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Research paper

Study of microRNAs-21/221 as potential breast cancer biomarkers in Egyptian women

Tarek Mohamed Kamal Motawi [a], Nermin Abdel Hamid Sadik [a,*], Olfat Gamil Shaker [b], Maha Rafik El Masry [c], Fady Mohareb [d]

[a] Biochemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Einy, Cairo, Egypt
[b] Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Cairo University, Egypt
[c] Biochemistry Department, Faculty of Dentistry, October University for Modern Sciences & Arts (MSA), Giza, Egypt
[d] The Bioinformatics Group, School of Energy, Environment and AgriFood, Cranfield University, Bedford MK43 0AL, UK

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ABSTRACT

microRNAs (miRNAs) play an important role in cancer prognosis. They are small molecules, approximately 17–25 nucleotides in length, and their high stability in human serum supports their use as novel diagnostic biomarkers of cancer and other pathological conditions. In this study, we analyzed the expression patterns of miR-21 and miR-221 in the serum from a total of 100 Egyptian female subjects with breast cancer, fibroadenoma, and healthy control subjects. Using microarray-based expression profiling followed by real-time polymerase chain reaction validation, we compared the levels of the two circulating miRNAs in the serum of patients with breast cancer (n = 50), fibroadenoma (n = 25), and healthy controls (n = 25). The miRNA SNORD68 was chosen as the housekeeping endogenous control. We found that the serum levels of miR-21 and miR-221 were significantly overexpressed in breast cancer patients compared to normal controls and fibroadenoma patients. Receiver Operating Characteristic (ROC) curve analysis revealed that miR-21 has greater potential in discriminating between breast cancer patients and the control group, while miR-221 has greater potential in discriminating between breast cancer and fibroadenoma patients. Classification models using k-Nearest Neighbor (kNN), Naïve Bayes (NB), and Random Forests (RF) were developed using expression levels of both miR-21 and miR-221. Best classification performance was achieved by NB Classification models, reaching 91% of correct classification. Furthermore, relative miR-221 expression was associated with histological tumor grades. Therefore, it may be concluded that both miR-21 and miR-221 can be used to differentiate between breast cancer patients and healthy controls, but that the diagnostic accuracy of serum miR-21 is superior to miR-221 for breast cancer prediction. miR-221 has more diagnostic power in discriminating between breast cancer and fibroadenoma patients. The overexpression of miR-221 has been associated with the breast cancer grade. We also demonstrated that the combined expression of miR-21 and miR-221can be successfully applied as breast cancer biomarkers.

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1. Introduction

Worldwide, breast cancer is the most diagnosed cancer affecting women (Hortobagyi et al., 2005; Gill et al., 2007). Approximately 1.67 million new cases of breast cancer were diagnosed in 2012, and by 2025, this figure is predicted to escalate to 19.3 million. Although the highest reported prevalence of breast cancer is in developed countries, an increasing incidence and lower survival rate in developing countries has been found. This trend has been attributed to the adoption of the Western lifestyle (Porter, 2008), lack of breast cancer awareness and poor access to screening and health care services (Beaglehole and Yach, 2003; Parkin and Fernández, 2006; Badar et al., 2007; Rizwan and Saadullah, 2009). Breast cancer is the most frequent cause of cancer death in women from less developed regions (324,000 deaths, 14.3% of the total), and it is a leading cause of cancer death in more developed regions (198,000 deaths, 15.4%), second only to lung cancer (Ferlay et al., 2013; Bray et al., 2008).

According to the Egyptian National Cancer Institute (NCI), breast cancer is the most common type of cancer among Egyptian women, representing 18.9% of total cancer cases (Elatar, 2002), with an age-adjusted rate of 49.6 per 100,000 people. However, this represents

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; BLBC, basal-like breast cancer; CDK, cyclin-dependent kinases; CI, confidence interval; Ct, cycle threshold; EMT, epithelial–mesenchymal transition; ER, estrogen receptor; HCC, hepatocellular carcinoma; kNN, k-Nearest Neighbor; maspin, mammary serine protease inhibitor; miR-21, microRNA-21; miR-221, microRNA-221; miRNAs, microRNAs; mRNA, messenger RNA; NB, Naïve Bayes; PBS, phosphate buffer saline; PCA, principle component analysis; PDCD4, programmed cell death 4; RF, Random Forest; ROC, Receiver Operating Characteristic; RT-PCR, real-time polymerase chain reaction; RT, reverse transcription; SD, standard deviation; TNBC, triple negative breast cancer; TPM1, tropomyosin 1.

* Corresponding author at: Faculty of Pharmacy, Cairo University, Kasr El-Einy Street, Cairo 11562, Egypt.
E-mail address: nerminsadik@yahoo.com (N.A.H. Sadik).

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hospital-based data from tertiary referral centers and does not represent all breast cancer cases in Egypt. According to the population-based cancer registry of Ghrabiah, Egypt, the median age at diagnosis is one decade younger than in Europe and North America, while most patients are premenopausal (Ibrahim et al., 2002; Omar et al., 2003). Data from GLOBOCAN 2012 also reports that breast cancer is the most prevalent cancer in Egyptian women (Ferlay et al., 2013). The estimated 5-year prevalence of all cancer types occurring in females is 49.2% (Bray et al., 2008).

Fibroadenoma is the most common benign tumor occurring in female breast tissue (Dixon, 1991; Fechner, 1988). It is normally diagnosed in young women, but may also occur in older women (Hunter et al., 1996). It may result from abnormal growth and hyperplasia of the breast lobular tissue.

microRNAs (miRNAs) are highly conserved noncoding RNA molecules that are approximately 17–25 nucleotides in length. They control gene expression at the posttranscriptional level by interacting with a specific target messenger RNA (mRNA) (Lagos-Quintana et al., 2001; Lau et al., 2001; Lee and Ambros, 2001). They also regulate a variety of cellular processes, such as proliferation, differentiation, metabolism, aging, and cell death. As such, the importance of miRNAs is increasingly recognized in almost all fields of biological and biomedical fields (Li et al., 2010). In humans, it has been estimated that there are more than 1000 miRNAs in the genome, which regulate approximately 30% of all protein-coding genes (Lewis et al., 2005). The importance of miRNAs in oncogenesis has also been recognized. Dysregulation of miRNA expression plays an important role in cancer development through various mechanisms, such as deletions, amplifications, epigenetic silencing, or mutations in miRNA loci (Kosaka et al., 2010). To date, an association between differentially expressed miRNAs and many clinicopathological features has been shown, including mRNA expression-based classification (Blenkiron et al., 2007), tumor grade, and breast cancer staging (Iorio et al., 2005).

miR-21 is one of the most important miRNAs that is deregulated and over-expressed in many malignant tumors, including breast cancers (Chan et al., 2005). Some studies have reported that miR-221 is also deregulated in breast cancer (Shah and Calin, 2011). miRNA therapy could also be a powerful tool for the treatment of poorly differentiated cancer (Lu et al., 2005).

This study aimed to evaluate the expression level and diagnostic potential of serum miR-21 and miR-221 from Egyptian female patients with breast cancer, fibroadenoma, and healthy control subjects, regardless of the age and also to identify the relationship between the clinicopathological features of breast cancer and the expression of these miRNAs.

2. Subjects and methods

2.1. Patients

A total of 50 female breast cancer patients (mean age ± SD: 53.5 ± 7.5) were assigned to the study. Patients were selected from the Kasr El-Einy Hospital, Faculty of Medicine in Cairo, Egypt. The serum samples were obtained from breast cancer patients who were recently diagnosed by mammogram and from untreated patients. All patients were subjected to a complete clinical examination, and a full clinical history was taken. Patients who had received chemotherapy/radiotherapy or who had an acute infection were excluded from the study, as well as patients who had cancer at any other site at the time of the selection. Chest radiology, liver ultrasound scanning, and bone scanning were used to exclude those with metastatic cancer. The clinicopathological characteristics of breast cancer patients, including the histological grade and hormone receptor status are shown in Table 1.Demographic and clinical features of study groups are shown in Table 2.

Table 1
Clinicopathological characteristics of breast cancer patients.

Parameters	Patients frequency
Total	50
Age	Range (37–70 years)
Less than 55	31
More than 55	19
Distant metastasis	
M0	50
Family history	
Positive	28
Negative	22
Tumor type	
Invasive ductal carcinoma	46
Invasive lobular carcinoma	4
Grading	
G I, II	37
G III	13
Tumor stage	
T2	35
T3	15
Lymph node metastasis	
N 1	6
N 2	30
N3	14
Estrogen receptor	
Positive	8
Negative	42
Progesterone receptor	
Positive	7
Negative	43

In addition, blood samples were collected from 25 female fibroadenoma patients (mean age ± SD: 32.1 ± 14.4) who were diagnosed by mammogram and breast ultrasound. In these patients, the fibroadenoma masses were solid, smooth, painless, and mobile, while aspiration cytology confirmed that the masses were benign in each patient. Additionally, a set of 25 blood samples from healthy female subjects (mean age ± SD: 28.6 ± 5.9) was collected from the outpatient clinic at El-Kasr El-Einy Hospital. None of these individuals had been previously diagnosed with malignancies, hypertension, diabetes, or any other diseases. All procedures involving blood samples collection were performed by trained technicians at the outpatient clinic at El-Kasr El-Einy, Faculty of Medicine, Cairo University, Cairo, Egypt. All participants had Egyptian ethnic origin. The study was performed with the approval of the Faculty of Pharmacy, Cairo University local ethics committee and carried out in compliance with the Helsinki Declaration (2008). Informed consent was obtained from all of the subjects enrolled in this study.

Table 2
Demographic and clinical features of study groups.

Parameters		Control N = 25	Fibroadenoma N = 25	Breast cancer N = 50	P value
Age		28.6 ± 8.5	32.1 ± 14.4	53.5 ± 7.5[a,b]	<0.001*
Menstrual history	Pre-menopause	23	21	13	<0.0001*
	Post-menopause	2	4	37	
Family history	Yes	–	5	28	<0.0001*
	No	25	20	22	
Diabetes	Yes	–	–	13	<0.001*
	No	25	25	37	
Hypertension	Yes	–	2	12	0.007*
	No	25	23	38	

Values are expressed as the means ± S.D (age) or frequency. *Indicates statistical significance. P values <0.05 are considered significant.
Clinical data were analyzed by a. ANOVA and b. Chi square test and Fisher's exact test.
[a] Statistical significance from the control group.
[b] Statistical significance from the fibroadenoma group.

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