumorigenesis, including cancer cell proliferation, migration, invasion, metastasis, apoptosis, epithelial-mesenchymal transition, and angiogenesis [20]. However, numerous studies have found that the role of miR-574-5p is significant but also controversial, acting as both a tumor suppressor and oncogene according to the tumor type and associated pathways. In recent years, the protective role of miR-574-5p in breast cancer, especially TNBC, has been gradually brought to light [21]. A study by other researchers reported that miR-574-5p could repress TNBC cell proliferation, migration, and epithelial-mesenchymal transition (EMT) in vitro while reducing tumor growth and metastasis in vivo [22]. It was demonstrated that miR-574-5p could simultaneously target SRY (sex-determining region Y)-box 2 (SOX2), B-cell lymphoma/leukemia 11A (BCL11A), and thus inhibit the SKIL/transcriptional co-activator with PDZ-binding motif (TAZ)/connective tissue growth factor (CTGF) axis to regulate malignant phenotypes in TNBC [23].

In examining the expression of miRNAs, genetic background is an important factor. Analysis of the importance of differential expression [24]. However, factors such as the presence of an independent promoter and different stability of Different miRNAs lead to differences in expression levels of miRNA and host gene [25]. Many studies indicate that the co-expression of miRNA and host gene with promoter is common In studies on the effect of the host gene and On the expression and evolution of

miRNAs in 15 genomic contexts It was found that the chromatin status of the host gene and Gene containing region, on the time and place of expression Intronic miRNAs act [26]. Limitations of the study include things like Even the problem of transfecting some breast cancer cells Through the lipofectamine method, it was pointed out that miR on different categories with features -6165 be checked differently. Also, tissue samples of the case The investigation was of the paraffin type, from which RNA was extracted The tissues are very difficult and the amount is less than the tissues it is fresh [27].

As TNBC tends to metastasize distantly to specific organs, such as the lungs, a high level of miR-574-5p inhibited the migration and invasion abilities in lung-metastatic TNBC cells, whereas its knockdown resulted in enhanced metastasis performance [28]. Further experiments demonstrated that miR-574-5p was the shared miRNA for the upstream linc-ZNF469-3 and downstream target ZEB1, highlighting the role of linc-ZNF469-3/miR-574-5p/ZEB1 in TNBC lung metastasis.

Conclusion

miR-574-5p increased in breast cancer cell lines with a role in the growth, metastasis, and migration of breast cancer.

Conflict of Interest

No conflicts of interest were declared by the authors.

Financial Disclosure

The authors declared that this study has received no financial support.

Ethics Statement

Not applicable.

Authors' contributions

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.

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