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EXPLORING MITOMIRS IN BREAST CANCER: AN *IN-VITRO* STUDY OF THEIR EMERGING ROLES

MEME KANSERİNDE MİTOMİR'LERİN TANIMLANMASI: *İN VİTRO* ÇALIŞMA İLE ROLLERİNİN GÖSTERİLMESİ

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ABSTRACT

Objective: Breast cancer is associated with a 5% genetic predisposition in women. Mitochondria play a role in important cellular events such as metabolism, cell death, and inflammation. Recent studies have highlighted precursor micro-RNA (pre-miRNA) to be located in mitochondria as well as mature miRNA. This study aims to reveal the occurrence of mitochondrial miRNA (mitomiR) in breast cancer cells.

Materials and Methods: The study has prepared the mitochondrial fractions using the magnetic-activated cell sorting (MACS) method and performed small RNA (sRNA) sequencing.

Results: The study has identified known and novel mitomiR sequences aligned to the mitochondrial genome.

Conclusion: Identifying new mitomiRs can provide significant contributions and illuminate the molecular mechanism underlying mitomiR biogenesis.

Keywords: mitomiRs, MACS, breast cancer, small RNA sequencing

ÖZET

Amaç: Kadınlarda meme kanseri %5 oranında genetik yatkınlıkla ilişkilidir. Mitokondri, metabolizma, hücre ölümü ve inflamasyon gibi önemli hücresel olaylarda rol oynar. Son çalışmalar, olgun miRNA'ların yanı sıra pre-miRNA'ların mitokondride bulunduğunu göstermişlerdir. Bu çalışmada meme kanseri hücrelerinde mitomiR'lerin varlığını ortaya koymayı amaçladık.

Gereç ve Yöntem: MACS yöntemi kullanılarak mitokondriyal fraksiyonlar hazırlandı ve küçük RNA dizilimi yapıldı.

Bulgular: Mitokondriyal genoma hizalanmış bilinen ve yeni mitomiR dizilerini belirledik.

Sonuç: Yeni mitomiR'lerin tanımlanması literatüre önemli katkılar sağlayabilir ve mitomiR biyogenezi moleküler mekanizmalar ile aydınlatılabilir.

Anahtar Kelimeler: mitomiR, MACS, meme kanseri, küçük RNA dizileme

INTRODUCTION

Breast cancer ranks first among cancer-related deaths in women and is associated with 5% genetic predisposition among women, with an autosomal dominant inheritance pattern having been observed (1). BRCA1 and BRCA2 gene mutations have also been associated with a very high risk of breast and ovarian cancer, with 65% -85% of women who carry these mutations bearing a lifelong risk for developing invasive breast cancer and a 15%-65% risk of contracting invasive ovarian cancer (1, 2).

Mitochondria are double-membrane organelles approximately 0.5–1 μm wide and 7 μm long. In addition to ATP production, these highly dynamic organelles play an essential role in regulation many physiological processes including metabolism, apoptosis, disease, and aging. While mitochondrial function is a key to cell survival and death, mitochondrial metabolism deregulation is also critical in the pathogenesis of many cancers (3, 4) and is also central to oxidative phosphorylation, with 95% of cellular energy being provided by oxidative phosphory-

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lation in mitochondria. This process includes the five different protein complexes, including complexes I-V. The electron transport chain (ETC) in the inner membrane is associated with coenzyme Q and cytochrome c electron transfer components. Beta-oxidation of fatty acids and Pyruvate oxidation pathways occur in the tricarboxylic acid cycle (TCA) of the mitochondrial matrix. A total of 91 polypeptides, including cytochrome c, are directly associated with oxidative phosphorylation (OXPHOS). Some of these are encoded as nuclear proteins and others as mitochondrial proteins (5).

Depending on the restricted coding capacity of mitochondrial DNA (mtDNA), nuclear genes are required for biological functions and structural components. In addition, nuclear-encoded genes regulate mtDNA replication, transcription, and translation. For this reason, the collaboration of nuclear genes and mtDNA is necessary for regulating OXPHOS capacity in response to several physiological and disease states (6, 7). Mammalian cells contain more than 1,000 mitochondria and approximately 10,000 copies of mtDNA. The mitochondrial genome is 16.6 kb in size, contains no introns, has a circular double-stranded structure, and contains a total of 37 genes, 2 rRNA, 22 tRNA, and 13 polypeptides coding for the ETC and non-coding RNAs. Although the mitochondria genome has only 13 protein-coding genes, it contains around 1,500 proteins. Replication and transcription of mtDNA begin in the mitochondrial D-loop, which is known as the small non-coding region. All other proteins associated with replication, transcription, and translation are encoded by nuclear genes and imported to mitochondria through special transport systems (4, 8-11).

Although mitochondrial RNA (mtRNA) are transcribed from both strands such as polycistronic precursor transcripts, the process leads to the release of non-coding RNA and coding RNA containing tRNA, rRNA, and mRNA (12). Similarly, RNA transport is very important for mitochondrial function, although the relevant mechanisms have yet to be elucidated.

Non-coding RNA (ncRNA) have many functions, from catalyzing biological reactions, cellular defense, and developmental processes to cellular response. In addition, ncRNA regulate transcriptional and post-transcriptional gene silencing and chromosome remodeling. Many non-coding RNA in mitochondria can also be encoded by mtDNA (13). However, many types of RNA are known to enter and exit mitochondria. The most common RNA in mitochondria (e.g., tRNAs, 5S rRNA, RNAse MRP and RNAse P) are nuclear-encoded and transferred to the mitochondria (14, 15). Mitochondria also contain many non-coding RNA, such as miRNA, snRNA, Piwi-interacting RNA (piRNA), signal recognition particle RNA (srpRNA), and small nucleolar RNA (snoRNA) (12, 16). Unlike nuclear-encoded miRNA, mitochondrial miRNA (mitomiR) biogenesis is not yet fully known, but several hypotheses are found in this regard. The miRNA encoded by the mitochondrial genome are assumed to act in three different ways: 1) Nuclear genome-encoded miRNA suppress mRNA translation in the cytosol by targeting nuclear-encoded mitochondrial proteins, thus affecting the transport of specific mitochondrial proteins to the mitochondria; 2) nuclear-encoded miRNA regulate the translation of mitochondria-encoded proteins; 3) mitochondrial genome-encoded miRNA regulate the translation of mitochondrial genome-encoded proteins (17). Some pre-miRNA are processed in the mitochondria and can synthesize the mature miRNA that are simultaneously activated on mitochondrial transcripts or sent to the cytosol in order to combine with genomic mRNA (18). Bandiera et al. (19) discovered the presence of miRNA with different expression profiles in the nucleus and mitochondria called mitomiR and showed mitomiR to have both nuclear- and mitochondrial-encoded targets (19). The miRNA detected in mitochondria may vary depending on the cell type (16, 20). MitomiR differ from other miRNA in terms of their thermodynamic properties and dimensions and are expressed in the loci of almost all nuclear-encoded genes related to mitochondrial function. Compared to the miRNA found in the cytosol, mitomiR appear to be unable to preferentially target nuclear-encoded mitochondrial genes.

By considering one of the causes of cancer formation to be defects in mitochondria, this study aims to identify mitomiR that have different expressions in the mitochondria of breast cancer cell lines and to determine their target genes.

MATERIALS AND METHODS

Cell culture

The MCF-10A cells have been cultured in the DMEM/F-12 (Sigma, Germany) medium containing 1% penicillin-streptomycin, L-glutamine, 5% heat-inactivated Horse Serum (GIBCO, USA), 10 µg/ml insulin (GIBCO, America), 20 ng/ml epidermal growth factor (EGF; Miltenyi Biotec, Germany), and 0.5 µg/ml hydrocortisone (Sigma, Germany); the MDA-MB-231 cells have been cultured in the RPMI1640 Medium (GIBCO, USA) containing 5% heat-inactivated fetal bovine serum (FBS; GIBCO, USA), 1% penicillin-streptomycin (GIBCO, USA), and 1% L-glutamine (GIBCO, USA); and the MCF-7 cells have been cultured in the DMEM Medium (SIGMA, USA) containing 5% heat-inactivated FBS (GIBCO, USA), 1% penicillin-streptomycin (GIBCO, USA), and 1% L-glutamine (GIBCO, USA) incubated at 37°C at 5% CO2 and 95% humidity.

Isolating mitochondria from cells using MACS technology

A mitochondria isolation kit (Miltenyi Biotec, Germany) has been used to obtain a high yield with purity and integrity of mitochondria isolated from cells. In short, the cells were disrupted with the help of a Dounce homogenizer, treated with 9 mL of the 1X lysate separation buffer and labeled with 50 µl anti-TOM22 coated beads, incubated for 1 hr at 40°C using a rotator. The labeled lysate was then transferred to LS Columns (Miltenyi Biotec, Ger-

many). The mitochondria were eluted after being washed five times with 3 mL of the 1X lysate separation buffer.

The RNase A (10 mg/ml, ABM, Canada) treatment was performed to eliminate genomic contamination in the mitochondrial fractions. The pellet was treated with TRIZOL (MRC, USA) immediately after processing to inactivate the RNase A and isolate the mtRNA.

Small RNA (sRNA) sequencing

The sRNA sequencing was performed using the Illumina platform. In short, the purity and quality of the mtRNA were checked using the NanoDrop (Thermo, America) and Agilent 2100 Bioanalyzer (Agilent Technologies, America) devices. Libraries were prepared from the 50ng mtRNA samples using the SMARTer smRNA for Illumina Kit (CloneTech, America). First, the poly(A) tail was added to the input RNA using Poly(A) Polymerase in order to facilitate oligo(dT)-primed cDNA synthesis. Next, adapter-linked miRNA fragments were transformed into cDNA fragments followed by the PCR purification. The sizes of the amplified cDNA fragments were checked using a Bioanalyzer DNA High Sensitivity Chip. The cDNA fragments were sequenced according to the read length using the sequence by synthesis method on the Illumina platform.

Bioinformatic analysis

After sequencing, the raw sequence reads were filtered, then the adapter sequences were clipped away from the raw sequence readings using the program Cutadapt version 4.1. The clipped reads were then clustered, and these clusters contain reads that match 100% of the sequence and read length. Clustered reads were then aligned with the reference genome (hg19) and precursor miRNA from the miRBase (v21; https://www.mirbase.org/) to identify the defined miRNA. The miRDeep2 algorithm (https://github.com/rajewsky-lab/mirdeep2/releases/latest) was used to predict the potential hairpin structures of the miRNA. To classify other RNA types, clustered reads were mapped to the reference genome in miRBase (v21) and the non-coding RNA database Rfam (v9.1; https://rfam.org/). The number of reads for each miRNA was transferred from the mapped miRNA, and the distribution of each miRNA has been reported.

The web-based DIANA bioinformatics analysis program (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=mirpath/index) was used to analyze the functions of the miRNA, and the Gene Ontology (GO; http://geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway (https://www.kegg.jp/kegg/pathway.html) analyses were performed to determine the biological processes, molecular functions, and biochemical pathways pertaining to these miRNAs. The Search Tool for the Retrieval of Interacting Genes / Proteins (STRING) database (v10.5; https://string-db.org/) was used to analyze the proteins that interact with the miRNA and gene regions that show homology.

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RESULTS

Mitochondrial RNA (mtRNA) isolation and mitochondrial gene enrichment analysis

The study grew $5x10^7$ cells on T125 flasks to prepare the purified mitochondrial fractions from MCF-7, MDA-MB-231 and MCF-10A cells. A mitochondria isolation kit using MACS technology enabled the isolation of mitochondria and cytosol fractions from the same cells. Thus, the study evaluated the genomic contamination in the mitochondrial fraction at the RNA level by comparing it to the cytosol fractions. The 16S rRNA gene was used as a calibrator for calculating the mitochondrial/nuclear RNA ratio (figures 1a, 1b).

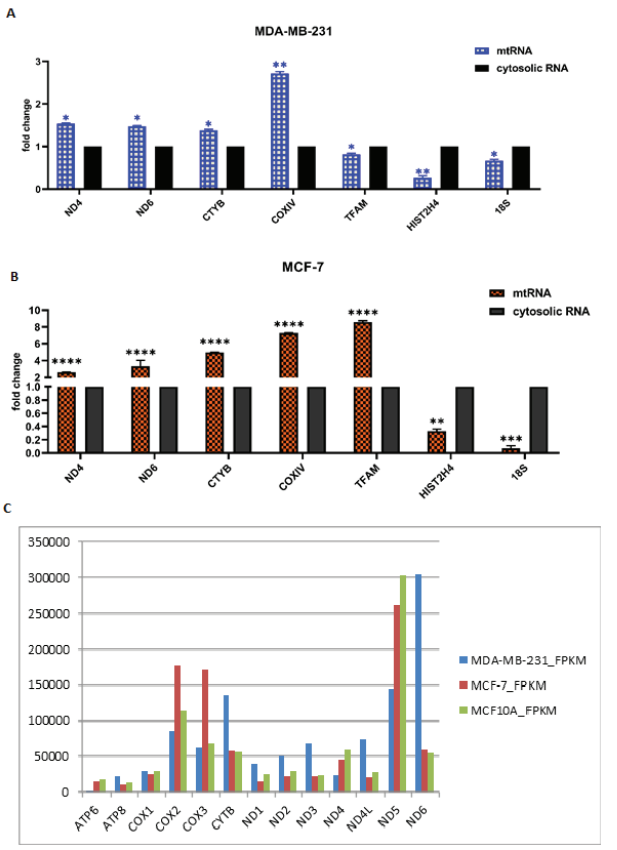

Figure 1: A, B) Mitochondrial genes were found to be significantly enriched in mitochondrial fractions compared to cytosolic fractions in both MCF-7 and MDA-MB-231 cells whereas HIST2H4, Nuclear gene histone cluster 2 H4 family, and 18S rRNA levels were vice versa. Two way ANOVA and Sidak's multiple comparisons test were used for statistical analysis. (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001) C) Fragments Per Kilobase of transcript per Million mapped reads (FPKM) of mitochondrial encoded genes as a result of small RNA sequencing of cells.

Enrichment of the mitochondrial genes in the mtRNA samples was also evaluated with the data obtained using the next-generation sequencing method (figure 1c). Thirteen mitochondria-encoded genes were evaluated and fragments per kilobase of exon per million mapped fragments (FPKM) were found to range between 320 and 303,493.

Mitochondria-associated sRNA library analysis

The study isolated the sRNA associated with mitochondria (18-30 nucleotides [nt]) and generated and sequenced libraries using the Illumina HT2500 platform. The peak seen around 175bp indicates the predicted size of the adapter-linked miRNA library, and libraries containing miRNA-derived sequences range in size from about 172-178 bp. The total base number, readings, GC percentage, Q20 (%) and Q30 (%) were calculated for each sample. Sequencing resulted in crude sequencing read lengths from the MDA-MB-231, MCF-7 and MCF-10A cells of 28,496,397, 32,712,495, and 35,790,420, respectively. The 3' adapter sequences of mature miRNAs with 24 bp length were removed using the program Cutadapt. By filtering sequences smaller than 15 nucleotides, the 24,781,073, 21,059,268, and 22,302,897 reads were then matched to the genome (hg19), respectively. The sRNA population including the miRNA, piRNA, and sRNA of respective lengths 21-22 nt, 30 nt, and 24 nt was categorized by performing a length analysis. The highest read rate in the samples was at 25 nt, which indicates the presence of miRNA, piRNA, and sRNA.

The clustered reads were mapped to the reference genome (hg19) and precursor miRNA separately in order to determine the defined miRNA. In addition, the Rfam database (v9.1) was used to identify the miRNA and other RNA species. The miRDeep2 score was chosen between±10. Figure 2 shows the small RNA class types in the samples.

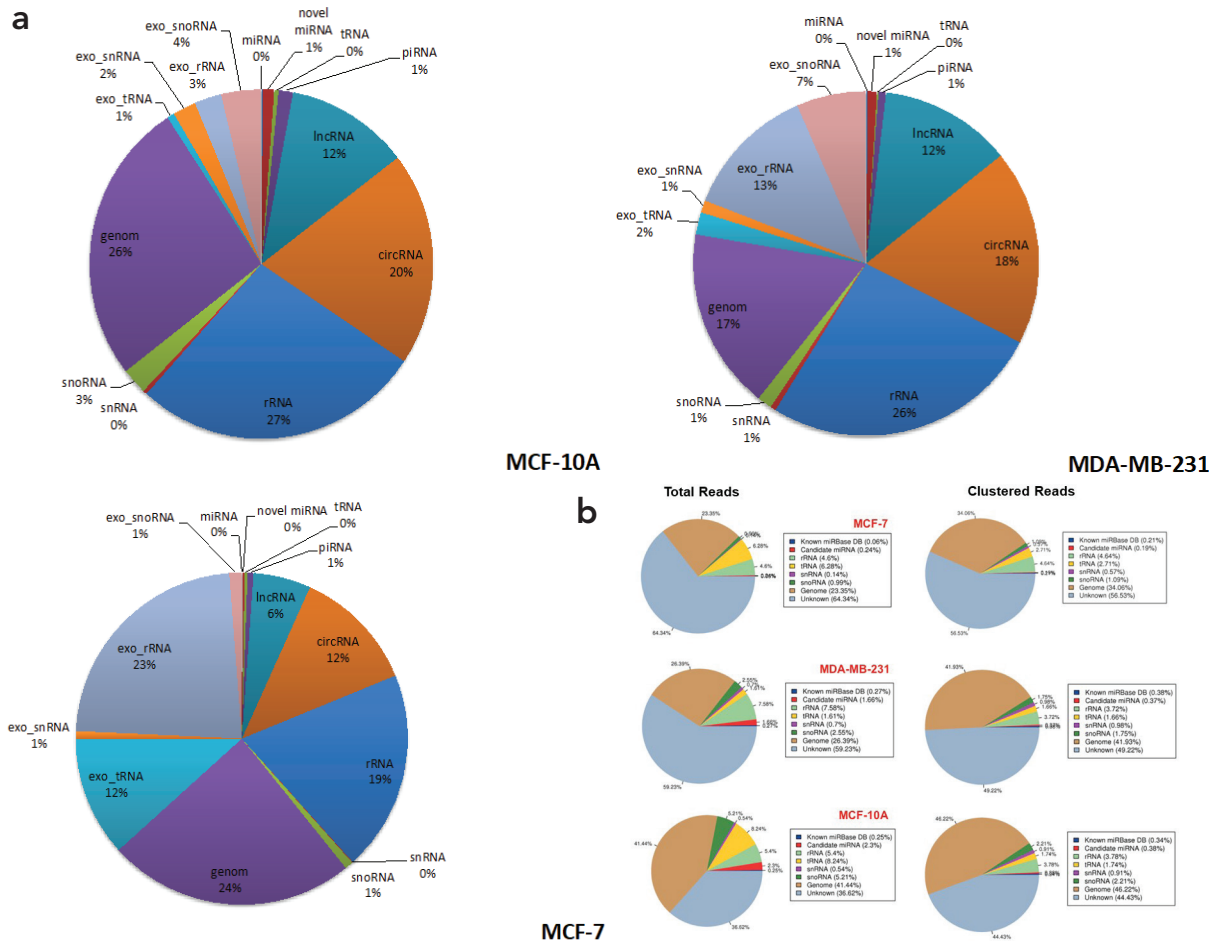

Figure 2: a) Pie charts corresponding to RNA contents in the cells. sRNA sequences are paired with RNAs from Genbank and Rfam databases in MCF-7, MDA-MB-231 and MCF-10A cells. b) Percentage of small RNA reads in the samples. smRNA class type (such as known miRBase, candidate miRNA, rRNA, tRNA, snRNA, snoRNA, Genome, Unknown) are shown for total reads (on the left) and clustered reads (on the right) respectively. Unique clustered reads are sequentially aligned to reference genome, miRBase v21 and run blast to non-coding RNA database, Rfam 9.1 to classify known miRNAs and other type of RNA such as tRNA, snRNA, snoRNA etc. in MCF-7, MDA-MB-231 and MCF-10A cells.

Identifying the mitochondria-associated miRNA

The numbers of miRNA frequencies in the mtRNA samples were determined to be between 1-12,384. According to the bioinformatic analysis results, 283 miRNAs in the MCF-10A cells, 234 miRNAs in the MCF-7 cells, and 220 miRNAs in the MDA-MB-231 cells were found to be compatible with the miRBase database (v21). The most frequently determined miRNA associated with mitochondria in the samples were hsa-miR-6087-5p, hsa-miR-3960-3p, hsa-miR-7641-5p, hsa-miR-3648-3p, hsa-miR-4488-5p, hsa- miR-4485-5p, hsa-miR-4449-3p, hsa-miR-4484, let-7 family members (let-7a, b, c, d, e, f, g, i), hsa-miR-1290 -3p, hsa-miR-423-5p, and hsa-miR-3687-3p. In addition, hsa-miR-1246-5p, hsa-miR-1275-5p, hsa-miR-663a-5p, miR-25-3p, miR-23a-3p, hsa- miR-423-5p, hsa-miR-320a-3p, hsa-miR-574-5p, and hsa-miR-7704-5p were also found to be associated with mitochondria (table 1).

In order to detect the presence of miRNA-targeting mitochondria-encoded genes among the miRNA, the precursor sequences were matched with the mitochondrial genome using the programs SerialCloner 2-6-1 (figure 3) and Mitowheel.

Table 1: Nuclear-encoded miRNAs detected in mitochondrial fractions of cells

miRBase ID	MCF-10A (number of reads)	MCF-7 (number of reads)	MDA-MB-231 (number of reads)
hsa-miR-6087-5p	11187	4175	1322
hsa-miR-3960-3p	5094	871	2438
hsa-miR-7641-5p	4851	232	408
hsa-miR-3648-3p	4620	1057	2405
hsa-miR-4449-3p	1709	234	154
let-7 family	1652	72	274
hsa-miR-1275-5p	86	7	25
hsa-miR-663a-5p	534	381	230
hsa-miR-423-5p	138	9	138
hsa-miR-320a-3p	138	62	76
hsa-miR-1296-3p	5	4	4
hsa-miR-574-5p	212	54	179
hsa-miR-221-3p	16	-	118
hsa-miR-3687-3p	636	202	256
hsa-miR-7704-5p	161	96	57
hsa-miR-664b-3p	102	37	56
hsa-miR-6724-5p	54	31	19
hsa-miR-193b-5p	48	9	5
hsa-miR-6126-5p	17	20	12

Figure 3: Matching defined miRNA (mitomiR) sequences with the mitochondrial genome using SerialCloner 2-6-1.

GO and KEGG pathway analysis of MitomiR and STRING protein-protein interaction analysis

Evaluations were made using Fisher's exact test analysis method in the DIANA database for the pathway analysis of the miRNA. The pathways in which the targets of the miRNA clusters are highly correlated were determined by considering the p-value. Figures 4 a and 4b summarize the gene-related pathways. This study performed

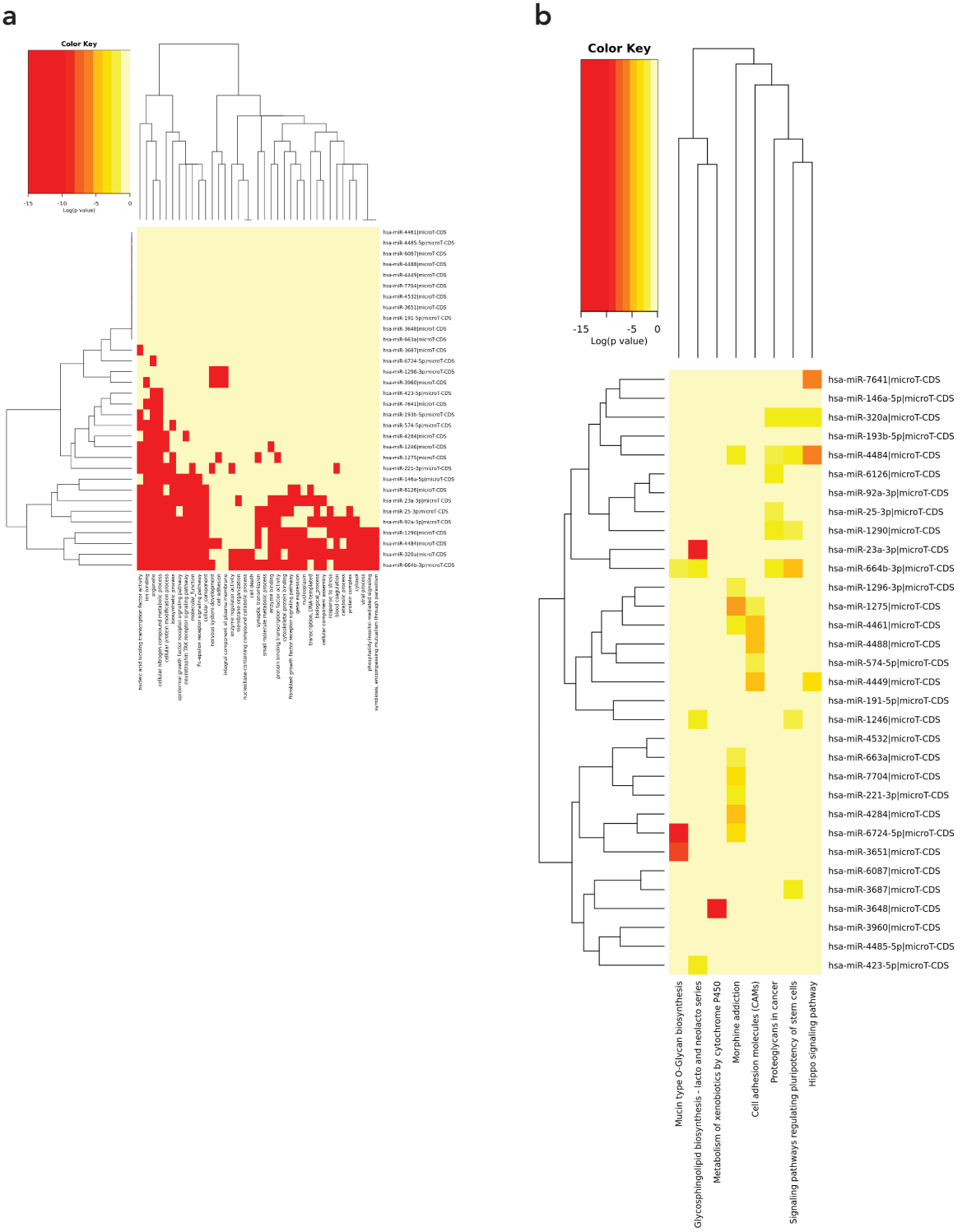

Figure 4: a) Heat-map analysis (DIANA database) of GO terms of the detected miRNAs b) Heat-map analysis (DIANA database) corresponding to KEGG pathways of the detected miRNAs

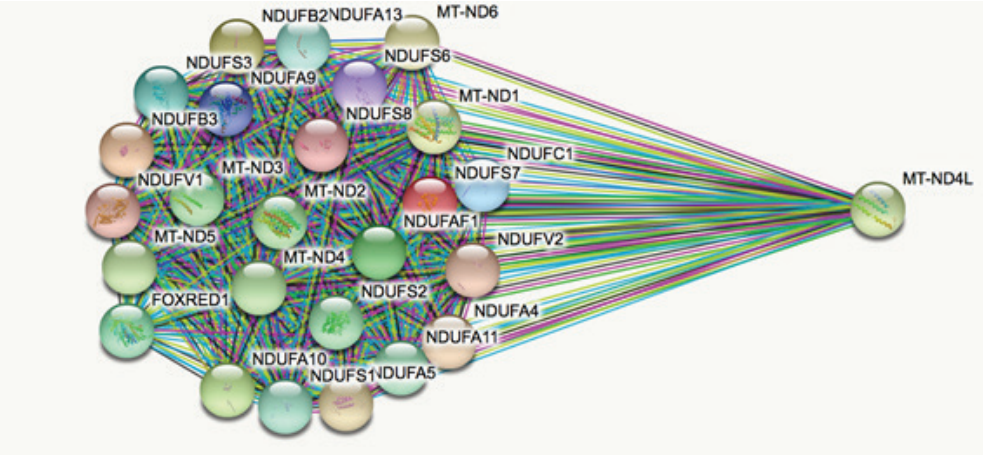

Figure 5: STRING analysis of hsa-miR-4461.

the protein-protein interaction analysis by detecting hsa-miR-4461 showing homology with the mitochondrial gene region MT-ND4L using the STRING v10.5 database (figure 5).

DISCUSSION

This study has determined the presence of miRNAs in mitochondrial fractions isolated from breast cancer cells and shown the identified miRNA targets' ability to regulate cellular pathways in which mitochondria play key roles. Because the MACS isolation method was shown to enable the isolation of mitochondria with a high purity and density equivalent to ultracentrifugation (21), the mitochondrial fraction was therefore first obtained from the cells using this technology. The most common problem in obtaining mitochondrial fractions is the presence of cytosolic contamination. The desired result removing the cytosolic contamination from the samples was achieved by using a method that enables the isolation of mitochondria using super magnetic beads conjugated with the outer membrane protein antibody anti-TOM22 and washing the isolated mitochondrial fractions with RNAse A.

Warburg hypothesized that increased aerobic glycolysis in tumor cells may be associated with impaired respiratory capacity in these cells. Although cancers are associated with reduced respiration in cells resulting in mitochondrial dysfunction, these defects do not cover OXPHOS as a whole. Some studies have shown the most aggressive breast cancer cells to have the most extensive OXPHOS defect, where malignant cells play a role in mitochondrial damage and result in OXPHOS deregulation (22). In the normal breast cell line (MCF-10A) and metastatic (MDA-MB-231) and non-metastatic (MCF-7) breast cancer cell lines used in the study, the expression of OXPHOS genes was primarily confirmed at the mRNA level.

The necessity for discovering the underlying molecular mechanisms for miRNA transport from the nucleus to the mitochondria has emerged alongside the discovery of the mitomiR. Many studies have shown the transport of nuclear-encoded RNA to the mitochondria to be able to occur through several transmission routes, mostly ATP-dependent. However, the molecular mechanisms of mitochondrial RNA transport can often show species-specific variability (23). Pre-miRNAs were previously shown to also be present in mitochondria as well as in mature miRNAs, and these findings increase the probability of mitochondrial miRNA biosynthesis (24). Some pre-RNA sequences are thought to be processed in mitochondria and to act on mitochondrial transcripts or form mature miRNAs that are transported to the cytosol to interact with genomically derived mRNAs. For this reason, the mitochondrial-processed miRNA are thought to contribute to the post-transcriptional regulation of gene expressions related to mitochondrial functions (4). This study identified sRNAs, especially miRNAs, in the MCF-10A, MCF-7, and MDA-MB-231 cell lines using sRNA sequencing and determined their relationship with mitochondria. Based on the idea that miRNA targets are able to regulate the critical cellular pathways in which mitochondria play a key role, bioinformatic analyses were performed as a result of the sRNA sequencing of the mtRNA samples, which enabled the identification of mature miRNAs in the samples. Library analyses revealed three mitochondria-encoded miRNAs (i.e., hsa-miR-4461, hsa-miR-4484, and hsa-miR-4485). Once the sequences of these three miRNAs were aligned with the mitochondrial genome, they showed respective homology with the ND4L, L-ORF, and 16S rRNA genes.

A dynamic relationship is known to exist between mitochondrial function and miRNA activity. The presence of the miRNA in mitochondria isolated from various tissues and cells, as well as the presence of the important proteins

AGO and Dicer (19, 21, 25) suggests that an active RNA interference (RNAi) mechanism may be present in mitochondria. According to other studies on miRNA, homology has been shown with mitochondrial genes in breast cancer cell lines, and the possible targets of hsa-miR-4485, which is localized on chromosome 11p15.4 and targets 16S rRNA, may involve many steps of tumor formation.

Sripada et al. demonstrated hsa-miR-4485's ability to inhibit glycolysis and reduce the clonogenic potential of breast cancer cells by using bioinformatics programs to determine the pathways that are associated with these targets to also be associated with tumor suppression, cancer cell migration, metabolic reprogramming, and cell cycle control (16). hsa-miR-4484 is localized on chromosome 10q26.2 and has been shown to regulate AATF by interacting with MRP3 K12/DLK, which is associated with apoptosis being triggered in cells. Hypoactivation of survival and proliferative pathways such as PI3K/AKT results in cancer onset that also affects mitochondria. PI3K also plays a role as a miR-4484-specific target (24). hsa-miR-4461 is localized on chromosome 5q31.1, and the nucleophosmin 1 (NPM1) gene, which can play a protective role against oxidative stress in hematopoietic stem cells, is one of its possible targets. Many cancer cells with high NPM1 expression are more resistant to ultraviolet or hypoxia-induced apoptosis (25). Anti-apoptotic functions were found to be associated with the ability of NPM1 to inhibit p53 localization in mitochondria (26).

In addition to the hsa-miR-4484, hsa-miR-4485, and hsa-miR-4461 mitomiRs that showing homology with the mitochondrial genome, the study also determined nuclear genome-encoded miRNA that are also associated with mitochondrial function (table 1).

As a result of the GO and KEGG pathway analyses made using the identified mitochondrial miRNA, the study also sheds light on which genes are targeted by targeting certain genes on specific pathways. Accordingly, GO terms such as transcription regulation (GO: 0006351), regulation of gene expression (GO: 0010467), stress response (GO: 0006950), cell death (GO: 0008219), biosynthetic process (GO: 0009058), and biological processes (GO: 0008150) were found to be quite meaningful. According to the KEGG analysis, the Hippo signaling pathway (hsa04390), proteoglycans (hsa0525), thyroid hormone signaling pathway (hsa04919), FoxO signaling pathway (hsa04068), TGF-β signaling pathway (hsa04350), mTOR signaling pathway (hsa04150), glycosphingolipids biosynthesis (hsaT30003), alanine-aspartate-glutamate metabolism (hsa00250), choline metabolism (hsa05231), PI3K-AKT signaling pathway (hsa04151), and insulin signaling pathway (hsa04910) are the possible targets.

In addition to the identified miRNA, other possible novel miRNA reads were also analyzed based on mature, star, and loop sequences using the RNAfold algorithm in the program miRDeep2, with 229 sequences from the MCF-10A cells, 112 sequences from the MCF-7 cells, and 139 sequences from the MDA-MB-231 cells being detected with the Randfold algorithm (table 2). Further analysis will be required in order to identify the possible functional roles of mitomiRs.

Table 2: Chromosomal locations of novel miRNA sequences

ID	Localization	Strand	Count	Sequence	Homology
MCF-10Am-0012	chr2:189355167..189355246	+	210	gggguuuggcagagaugu	locus MT-OHR
MCF-10Am-0013	chr2:191627126..191627186	+	24	gaggugaugauggag-gug	MTND5
MCF-10Am-0025	chr8:57649272..57649312	-	12	gagguugaagugagaggu	MTND5
MCF-10Am-0026	chr22:35822054..35822114	+	9	guagguggccugacuggc	MT-CO1
MCF7-m001	chr2:189355167..189355246	+	15	gggguuuggcagagaugu	locus MT-OHR
MCF7-m002	chr22:35822054..35822114	+	7	guagguggccugacuggc	MT-CO1
MDA-MB-231-0001	chrM:8293..8364	-	27	cacuguaaagaggu-guugg	ATP8
MDA-MB-231-0006	chr1:568841..568912	-	27	cacuguaaagaggu-guugg	MT-TK
MDA-MB-231-0014	chr2:189355166..189355247	+	28	gggguuuggca-gagaugug	locus MT-OHR
MDA-MB-231-0032	chr5:134260622..134260688	+	54	guugguuagguaguugag	MTND5
MDA-MB-231-0035	chr5:134263745..134263786	-	44	auggccuagacuacguac	MTND4L
MDA-MB-231-0036	chr22:35822054..35822114	+	56	guagguggccugacuggc	MT-CO1

CONCLUSIONS

Identifying new mitomiRs may provide significant contributions and possibly enlighten the molecular mechanism underlying mitomiR biogenesis.

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Conflict of Interest: The authors have no conflict of interest to declare.

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