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CLINICAL STUDY

Investigation of the miR-637 and miR-523-5p as candidate biomarkers in breast cancer

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ABSTRACT
OBJECTIVES: The distinction of benign lesions from malign tumors is crucial for the diagnosis and treatment of breast cancers.
BACKGROUND: The aim of this study was to investigate the use of miRNAs as plasma biomarkers for the discrimination of malign and benign breast tumors.
METHODS: Whole blood samples obtained from 40 individuals in 3 groups designated as invasive ductal carcinoma group, fibroadenoma group and healthy controls were included in this study. The expression levels of 372 miRNAs were determined using RT-PCR.
RESULTS: The comparison of fibroadenoma group with healthy controls revealed an upregulation of thirty miRNAs and downregulation of twenty-nine miRNAs. The comparison of invasive ductal carcinoma (IDC) group with controls has shown that eight miRNAs were upregulated while eleven miRNAs were downregulated. When comparing IDC and fibroadenoma groups, 15 miRNAs were found to be upregulated, while 10 miRNAs were downregulated. Further analysis of these miRNAs aimed to determine their power in distinguishing IDCs from fibroadenomas. Among the miRNAs analyzed, seven miRNAs have shown sufficient discriminative power, of which three miRNAs, namely miR-637, miR-523-5p and miR-490-3p, have shown a significantly high discriminative power.
CONCLUSIONS: Circulating miR-637 and miR-523-5p combination maybe used to discriminate between invasive ductal carcinomas and fibroadenomas. *(Tab. 9, Fig. 4, Ref. 30).* Text in PDF *www.elis.sk*
KEYWORDS: breast cancer; fibroadenoma; plasma; biomarker; microRNA

Introduction

Breast cancers develop as a result of uncontrolled growth of breast tissue cells. Usually, the first signs are lumps and deformations of the breast. Most common types of breast cancers are lobular carcinomas developing in the mammary glands and ductal carcinomas developing in the milk ducts [1]. Breast cancers which spread to neighboring tissues are called invasive breast cancers. Invasive ductal carcinomas comprise the most common type of breast cancers [2]. Breast cancers account for 25% of all deaths in women worldwide, resulting in 500,000 deaths in 2012 [3].

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Breast cancers are categorized into diverse types according to their stage, grade, receptor positivity and histology. The choice of treatment is based upon this classification. Molecular studies have shown that breast cancers can also be categorized according to their molecular profile. This classification is based on detection of gene expression levels [4]. One of the major drawbacks of molecular profiling of breast tumors is the requirement of fresh biopsy samples instead of paraffin- embedded tissue samples. The most common molecular subtype is Luminal A with a frequency of 60% [5].

Among benign lessons of breast fibroadenomas, the most common benign tumors occur with a frequency of 25% [6]. Fibroadenomas usually occur before the age 35 where estrogens are thought to play a crucial role. Recent studies have shown that microRNAs (miRNAs) are essential to the pathophysiology of breast cancers and fibroadenomas [7-9].

miRNAs function as post-transcriptional regulators and are frequently deregulated in various cancers [10] due to polymorphisms, epigenetic alterations and copy number changes [11].

Following the discovery of their significance in carcinogenesis, many studies focused on miRNAs as potential circulating biomarkers for early detection of cancers. The first study to investigate the serum miRNA levels has shown that miR-21 serum levels were associated with survival in lymphoma patients [12]. Other studies

have revealed their potential as circulating biomarkers for lung, prostate, kidney, colorectal cancers and glioblastomas [13-17].

The ability to discriminate malign breast tumors from benign tumors via circulating biomarkers can be beneficial for differential diagnosis.

In the present study, the plasma levels of 372 miRNAs were measured in 3 different groups as follows: invasive ductal carcinoma (IDC), fibroadenoma and healthy controls. The was to identify circulating miRNAs and examine their potential use as biomarkers for differentiating between benign and malign breast tumors. Following the determination of miRNA plasma levels, their correlation with Ki67 index and discriminative power were calculated. Lastly, *in silico* target recognition was used to facilitate the determination of targeted pathways in IDC pathogenesis.

Materials and Methods

Study design

In total, 40 samples, of which10 healthy controls, 10 IDC patients and 20 fibroadenoma patients were included in the study. Fibroadenoma and IDC blood samples were obtained from patients enrolled in the Department of General Surgery, Medical Faculty, XXX University during the period of 01.04.2014-31.01.2016. Control group samples were obtained from age- and sex-matched healthy individuals. All individuals included in the study provided an informed consent form. The study was approved by a local Ethics Committee. (06.09.2013/15).

MicroRNA Isolation and cDNA synthesis

A volume of 2 ml of whole blood was collected in sterile tubes and centrifuged for 10 minutes at 3,000 rpm for plasma separation. Separated plasma was stored at -80^{0}C until miRNA isolation. MicroRNA was isolated using miRNeasy Serum Plasma miRNA isolation kit (Qiagen, Inc.) with spike-in *C. elegans* miR-39 control according to manufacturer's instructions. In order to reduce the background signal, the miScript RT II kit (Qiagen, Inc.) was used for cDNA synthesis which facilitates cDNA synthesis from miRNAs .

RT-PCR

The miScript miRNA PCR array plasma kit (Qiagen, Inc.) with miScript SYBR Green was used for real-time PCR to determine the miRNA expression levels. The miScript miRNA PCR array plasma kit is a plate with 384 wells, of which 372 wells contain specific primers for different miRNAs and the remainder 12 wells contain primers for 2 synthetic miRNA controls (*C. elegans* miR-39), 6 reference controls (SNORD61, SNORD68, SNORD72, SNORD95, SNORD96A, RNU6B), 2 reverse transcription controls and 2 positive PCR controls.

Raw Ct results were normalized with the global normalization method. The use of this method is advised for quantification of extracellular miRNA levels where normalization with a housekeeping gene is not possible [18]. Calculations from raw Ct values were performed via GeneGlobe Data Analysis Center, a web-based analysis software developed by SAbiosciences.

Statistical analysis

SPSS 21.0 was used for statistical analysis. Significance of miRNA expression levels was calculated by performing a t-test on $2^{-\Delta ct}$ values for each different miRNA. For the analysis of continuous variables, Shapiro-Wilk test and Levene's test were employed to investigate the distribution and variance homogeneity. Parametric continuous values were compared with t-test and ANOVA while non-parametric variables were compared with Mann-Whitney U and Kruskal-Wallis tests. Significant differences in non-parametric comparisons were assessed with the Tukey test. Categorical variables were analyzed by chi square tests and logistic regression. Significant correlations between the expression data and clinical parameters were assessed by calculating the Pearson correlation coefficient. The discriminative power of miRNAs with a significantly different expression levels were analyzed with receiver operator characteristic analysis. The Alpha level was set at 0.05 for all statistical analyses. The target genes and pathways of significantly upregulated miRNAs were determined with miRSystem, a web-based service which facilitates all known miRNA and pathway databases [19].

Results

Clinical and Demographic Characteristics

The mean ages for invasive ductal carcinoma, fibroadenoma and healthy control groups were 45.95 ± 8.77, 28.33 ± 11.02 and 45.4 ± 5.02, respectively. A proportion of 70% of tumors had a Ki67 index of more than 20 while 30% had a Ki67 index of 20 or

Tab. 1. Upregulated miRNAs in fibroadenoma patients

miRNA	Fold Regulation	p value	miRNA	Fold Regulation	p value	miRNA	Fold Regulation	p value
miR-301a-3p	7.5536	0.000002	miR-214-3p	15.4604	0.00006	miR-373-3p	6.3518	0.000871
miR-19b-3p	9.0453	0.000002	miR-143-3p	5.1	0.000069	miR-142-5p	13.3661	0.000969
miR-590-5p	5.5938	0.000004	miR-199a-5p	4.3084	0.000083	miR-96-5p	15.0724	0.001229
miR-16-5p	5.6719	0.000004	miR-101-3p	8.9828	0.000122	miR-142-3p	5.6719	0.001522
miR-19a-3p	11.9353	0.000005	miR-301b-3p	5.3784	0.00016	miR-425-5p	5.2072	0.001639
miR-144-3p	12.4135	0.000009	miR-30b-5p	4.1521	0.000338	miR-136-3p	4.181	0.003199
miR-199b-5p	4.7365	0.000023	miR-326	5.8179	0.000436	miR-424-5p	4.0199	0.003936
miR-27a-3p	4.8138	0.000028	miR-195-5p	6.4108	0.000447	miR-1277-3p	9.8298	0.006158
miR-29a-3p	4.1906	0.000049	miR-345-5p	4.3585	0.000601	miR-551a	9.1716	0.006976
miR-376c-3p	4.7585	0.000053	miR-15a-5p	4.1521	0.00071	miR-140-5p	5.0066	0.03468

Tab. 2. Downregulated miRNAs in fibroadenoma patients

miRNA	Fold Regulation	p value	miRNA	Fold Regulation	p value	miRNA	Fold Regulation	p value
miR-181c-3p	-5.2519	0.000012	miR-4688	-9.3146	0.000306	miR-615-5p	-6.756	0.00215
miR-637	-4.8216	0.000033	miR-124-3p	-6.2456	0.00056	miR-489-3p	-6.0328	0.002609
miR-206	-4.4265	0.000044	miR-490-3p	-6.0608	0.000709	miR-1225-3p	-4.9002	0.002794
miR-1193	-4.4779	0.000047	miR-4687-5p	-5.6811	0.000762	miR-675-3p	-4.6466	0.00653
miR-183-3p	-5.5642	0.000052	miR-3923	-5.4749	0.000793	miR-3159	-6.1738	0.007468
miR-2276-3p	-5.9223	0.000086	miR-3131	-6.4063	0.000901	miR-1286	-5.1438	0.020527
miR-375	-4.5509	0.000095	miR-4538	-5.2156	0.000966	miR-22-3p	-7.4962	0.032289
miR-34c-3p	-6.3036	0.000114	miR-631	-5.073	0.001137	miR-379-5p	-5.3622	0.04047
miR-1539	-4.1301	0.000122	miR-4267	-4.9916	0.001264	miR-203a-3p	-18.5006	0.041562
miR-4422	-4.6038	0.000252	miR-3689e	-5.1319	0.001312			

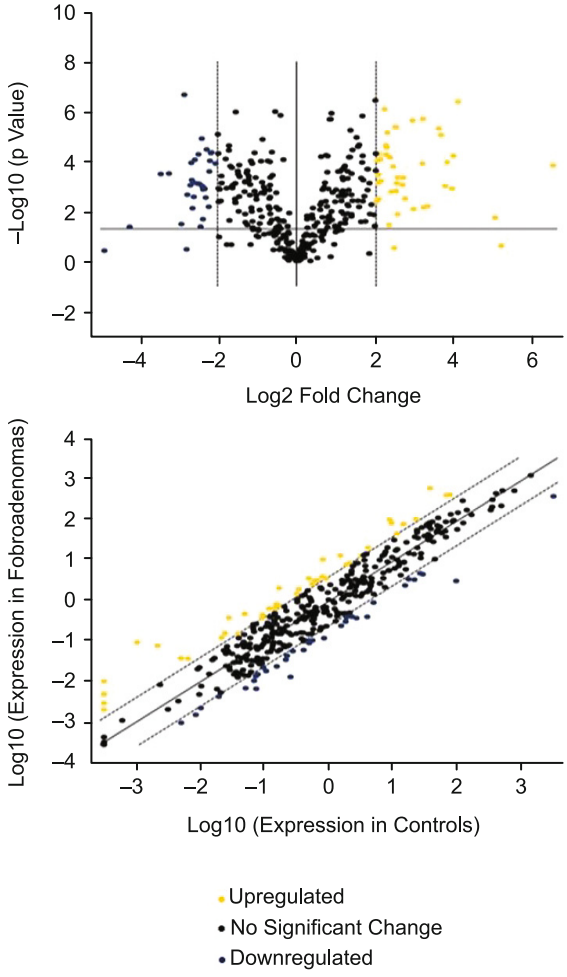

Fig. 1. Volcano and scatter plots for differentially expressed miRNAs in sera of fibroadenoma patients in comparison to healthy controls

lower. There were 5 patients with a tumor with a diameter of <20 mm, 3 with a tumor diameter of 20-30 mm and 2 with a tumor diameter of >30 mm.

Differential Expression in Fibroadenoma Group in Comparison to Healthy Controls

There were 59 miRNAsshowing significant deregulation in fibroadenoma samples in comparison to controls (Figure 1), of which 30 were upregulated 4-fold or more. Most prominently upregulated miRNAs were miR-301a-3p, miR-19b-3p and miR-590-5p ($FC_{301a-3p}$=7.5 , $p_{301a-3p}$=0.000002 ; FC_{19b-3p}=9.04 , p_{19b-3p}= 0.00002 ; FC_{590-5p}= 5.6 p_{590-5p}= 0.000004). Largest fold changes were observed in miR-214-3p, miR-96-5p and miR-142-5p ($FC_{214a-3p}$=15.5 , $p_{214a-3p}$=0.00006; FC_{96-5p}=15.07 , p_{96-5p} = 0.001229 ; FC_{142-5p}=13.4 , p_{142-5p}=0.000969) (Tab. 1.).

There were 29 miRNAs showing a downregulation of 4-fold or greater in fibroadenoma samples in comparison to controls (Table 2). Most prominent, downregulated miRNAs were miR-181c-3p, miR-637 and miR-206 ($FC_{181c-3p}$=-5.25 , $p_{181c-3p}$=0.000012; FC_{637}=-4.82 , p_{637}= 0.000033; KR_{206}= -4.42 p_{206}= 0.00044). Highest fold changes among downregulated miRNAs were observed in miR-203a-3p, miR-4688 and miR-22-3p ($FC_{203a-3p}$=-18.5 , $p_{203a-3p}$ =0.041562 ; FC_{4688}=-9.3 , p_{4688}= 0.000306 ; FC_{22-3p}= -7.5 p_{206}= 0.032289).

Differential Expression in IDC Group in Comparison to Healthy Controls

In comparison to the control group, 19 miRNAs have shown significant dysregulation in IDC group (Figure 2). Out of these 19 miRNAs, 8 were upregulated (Table 3) while 11 miRNAs were downregulated (Table 4). The most substantially upregulated miRNAs were miR-98-5p, miR-199b-3p and miR29a-3p'dir (FC_{98-5p}=2.5 , p_{98-5p}=0.003278 ; $FC_{199b-3p}$=2.02 , $p_{199b-3p}$= 0.020376 ; FC_{29a-3p}= 2.02 p_{29a-3p}= 0.032619), while miR-19b-3p, miR-424-5p and miR-98-5p have shown the greatest fold change (FC_{19b-3p}=3.8, p_{19b-3p}=0.047818 ; FC_{424-5p}=3.06 , p_{424-5p}= 0.033694 ; FC_{98-5p}= 2.5, p_{98-5p}= 0.003278).

In comparison to controls, miR-1247-5p, miR-3135b and miR-1287-5p were most significantly downregulated miRNAs in IDC group ($FC_{1247-5p}$=-2.8, $p_{1247-5p}$=0.000262; FC_{3135b}=-2.8, p_{3135b}= 0.00047; $FC_{1287-5p}$= 2.2, $p_{1287-5p}$= 0.000693). Based on the same comparison, miR-4687-5p, miR-197-3p and miR-1247-5p were the most downregulated miRNAs ($FC_{4687-5p}$=-4.6, $p_{4687-5p}$=0.005159; FC_{197-3p}=-4.1, p_{197-3p}= 0.022644; $FC_{1247-5p}$= 2.8, $p_{1247-5p}$= 0.000262).

Dysregulated miRNAs in IDC Group in Comparison to Fibroadenoma Group

There were 25 miRNAs showing at least a 4-fold change in expression levels when compared to fibroadenoma group (Figure 3), while 15 miRNAs were upregulated and 10 were downregulated (Table 5). The microRNAs, miR-615-5p, miR-375 and miR-523-5p

Tab. 3. Upregulated miRNAs in IDC Group

miRNA	Fold Regulation	p value
miR-98-5p	2.4828	0.003278
miR-199b-3p	2.019	0.020376
miR-29a-3p	2.0237	0.032619
miR-424-5p	3.0549	0.033694
miR-101-3p	2.3982	0.040316
miR-542-3p	2.2912	0.041642
miR-19b-3p	3.7589	0.047818
miR-374c-5p	2.1096	0.049059

Tab. 4. Downregulated miRNAs in IDC Group

miRNA	Fold Regulation	p value
miR-1247-5p	-2.8132	0.000262
miR-3135b	-2.7921	0.00047
miR-1287-5p	-2.2021	0.000693
miR-4687-5p	-4.6231	0.005159
miR-4301	-2.6676	0.006562
miR-4770	-2.4153	0.010247
miR-671-3p	-2.5354	0.013627
miR-4732-5p	-2.0905	0.015115
miR-197-3p	-4.0903	0.022644
miR-126-5p	-2.0522	0.029184
miR-19b-1-5p	-2.3752	0.047464

were most prominently upregulated ($FC_{615\text{-}5p}$=9.9 , $p_{615\text{-}5p}$=0.00116; FC_{375}=6.2 , p_{375}= 0.0021424; $FC_{523\text{-}5p}$= 5.1 , $p_{523\text{-}5p}$= 0.001543), while miR-203a-3p, miR-615-5p and miR-490-3p were most upregulated ($FC_{203a\text{-}3p}$=13.8 , $p_{203a\text{-}3p}$=0.006919 ; $FC_{615\text{-}5p}$=9.9 , $p_{615\text{-}5p}$= 0.00116; $FC_{490\text{-}3p}$= 9.3 , $p_{490\text{-}3p}$= 0.004741).

MicroRNAs, miR-143-3p, miR-181c-5p and miR-144-3p were the most significantly downregulated miRNAs in IDC group in comparison to controls ($FC_{143\text{-}3p}$=-7.5 , $p_{143\text{-}3p}$=0.000005 ; $FC_{181c\text{-}5p}$ =-4.9 , $p_{181c\text{-}5p}$= 0. 000005 ; $FC_{144\text{-}3p}$= -9.85,1 , $p_{144\text{-}3p}$= 0.000041) (Table 6). miR-144-3p, miR-142-5p and miR-214-3p were the most downregulated miRNAs ($FC_{\text{-}3p}$=-9.9 , $p_{144\text{-}3p}$=0.000041 ; $FC_{142\text{-}5p}$=-8.6 , $p_{142\text{-}5p}$= 0.000176 ; $FC_{214\text{-}3p}$= -8.1 , $p_{214\text{-}3p}$= 0.044356).

Correlation of miRNA Expression Levels with Ki67 Index

None of the upregulated miRNAs in the IDC group in comparison to controls were significantly correlated with Ki67 index (minimum=10, maximum=80, mean=39.15, Standard Deviation =21.58) ($r<0.5$, $p>0.05$).

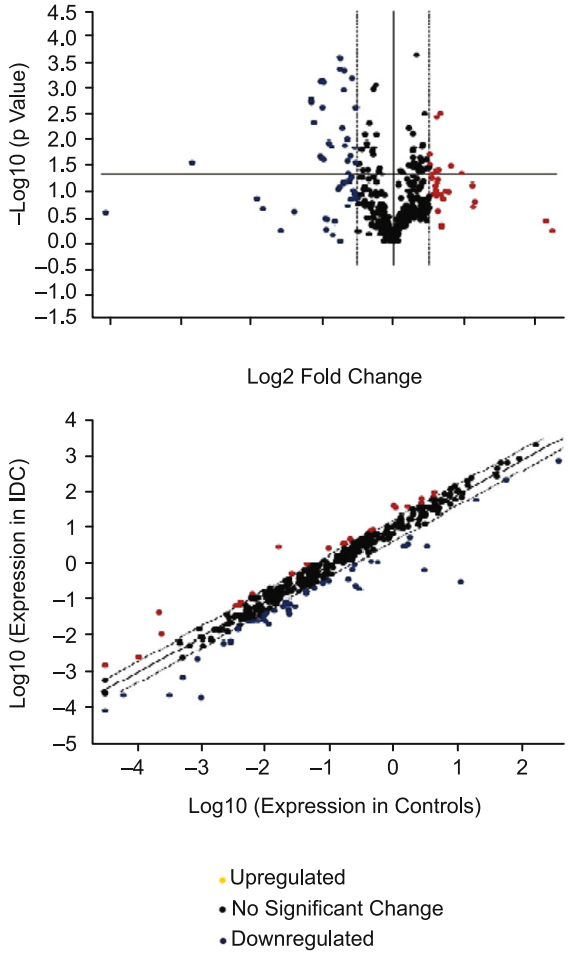

Fig. 2. Volcano and scatter plots for differentially expressed miRNAs in sera of IDC group in comparison to healthy controls

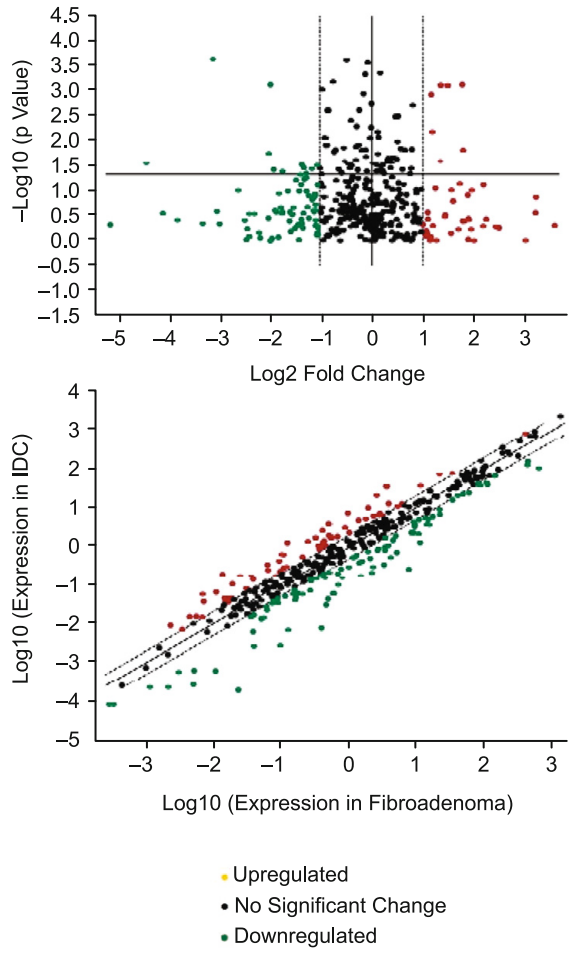

Fig. 3. Volcano and scatter plots for differentially expressed miRNAs in IDC group in comparison to fibroadenoma group

Tab. 5. Upregulated miRNAs in the sera of IDC patients in comparison to fibroadenoma patients

miRNA	Fold Regulation	p value
miR-615-5p	9.9437	0.00116
miR-375	6.2281	0.001424
miR-523-5p	5.1412	0.001543
miR-637	9.1079	0.002334
miR-4538	7.3341	0.00289
miR-490-3p	9.3748	0.004741
miR-203a-3p	13.8449	0.006919
miR-124-3p	6.8707	0.00695
miR-532-3p	5.0939	0.00805
miR-3131	5.3472	0.013172
miR-489-3p	7.6367	0.01934
miR-4302	7.8017	0.031092
miR-219a-1-3p	4.8611	0.035044
miR-181c-3p	4.4861	0.041443
miR-3159	5.4156	0.049431

Tab. 6. Downregulated miRNAs in the sera of IDC patients in comparison to fibroadenoma patients

miRNA	Fold Regulation	p value
miR-143-3p	-7.5182	0.000005
miR-181c-5p	-4.9373	0.000005
miR-144-3p	-9.8803	0.000041
miR-451a	-6.0855	0.00007
miR-345-5p	-5.079	0.000076
miR-326	-4.0173	0.000104
miR-145-5p	-4.1951	0.00015
miR-142-5p	-8.5914	0.000176
miR-301b-3p	-4.0336	0.001121
miR-214-3p	-8.1373	0.044356

Tab. 7. ROC analysis results for IDC / healthy control discrimination

miRNA	AUC	95%CI
miR-374c-5p	0.758	0.320-0.998
miR-98-5p	0.750	0.326-1.0
miR-101-3p	0.374	0.012-0.752
miR-19b-3p	0.365	0.055-0.767
miR-29a-3p	0.325	0.137-0.853
miR-542-3p	0.583	0.202-0.965
miR-424-5p	0.521	0.119-0.922
miR-199b-3p	0.500	0.103-0.897

Tab. 8. ROC analysis results for IDC / fibroadenoma discrimination

miRNA	AUC	95%CI	p value
miR-637	0.958	0.8810-1.00	0.020
miR-523-5p	0.937	0.8690-1.00	0.023
miR-490-3p	0.924	0.8440-1.00	0.026
miR-532-3p	0.899	0.7530-1.00	0.029
miR-489-3p	0.871	0.7310-1.00	0.030
miR-375	0.888	0.7970-1.00	0.039
miR-615-5p	0.768	0.6890-1.00	0.040
miR-4538	0.745	0.651-1.00	0.059
miR-124-3p	0.612	0.632-1.00	0.061
miR-3131	0.560	0.535-1.00	0.075
miR-203a-3p	0.830	0.421-1.00	0.098
miR-4302	0.647	0.435-0.944	0.127
miR-3159	0.750	0.324-1.00	0.201
miR-181c-3p	0.667	0.289-0.960	0.394
miR-219a-1-3p	0.500	0.112-0.612	0.845

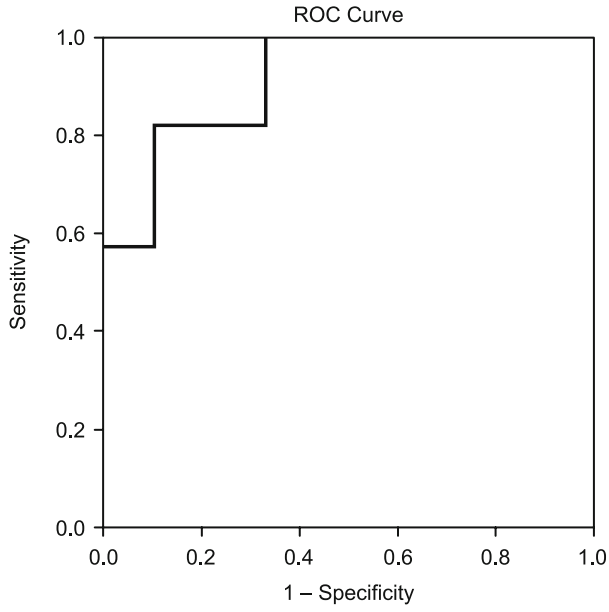

Fig. 4. ROC Curve for miR-637 + miR-523-5p Combination

Receiver Operator Characteristics Analysis for Upregulated miRNAs in IDC Group

When the discriminative power of miRNAs with increased expression in IDC group (Table 3) was analyzed by receiver operator characteristics curve analysis, none of these miRNAs showed sufficient power for IDC, fibroadenoma discrimination (Table 7). When the analysis was repeated using miRNA combinations in order to increase the discriminative power, the miR-374c-5p and miR-98-5p combination has shown an increase in power, albeit not statistically significant (AUC: 0.875, 95%CI: 0.563 – 1.00, p: 0.165).

When the same analysis was performed for upregulated miRNAs in IDC group in comparison to the fibroadenoma group (Table 8), 7 miRAs have shown a significantly high discriminative power (Table 8). The combination of miR-637 and mir-523-5p had the highest discriminative power when the analysis was performed for different miRNA combinations (AUC=0.982, %95%CI:0.895-1.00, p value=0.011) (Figure 4).

Pathway Analysis of Upregulated miRNAs According to IDC – Fibroadenoma Comparison

The pathway analysis indicates MAPK pathway as the target pathway with 8 out of 15 miRNAs targeting 35 genes out of 87 genes in this pathway (Pathway Score= 1.836). SP1 and MAP3K3 were identified as the most common targets of these miRNAs (Table 9).

Discussion

The MAPK pathway, which was indicated as a target for upregulated miRNAs in IDC in comparison to fibroadenomas, plays

Tab. 9. Target genes in MAPK pathway

Target Genes in MAPK Pathway	Number of Targeting miRNAs	Targeting miRNAs
SP1	4	miR-124-3p, miR-203a-3p, miR-375, miR-637
MAP3K3	3	miR-124-3p, miR-490-3p, miR-637
MAPK4	2	miR-124-3p, miR-532-3p
TGFBR1	2	miR-124-3p, miR-490-3p
SHC1	2	miR-124-3p, miR-203a-3p
JUN	2	miR-203a-3p, miR-637
RPS6KB1	2	miR-124-3p, miR-203a-3p
RPS6KA3	2	miR-203a-3p, miR-490-3p
MAP3K1	2	miR-124-3p, miR-203a-3p
RAPGEF2	2	miR-124-3p, miR-203a-3p
CREB1	2	miR-203a-3p, miR-489-3p
MAP3K5	2	miR-203a-3p, miR-375
MAP4K3	2	miR-203a-3p, miR-490-3p
MEF2D	2	miR-490-3p, miR-637
MAPK10	2	miR-203a-3p, miR-615-5p
MEF2C	2	miR-203a-3p, miR-615-5p
MAPK14	2	miR-124-3p, miR-489-3p
MAPK9	2	miR-124-3p, miR-615-5p

a crucial role together with the PI3K/AKT pathway in many cancer types. The amplifications and mutations in PI3K/AKT pathway are mutually exclusive with alterations in receptor tyrosine kinases [20]. This indicates that tyrosine kinase amplifications are aimed at activating PI3K/AKT pathway [21]. In accordance with this, the receptor tyrosine kinases are supressed in normal breast epithelium and the propagating signals usually target the JUN/MAPK pathway. In contrast to this, in breast cancer cells, the receptor tyrosine kinases activate PI3K/AKT pathway [22].

These findings imply that the mutations, copy number changes and expression alterations observed in breast cancer cells facilitate the switch from a state where MAPK pathway is active and PI3K/AKT pathway is inactive to a reverse state where PI3K/AKT pathway is active and MAPK pathway is inactive.

This hypothesis was investigated by Guille A. et al., who showed the opposite effects of PI3K/AKT and MAPK pathways where AKT activation was responsible for the inhibition of MAP2K4 synthesis [23]. According to this hypothesis the pathway-switch occurs during tumorigenesis in breast cancers where MAPK pathway is inhibited and PI3K/AKT pathway is activated, resulting in the acquisition of the neoplastic phenotype.

The pathway analysis results indicate that the miRNAs which were found to be upregulated in the IDC / fibroadenoma comparison in this study, target the MAPK pathway, especially MAP3K3 and MAPK4. These results corraborate the "pathway-switch" hypothesis. In the light of these findings, it is possible that miRNAs may play a role in the PI3K/AKT - JUN/MAPK switch and acquisiton of neoplastic phenotype in addition to genomic alterations. *In silico* target recognition results supported with experimental evidence, highlight miR-124-3p, miR-203a-3p, miR-637, miR-615-5p, miR-375, miR-523-5p, miR-4538, miR-490-3p, miR-532-3p, miR-3131, miR-489-3p, miR-4302, miR-219a-1-3p, miR-181c-3p and miR-3159 as the most prominent miRNAs in the supression of MAPK pathway.

Analyses of cellular miRNA profiles in breast cancer have determined that many miRNAs display expression patterns linked to molecular subtype as well as tumor grade and other tumor-related processes [24]. Especially for breast cancer, the levels of tissue-expressed and circulating miRNAs are highly correlated and circulating miRNA levels returned to their normally expected levels after surgical removal of the tumor, suggesting that plasma miRNA levels may reflect tissue expression levels [25-29]. Heneghan et al. [27] surveyed a panel of seven candidate miRNAs in whole blood RNAs from 148 breast cancer patients and 44 age-matched and disease-free controls. They found out that the expression of miR-195 was significantly elevated in breast cancer patients. Additionally, they observed a significant reduction in miR-195 in post-operative whole blood compared to the pre-operative samples of the same patients.

Menha et al. [30] determined serum miRNA expression levels using quantitative real-time polymerase chain reaction (qPCR) in their study with primary breast cancer patients (n=137), benign breast lesion patients (n=60) and a healthy control group (n=38). They found that miRNA-21 and miRNA-373 levels were statistically significantly higher in invasive duct carcinoma (IDC) compared to non-IDC. In this study, when we compared miRNA levels between invasive canal carcinoma (IDC) and healthy control groups, 8 miRNAs (miR-98-5p, miR-199b-3p, miR-29a-3p, miR-424-5p, miR-101-3p, miR-542-3p, miR-19b-3p and miR-374c-5p) were up-regulated, and 11 miRNAs (miR-1247-5p, miR-3135b, miR-1287-5p, miR4687-5p, miR-4301, miR-4770, miR-671-3p, miR-4732-5p, miR-197-3p, miR-126-5p and miR-19b-1-5p) were found to be down-regulated.

The main aim of this study was to identify a circulating miRNA signature which could facilitate the discrimination of fibroadenomas from invasive ductal carcinoma and support the decision for a biopsy. When the upregulated miRNAs in IDC samples were analysed in comparison to fibroadenoma samples, 9 miRNAs (miR-615-5p, miR-375, miR-523-5p, miR-637, miR-4538, miR-490-3p, miR-203a-3p, miR-124-3p and miR-532-3p) had sufficient discriminative power. The combination of miR-637 and miR-523-5p had the highest discriminative power.

Using this study as a starting point may enable the discovery of a test to discriminate between benign and malign breast tumors. This process should ideally follow the biomarker discovery pipeline, where each step is validated while the number of measured miRNAs is decreased and the number of samples is increased.

Learning Points

The combination of circulating miR-637 and miR-523-5p maybe used to discriminate between invasive ductal carcinomas and fibroadenomas. The microRNAs, miR-124-3p, miR-203a-3p and miR-637, may play a crucial role in the neoplastic pathway switch.

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