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Using miR-125b in the Prediction of Aromatase Inhibitors Resistance in Metastatic Breast Cancer

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AZ, AR and AM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DIAER and HB did the laboratory analysis. Authors AI, AR and AM managed the analyses of the study. Author AM collected the patients data and followed them up. Author AI did the literature searches and wrote the final draft. All authors read and approved the final manuscript.

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ABSTRACT

Background: Several studies investigated the miRNAs in cancer trying to assess the prognosis or to predict the response to certain treatment .One of these miRNAs are miR 125b , it was suggested by previous results as a good marker for prediction of aromatase inhibitors (AI) response. So, this study was conducted to assess the value using miRNA125b as a predictive factor to AI response.
Patients and Methods: A total of 90 patients of postmenopausal HR+ve metastatic breast cancer who attended to medical oncology department outpatient's clinic in SECI, Assiut university Egypt, from May 2017 to September 2018. Patients presented with metastasis at diagnosis as well as patients who relapsed only after 3 years of adjuvant hormonal treatment with SERM were included. All patients received AI as first line treatment for metastatic, miRNA 125b was isolated from

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peripheral blood samples and measured by using (q PCR).The response of patients was assessed by RECIST criteria and correlated with its expression levels.
Results: Expression of the miR125-b was significantly higher in patients than control p= 0.000. However, no significant difference in its expression between those with single of multiple metastases or even between the site of metastases. We didn't find also any significant difference in response p=0.648 and survival either PFS p=0.406 or OS p=0.384 between those patients with high expression vs. low expression.
Conclusion: Our results suggest that miR125b could be used as a diagnostic marker as it is significantly increased in patients than control. However, we don't recommend using miR125b as a marker to predict the AI resistance as further studies with large sample size are needed to confirm these results.

Keywords: Breast cancer; miR125b; predictive factor; aromatase inhibitors.

ABBREVIATIONS

AI : Aromatase inhibitor
DLk1-DIO3P : Delta-like Homolog 1gene and the type III Iodothyronine Deiodinase gene
ER : Estrogen receptor
KEGG : Kyoto Encyclopedia of Genes and Genomes
Let-7 : The lethal-7 (let-7) gene
miRNA : microRNA
mTOR : Mammalian target of rapamycin
PI3K : Phosphatidylinositol 3 kinase
PR : Progesterone receptor
RECIST criteria : Response Evaluation Criteria In Solid tumors
RTQ-PCR : Real-time quantitative PCR
TCGA : The Cancer Genome Atlas

1. INTRODUCTION

Breast cancer is a heterogeneous disease with several types and several lines of treatment. In approximately 70% of postmenopausal patients, breast cancer is a hormone-dependent disease that relies on estrogen to drive carcinogenesis. Endocrine therapies, including estrogen receptor modulators and aromatase inhibitors (AIs), are the most suitable treatment for estrogen receptor positive patients [1]. Up to now, acquired resistance to endocrine treatment is revealed to be a progressive event whereby breast cancer cells are converted to a nonresponsive phenotype and eventually to an estrogen-independent phenotype. Among the molecular mechanisms involved in the achievement of endocrine resistance, which is confirmed by recent studies, as they demonstrated the activation of the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) and/or mitogen-activated protein kinase (MAPK) pathways, both in breast cancer cell lines and in breast tumors [2-8]. activation of these survival pathways by stimuli may lead to endocrine resistance via the stimulation of kinases in an Estrogen Receptor dependent [9] and Estrogen Receptor independent fashion [2,10].

Recently, several investigation and researches support an involvement of miRNAs in estrogen action and/or in endocrine resistance. However, most studies devoted to Tamoxifen or fulvestrant resistance and few studies focused in aromatase inhibitors. MicroRNAs (miRNA) are small, non-coding RNAs that control the expression of the gene that targeting messenger RNA, resulting in either repression of translation or degradation of DNA [11]. There are over 4000 miRNAs have been identified, they are expected to regulate up to 30% of all human genes [12]. miRNAs can operate as tumor-suppressors or tumor-promoters and their dysregulation is intricately linked to cellular processes involved in the metastatic cascade, such as sustained proliferation, angiogenesis, and epithelial-mesenchymal transition (EMT) [13,14]. Recent evidence shows that miRNAs are also involved in the development of the resistance to currently employed treatments with anthracyclines, 5-fluorororacil, cyclophosphamide, carboplatine, taxanes, endocrine, and targeted so they are probably could be useful as predictors of resistance towards a given treatment [4].

Previous study by Vilquin P et al. [15] using cell extraction from tumor tissue showed that ectopic overexpression of e miR-125b in sensitive ER+ MCF-7 cells was sufficient to trigger activation of the AKT/mTOR pathway to develop de novo resistance to both letrozole and anastrozole.

This mechanism of action has been confirmed by several studies which is different from other mechanism of breast cancer drugs resistance triggered by other type of mi RNA (Fig. 1) [4].

Recently circulating miRNAs using extraction miRNA from peripheral blood instead from tissue show great promise in contributing to the diagnosis, prognosis, and evaluation of response to therapy and treatment of breast cancer [16–18]. miRNAs are constant in circulation and can be measured relatively simply and inexpensively (using real-time quantitative reverse transcriptase PCR; RT-qPCR) [19–22].

The aim of our study was to identify the validity of using circulating miRNA-125b as a simple predictor marker to aromatase inhibitors response in metastatic postmenopausal breast cancer.

2. PATIENTS AND METHODS

We prospectively recruited 90 post-menopausal patients with lung or bone metastatic breast cancer. We included patients who presented to Medical oncology department out patient's clinic, South Egypt cancer Institute, Assiut university, Egypt, between May 2017 and September 2018. We included only post-menopausal patients who presented with metastasis at the time of their diagnosis. We excluded patients with her2neu positive disease and patients who received chemotherapy as a first line metastasis.

Twenty-age matched healthy women with no history of cancer and in good health condition were recruited as controls.

For all patients we used nonsteridal aromatase inhibitors (letrazole) 2.5 mg tablet administered once a day, before starting the treatment, patients received a baseline blood draw for miRNA expression profiling. Tumor assessment was performed every 3 months by CT-scan. Treatment response was assessed by Response Evaluation Criteria In Solid Tumors RECIST criteria [22]. Patients reached complete (CR) or partial (PR) response were considered as responder; disease stabilization (SD) or disease progression (PD) considered as non-responder. The overall response is defined by summation of CR and PR.

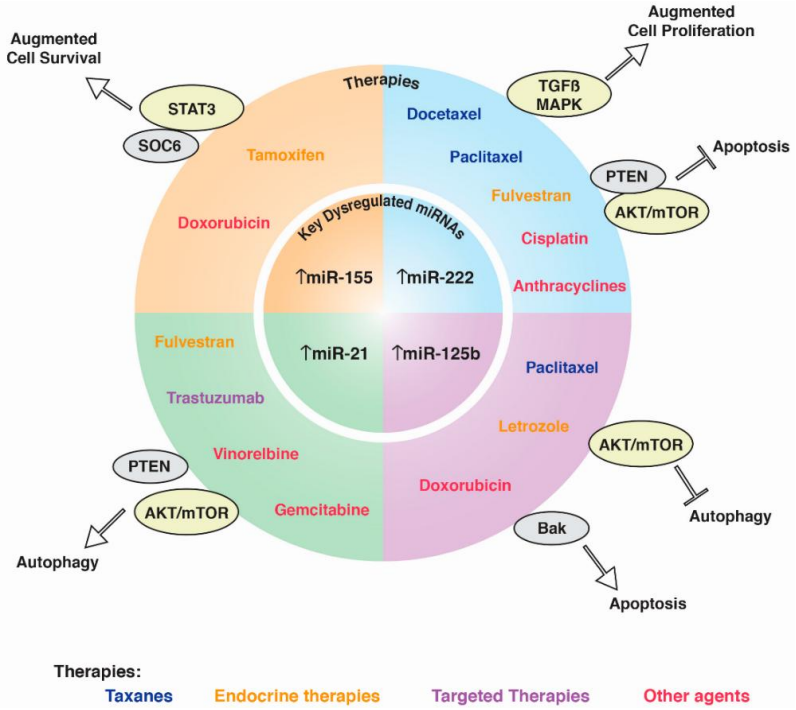

Fig. 1. Key miRNAs in the resistance to BC treatment

Illustrated by Campos-Parra AD et al., 2017 upregulation of four key miRNAs—miR- 155, miR-222, miR-125b, and miR-21—is associated with the resistance to systemic therapy: Taxanes, endocrine therapies, Targeted therapies, and other agents

This study strictly conformed to the principles outlined in the Declaration of Helsinki and it was approved by the ethics committee of South Egypt Cancer Institute SCI-IRBIORG0006563 N0=364.

2.1 miRNA 125b Measurement

From all patients and volunteers, miRNA was isolated from peripheral venous blood samples by using the miRNA easy Mini kit (Qiagen, Hilden, Germany).

Isolated miRNA is converted to its complementary DNA (CDNA) by using TaqMan miRNA Reverse Transcription kit (Qiagen, Hilden, Germany).

Then relative quantification of miRNA expression is measured by performing Q-PCR amplification using Taqman Universal PCR Master MixII(2x) and Taqman small RNA Assay(20x) by 7500 fast real time PCR system, Thermo Fisher Scientific, USA.

2.2 Analysis

To calculate relative expression in RT-qPCR.

A- The gene being tested: in control and experimental samples.
B- The housekeeping gene: in control and experimental samples.

- Take the mediocre of the cycle threshold (Ct) values for the housekeeping gene and the gene being tested in the experimental and control samples, returning 4 values. The 4 values are Gene being Tested Experimental (TE), Gene being Tested Control (TC), Housekeeping Gene Experimental (HE), and Housekeeping Gene Control (HC).

- Calculate ΔCt values for the experimental (ΔCTE) and control (ΔCTC) conditions. ΔCTE is the differences between TE and HE (TE-HE) and ΔCTC is the difference between TC and HC (TC-HC).

- Then, calculate ΔΔCt which is the difference between ΔCTE and ΔCTC (ΔCTE-ΔCTC) to arrive at the Double Delta Ct Value (ΔΔCt).

- Calculate fold change: Relative quantification (RQ) which is the value of 2^-ΔΔCt to get the expression status.

- We interpreted the results as following; If RQ is (more than 1) means expression of target is more than the control included (up expressed) and if RQ is (less than 1) means expression of target is less than the control (down expressed).

2.3 Estrogen and Progesterone Receptors Staining and Interpretation

The tissues were fixed in 10% neutral formol and included in paraffin blocks. Serial sections, initially colored HE, were made that were later imunohistochemically processed.

To evaluate imunohistochemical results only the nuclear marking was taken into consideration. To quantify the hormonal status the Allred score was used. This takes into consideration both the proportion of marked cells and the medium intensity of the nuclear marking. The Allred score [23] is the sum of the proportion score (proportion of marked cells) and the intensity score (marking intensity).

Table 1. Allred score

Proportion of positive cells proportion	Proportion score
0	0
0-1%	1
1%-10%	2
11%-33%	3
34%-66%	4
67%-100%	5
Staining intensity score	
Marking intensity	**Intensity score**
Lack of marking	0
Low intensity	1
Moderate intensity	2
High intensity	3

Allred score=PP+SI if ≤ 2 were considered negative, and the ones that had an Allred > 2-8 score were considered positive.

2.4 Statistical Analysis

All analyses were conducted using SPSS software version 21. Univariant factors were analyzed using the Chi-square test for categorical variables, difference was considered statistically significant at $P<0.05$, the survival progression free survival (PFS) and overall survival (OS) was calculated according to Kaplan Meier method [24].

3. RESULTS

The study included 90 postmenopausal patients with metastatic breast cancer patient. The median age at diagnosis was 55 years ranged from 47- 78.

According to site of metastasis, our study included, 50 patients of them (55%) had bone metastasis. Forty (45%) patients presented with lung metastasis. We also stratified the hormonal receptors status expression in each patients by Allred score [23] Table 2.

3.1 Expression of miR-125b in Controls and Breast Cancer Patients

In control group, *miR-125b* expression values ranged between 0.6 and 1 with a mean value of 0.7± 0.31 and median value of 0.92, while in metastatic BC patients it ranged between 0.88 and 706.8 with a mean value of 85.57± 148 and median value of 19.26. ($p < 0.001$).

miR-125b expression levels in lung metastases were 0.93-706.59 with a mean value of 120.49±200.75 and median value of 43.51 .While patients who presented with bone metastasis their *miR-125b* expression ranged 0.88-300.321 with a mean value of 90.88±190.044 and median value of 39.3. p- value 0.967 *miR-125b* expression levels in patient with single metastasis - 15 patients ranged 0.93-450.31 and mean value 150.99±265.34 with median 67.2, whereas in patients with multiple metastasis - 75 patients , ranged 0.85-706.59 with a mean value of 181.95±266.29 and median value 77.8. p value 0.065 Table 3.

3.2 Response to Aromatase Inhibitors

After median 12 months of follow up, the overall response was 68%, 24 patients had partial response while 38 patients had stationary course only 28 patients progressed on treatment. We found significance association between the number of metastasis and the response to aromatase inhibitors as patients with single metastasis lesion had better response to AI compared to patients with multiple organ metastasis $p < 0.001$, and response was significantly better in patients with high score of estrogen and progesterone receptors levels $p<0001$,$p <0.001$ respectively.

However, we did not find any significance difference associated in patients regarding site of metastasis (bone or lung) (p- value 0.101).

3.3 Correlation of MiR125b Expression with Response and Survival

Regarding miR125b expression levels, we did not find any significance association between high expression level of miRNA 125b and the response to aromatase inhibitors. Also, we did not find any significant bad impact on survival either PFS (p- value 0.406 or OS p- value 0.384).

Table 2. Patients and pathological characteristic

Item name		N= 90 N (%)	
Age Median (rang)	55 (47-75)		
Site of metastasis	Bone metastasis	50	(55)
	Lung metastasis	40	(45)
ER Expression	Weak	22	(24)
	Moderate	32	(36)
	Strong	36	(40)
PR Expression	Weak	8	(9)
	Moderate	36	(40)
	Strong	46	(51)

Table 3. MiR125b expression levels

Groups	Range	Median	Mean± SD	p. value
Healthy controls	0.6-1	0.92	0.7±0.31	0.001
Metastatic breast cancer patients	0.88-706.59	19.26	85.57±148.06	
Patient with multiple metastasis.	0.85-706.59	77.84	181.95±266.29	0.065
Patients with single metastasis.	0.93-250.31	59.218	150.99±265.34	

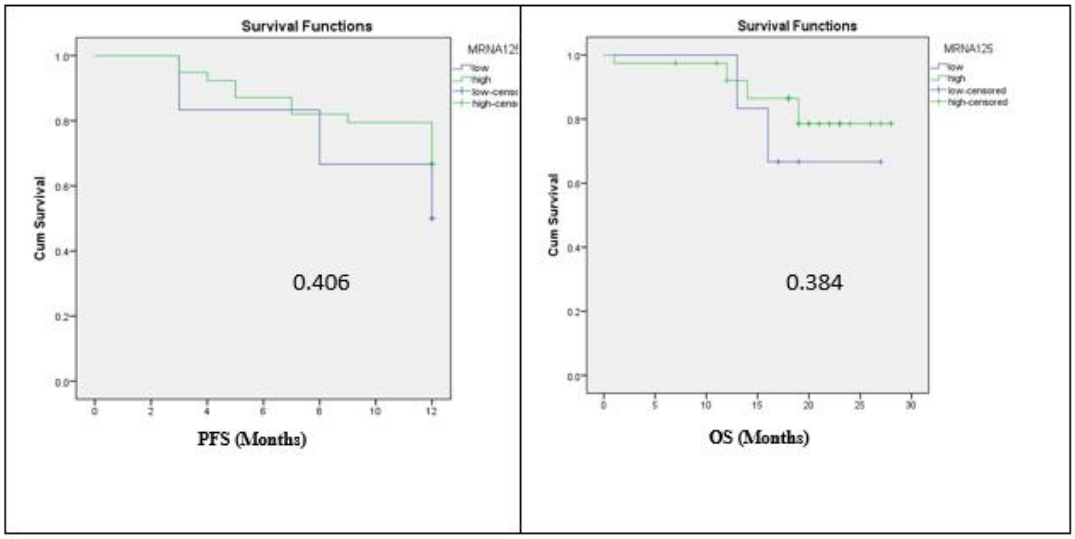

Fig. 2. Expression of miRNA 125b and survival (PFS and OS)

4. DISCUSSION

In the era of tailored breast cancer management, clinicians need multiple tests, allowing disease detection/screening, helping in treatment choice, and to monitor disease response, ultimately to achieve the optimal outcome for patients. Circulating miRNAs are an interesting aide to conventional diagnostic and prognostic modalities, as they are stable in circulation, easily quantifiable and can reveal further information of the underlying biology of the tumor [25].

Although the clinical application of serum miRNAs as a noninvasive strategy is promising, but the data about miRNA signatures in BC patients still contradicted.

In the current study, we analyzed the serum level of miR-125b in 90 patients with metastatic breast cancer females and 20 age matched healthy volunteers. We choose to investigate the miR 125b, which is a tumor suppressor in breast tumorigenesis and has been proved in a previous study by Vilquin [15] as strong predictor to AI response. We found that miR-125b expression levels were significantly higher in metastatic BC patients compared to controls.

Our results probably disagree with Han et al. [26] reported that the serum levels of miR-125b showed no significant difference between BC patients and healthy controls. But our results are matched with Wang et al. [27] who found that early stage BC patients had comparable miR-125b level to healthy controls, late stage patients had on average 3.5-fold higher mean values of miR-125. Our results didn't show any significant difference in response to letrazol in patients with high expression of miR125b compared to the patients who had low expression also we didn't find any significant effect on survival either progression free survival or overall survival which contradict the results of Vilquin et al. [15] who demonstrated that ectopic overexpression of miR-125b is sufficient to confer MCF-7 cell resistance to letrozole by targeting and activating the AKT/mammalian target of rapamycin pathway, which appears to be estrogen-independent. However recently 2 studies one conducted by Bailey ST et al. [28], other conducted by Hoppe R et al. [29] their results revealed that miR-125b was one of the lowest levels of regulated miRNAs in both of AI resistance models, they suggested that other miRNAs such as miR-99a [30] and miRNAs of the DLK1-DIO3 locus [31] may inhibit with the mTOR pathway during the reprogramming of growth signaling. A suppressive role of the DLK1-DIO3 miRNA mega-cluster on the PI3K-mTOR pathway has been identified in hematopoetic stem cells resulting in a decrease of mitochondrial biosynthesis and metabolic activity and the protection of these cells from excessive production of reactive oxygen species [31]. Moreover, they found by using NanoString technolog, the expression level of miR-125b in cluster with let-7c and miR99a was downregulated during the progression to

endocrine resistance in luminal B BC; moreover, it was shown that miR-125b and let-7 c regulate HER2 expression. These findings were correlated with data from samples profiled in The TCGA (Cancer Genome Atlas), and it was recognized that the let-7c and miR-99a expression level was reduced in luminal B compared with luminal A tumors and only a leaning toward reduced miR-125b expression was observed in these same subgroups. Thus, the authors suggested the use of this cluster as a biomarker of a poor outcome in ER positive BC patients [28]. So a probable of more complicated mechanism other than one miR125b are responsible for AI resistance and further studies are still needed to clarify this mechanism.

5. CONCLUSION

Our results do not recommend to using miR125b as a marker to predict the AI resistance however, further study with large sample size still needed to confirm our results.

CONSENT

Written informed consent was obtained from all the patients.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Burstein HJ, Lacchetti C, Anderson H, Buchholz TA, Davidson NE, Gelmon KE, Giordano SH, Hudis CA, Solky AJ, Stearns V, Winer EP, Griggs JJ. Adjuvant endocrine therapy for women with hormone receptor-positive breast cancer: American society of clinical oncology clinical practice guideline update on ovarian suppression. J Clin Oncol. 2016; 34(14):1689-701.
DOI: 10.1200/JCO.2015.65.9573
Epub 2016 Feb 16.
2. Cavazzoni A, Bonelli MA, Fumarola C, La Monica S, Airoud K, Bertoni R, et al. Overcoming acquired resistance to letrozole by targeting the PI3K/AKT/mTOR pathway in breast cancer cell clones. Cancer Lett. 2012;323:77–87.
DOI: 10.1016/j.canlet.2012.03.034
Epub 2012 Apr 3.
3. Ghayad SE, Vendrell JA, Ben Larbi S, Dumontet C, Bieche I, Cohen PA. Endocrine resistance associated with activated ErbB system in breast cancer cells is reversed by inhibiting MAPK or PI3K/Akt signaling pathways. Int J Cancer. 2010;126:545–62.
DOI:10.1002/ijc.24750
4. Campos-Parra AD, Mitznahuatl GC, Pedroza-Torres A, Romo RV , Reyes FI, López-Urrutia E, Pérez-Plasencia C. Micro-RNAs as potential predictors of response to breast cancer systemic therapy: Future clinical implications. Int. J. Mol. Sci. 2017;18(6):1182.
Available:https://doi.org/10.3390/ijms18061182
5. Macedo LF, Sabnis GJ, Goloubeva OG, Brodie A. Combination of anastrozole with fulvestrant in the intratumoral aromatase xenograft model. Cancer Res. 2008;68: 3516–22.
DOI:10.1158/0008-5472.CAN-07-6807
6. Vilquin P, Villedieu M, Grisard E, Larbi SB, Ghayad SE, Heudel PE, et al. Molecular characterization of anastrozole resistance in breast cancer: Pivotal role of the Akt/mTOR pathway in the emergence of de novo or acquired resistance and importance of combining the allosteric Akt inhibitor MK-2206 with an aromatase inhibitor. Int J Cancer. 2013;133:1589–602.
DOI:10.1002/ijc.28182
Epub 2013 May 2.
7. Osborne CK, Schiff R. Mechanisms of endocrine resistance in breast cancer. Annu Rev Med. 2011;62:233–47.
DOI: 10.1146/annurev-med-0709091829-17
8. Ghayad SE, Bieche I, Vendrell JA, Keime C, Lidereau R, Dumontet C, et al. mTOR inhibition reverses acquired endocrine therapy resistance of breast cancer cells at the cell proliferation and gene-expression levels. Cancer Sci. 2008;99:1992–2003.
DOI: 10.1111/j.1349-7006.2008.00955.x

9. Le Romancer M, Poulard C, Cohen P, Sentis S, Renoir JM, Corbo L. Cracking the estrogen receptor's posttranslational code in breast tumors. Endocr Rev. 2011;32: 597–622.
DOI: 10.1210/er.2010-0016
Epub 2011 Jun 15.
10. Nicholson RI, Staka C, Boyns F, Hutcheson IR, Gee JM. Growth factor-driven mechanisms associated with resistance to estrogen deprivation in breast cancer: new opportunities for therapy. Endocr Relat Cancer. 2004;11: 623–41.
DOI: 10.1677/erc.1.00778
11. Zhang B, Pan X, Cobb GP, Anderson TA. microRNAs as oncogenes and tumor suppressors. Dev Biol. 2007;302:1–12.
DOI: 10.1016/j.ydbio.2006.08.028
12. Erson AE, Petty EM. MicroRNAs in development and disease. Clin Genet. 2008;74:296–306.
Available:https://doi.org/10.1152/physrev.00006.2010
13. Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature. 2007;449:682–8.
DOI:10.1038/nature06174
14. Casey MC, Sweeney KJ, Brown JAL, Kerin MJ. Exploring circulating micro-RNA in the neoadjuvant treatment of breast cancer. Int J Cancer. 2016;139:12–22.
Available:https://doi.org/10.1002/ijc.29985
15. Vilquin P, Donini CF, Villedieu M, Grisard E, Corbo L, Bachelot T, Vendrell JA, Cohen PA. MicroRNA-125b upregulation confers aromatase inhibitor resistance and is a novel marker of poor prognosis in breast cancer. Breast Cancer Res. 2015; 30;(17):13-22.
DOI: 10.1186/s13058-015-0515-1
16. McAnena P, Lowery A, Kerin MJ. Role of micro-RNAs in breast cancer surgery. Br J Surg. 2018;105:19–30.
Available:https://doi.org/10.1002/bjs.10790
17. Pritchard CC, Cheng HH, Tewari M. MicroRNA profiling: Approaches and considerations. Nat Rev Genet. 2012;13: 358–69.
DOI: 10.1038/nrg3198
18. Freedman JE, Gerstein M, Mick E, Rozowsky J, Levy D, Kitchen R, et al. Diverse human extracellular RNAs are widely detected in human plasma. Nat Commun. 2016;7:11106.
DOI: 10.1038/ncomms11106
19. Heneghan HM, Miller N, Lowery AJ, Sweeney KJ, Kerin MJ, et al. Circulating microRNAs as novel minimally invasive biomarkers for breast Cancer. Ann Surg. 2010;251:499–505.
DOI: 10.1097/SLA.0b013e3181cc939f
20. Cuk K, Zucknick M, Heil J, Madhavan D, Schott S, Turchinovich A, et al. Circulating microRNAs in plasma as early detection markers for breast cancer. Int J Cancer. 2013;132:1602–12.
DOI:10.1002/ijc.27799
21. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA. 2008; 105:10513–8.
Available:https://doi.org/10.1073/pnas.0804549105
22. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2): 228-47.
DOI: 10.1016/j.ejca.2008.10.026
23. Allred C, Harvely J, Berado M, et al. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol. 1999;11:155–168.
24. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958;53: 457-81.
25. Kassem NM, Makar WS, Kassem HA, Talima S, Tarek M, Hesham H, El-Desouky MA. Circulating miR-34a and miR-125b as promising non invasive biomarkers in Egyptian locally advanced breast cancer patients. Asian Pac J Cancer Prev. 2019;20(9): 2749-2755.
DOI: 10.31557/APJCP.2019.20.9.2749
26. Han J, Jiang Y, Zhang C, et al. A novel panel of serum miR-21/miR-155/miR-365 as a potential diagnostic biomarker for breast cancer. Ann Surg Treat Res. 2017; 92:55–66.
Available:https://doi.org/10.4174/astr.2017.92.2.55
27. Wang H, Tan G, Dong L, et al. Circulating MiR-125b as a marker predicting chemoresistance in breast cancer. PLoS One. 2012;7:34210.

Available:https://doi.org/10.1371/journal.pone.0034210
28. Bailey ST, Westerling T, Brown M. Loss of estrogen-regulated microRNA expression increases HER2 signaling and is prognostic of poor outcome in luminal breast cancer. Cancer Res. 2015;75:436-45.
DOI: 10.1158/0008-5472.CAN-14-1041
29. Hoppe R, Fan P, Büttner F, Winter S, Tyagi AK, Cunliffe H, Jordan VC, Brauch H. Profiles of miRNAs matched to biology in aromatase inhibitor resistant breast cancer Oncotarget. 2016;7(44):71235-71254.
DOI: 10.18632/oncotarget.12103
30. Yang Z, Han Y, Cheng K, Zhang G, Wang X. miR-99a directly targets the mTOR signaling pathway in breast cancer side population cells. Cell Prolif. 2014;47:587-95.
DOI: 10.1111/cpr.12146
Epub 2014 Oct 27.
31. Qian P, He XC, Paulson A, Li Z, Tao F, Perry JM, Guo F, Zhao M, Zhi L, Venkatraman A, Haug JS, Parmely T, Li H, et al. The Dlk1-Gtl2 locus preserves LT-HSC function by inhibiting the PI3K-mTOR pathway to restrict mitochondrial metabolism. C Stem Cell. 2016;18(2):214-28.
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