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Research paper

Prognostic and biological significance of microRNA-221 in breast cancer

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ABSTRACT

Introduction: Breast cancer (BC) is the most notorious cancer between females with high rates of morbidity and mortality. The aim of this study was to determine the differential expression of breast tissues microRNA-221 (miR-221) and assess its prognostic and biological significance in breast cancer (BC).
Methods: A quantitative reverse transcription PCR (qPCR) assay was performed to detect the expression of breast tissue miR-221 in different subtypes of BC (n = 76) and controls (n = 36) and its correlations with clinicopathological factors of patients. Univariate and multivariate analyses using the Cox proportional hazards model were performed to analyze the prognostic significance of miR-221 expression.
Result: Our data indicated that the relative level of miR-221 expression in BC tissues was significantly higher than that in noncancerous breast tissues ($p < 0.01$). Of 76 BC patients, 62 (81.6%) were positive cases. By statistical analyses, high miR-221 expression was observed to be closely correlated with advanced clinical stage ($p < 0.01$). Moreover, patients with high miR-221 expression had worse 5-year relapse free survival ($p = 0.0124$). Univariate and multivariate analyses indicated that high miR-221 expression was an independent poor prognostic factor for BC patients.
Conclusion: miR-221 is a potential biomarker for predicting the survival of BC patients and may be a molecular therapeutic target for BC.

1. Introduction

Breast cancer (BC) is considered the top cancer in women in both the developed and the developing countries. BC is a heterogeneous disease regarding molecular alteration and clinical outcome (Siegel et al., 2013; Polyak, 2011). TNM staging is considered the standard method in routine use for identifying BC prognosis, for the purpose of identifying patients of BC high risk mortality, and also who could respond to treatment (Wiseman et al., 2005). Recently, molecular techniques, especially gene expression profiling, have been used increasingly, in order to improve BC classification and to determine patient prognosis and response to therapy (Cava et al., 2014).

BC is a multistep process characterized by genetic and epigenetic alterations that affect the main cellular pathways involved in growth and development. Moreover, a better understanding of the molecular mechanisms involved in BC development may contribute to exploit potential helpful prognostic biomarker and therapeutic target for BC (Hayes et al., 2001).

MiRs are a class of small non-coding RNAs (18–24 nucleotides) which are involved in epigeneticposttranscriptional regulation of target mRNAs. Deregulated miRs have been considered as a hallmark of tumorigenesis (Iorio and Croce, 2009). MiRs hold great potential as therapeutic targets in cancer; however, more insights into the efficiency of miR inhibition for patient survival need to be elucidated (Iorio et al., 2005). miR-221/222 have been pointed out to be oncogenes or tumor suppressors, according to type of cancer (Croce, 2009). Rao and his colleagues demonstrated that miR-221/222 overexpression is associated with tamoxifen resistance but marked upregulation of miR-221/222 is characteristic of fulvestrant resistance (Rao et al., 2011). Some reports confer the association between miR-221 and tamoxifen resistance in BC (Miller et al., 2008; Zhao et al., 2008). Brognara et al. (2012), assessed the role of peptide nucleic acid targeting miR-221 through modulating p27Kip1 expression in breast cancer cells. Li et al. (2013), demonstrated the oncogenic role of miR-221 in targeting ARHI gene in BC.

The aim of this study was to evaluate miR-221 expression by A quantitative reverse transcription PCR (qPCR) in BC, to explore its clinical

Abbreviations: miRNA, Micro-ribonucleic acids; BC, Breast cancer; BMI, Body mass index; LN, Lymph node; ER, Estrogen receptor; PR, Progesterone receptor; Her-2 neu, Human epidermal growth factor receptor 2; IDC, Invasive duct carcinoma; ILC, Invasive lobular carcinoma; OCT, Oral contraceptive therapy; HT, Hormonal therapy.

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significance and to assess the potential value of miR-221 as a prognostic marker.

2. Patients and methods

2.1. Patients and clinical samples

Each research participant who agreed to donate her breast tissues left over after the performed diagnostic or therapeutic procedures for in vitro diagnostics, provided a signed copy of informed consent in accordance with the guidelines of institutional review board of the Faculty of Medicine ethical committee, Ain Shams University, Cairo, Egypt. All the patients were recruited from the General Surgery department, Ain Shams University Hospitals, began from May 2009 till January 2010. The patients were followed up for recurrence till January 2015.

We enrolled 78 breast cancer patients and age matched 18 control including; benign breast lesions (10 fibroadenoma, and 8 fat necrosis) and 18 healthy volunteers. All breast cancer patients who underwent primary breast tumor surgery were eligible for this study. Inclusion criteria were women aged >18 years and had unilateral breast cancer, received breast-conserving surgery or mastectomy. Exclusion criteria of cancer patients were; patients receiving either chemotherapy or radiotherapy; as well as those who had inflammatory mastitis or other types of cancer. Table 1 provides a summary of the clinical and biologic characteristics of the study.

The follow-up data was obtained through the hospital visits and considered from the first day after surgery. Recurrence after 90 days were considered events, dated and reviewed by at least 3 expert oncologists.

After surgery, all breast tissue samples were, immediately, flash frozen in liquid nitrogen and stored at −80 °C until used. Pathological and clinical information documented at the time of surgery included the stage of the cancer, grade and type of the tumor. Tumors were staged according to the American Joint Committee on Cancer (AJCC) TNM staging (NCCN, 2013; Compton et al., 2012). Histologic classification was based on the WHO classification system (World Health Organization, 2003) and was confirmed by two pathologists independently. For each breast tissue sample a 5 μm eosin/hematoxylin stained section was prepared to confirm that the tumor sample contained more than 70% of cancer cells and to ensure the absence of tumor cells in the normal specimen that were obtained from patients who underwent breast reduction surgery. Patients assessed for the expression of ER, PR and HER2/neu by immunohistochemistry. The pathologist was blinded to the participant's clinical history and the results of the immunohistochemistry.

2.2. miRNA isolation from breast tissue samples

miRNA was extracted from the breast tissue samples using miRNEasy RNA isolation kit (Qiagen, MD) according to the manufacturer's instructions to obtain the miRNAsfrom 30–50 mg total RNA samples. The concentration and quality of RNA was measured by NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, DE, USA). A ratio of absorbance at 260 nm and 280 nm ~2.0 is generally accepted as "pure" for RNA. Afterwards, the RNA was kept in −70 °C till its use in the reverse transcription polymerase chain reaction.

2.3. qRT-PCR analysis of miR-221 expression

1 μg miRNA was used in reverse transcription with a miScript II RT Kit (Qiagen/SABiosciences Corporation, Frederick, MD). Quantitative RT-PCR was carried out using Step OnePlus™ System (Applied Biosystems Inc., Foster, CA). SNORD-68 was used as the internal control. The PCR primers

Table 1
Study population demographic and clinicopathological characteristics in the study (N = 112). Add the total number of each group.

Clinico pathological factors		Group			χ^2 (p)
		Malignant No. (%)	Benign No. (%)	Normal No.(%)	
Age, mean ± SD		52.28 ± 12	49.44 ± 13.2	50.39 ± 11.16	F:0.861[a] p: (0.678)
Parity	Mutipara	63 (82.9%)	14 (77.8%)	18 (100%)	4.134[b]
	Nullipara	13 (17.1%)	4 (22.2%)	0 (0%)	p: (0.127)
Menopausal	Premenopausal	30 (39.5%)	6 (33.3%)	9 (50%)	1.089[b]
	Postmenopausal	46 (60.5%)	12 (66.7%)	9 (50%)	p:(0.58)
Family history	Positive	30 (39.5%)	1 (5.6%)	0 (0%)	16.572[b]
	Negative	46 (60.5%)	17 (94.4%)	18 (100%)	p: (0.001)[c]
BMI	Normal	17 (22.4%)	11 (61.1%)	11 (61.1%)	24.311[b]
	Overweight	21 (27.6%)	6 (33.3%)	6 (33.3%)	p: (0.001)[c]
	Obese	38 (50%)	1 (5.6%)	1 (5.6%)	
OCT	Past administration	31 (40.8%)	11 (61.1%)	12 (66.7%)	5.332[b]
	Never	45 (59.2%)	7 (38.9%)	6 (33.3%)	p: (0.07)
HT	Past administration	30 (39.5%)	13 (72.2%)	15 (83.3%)	14.8[b]
	Never	46 (60.5%)	5 (27.8%)	3 (16.7%)	p: (0.001)[d]
Molecular Subtype	Luminal A	35 (46.1%)	–	–	
	Luminal B	12 (15.8%)			
	Basal (triple negative)	19 (25%)			
	Her-2 overexpressing	10 (13.2%)			
Histological type	IDC	56 (73.7%)	–	–	
	Mixed	10 (13.2%)			
	Other	10 (13.2%)			
Stage	I	31 (40.8%)	–	–	
	II	38 (50%)			
	III	7 (9.2%)			
Grade	1	15 (19.7%)	–	–	
	2	45 (59.2%)			
	3	16 (21.1)			

BMI: body mass index, OCT; oral contraceptive therapy, HT: hormonal therapy, IDC: invasive duct carcinoma, ILC: invasive lobular carcinoma.
[a] One way Anova Test.
[b] Chi-square test.
[c] Highly significant.
[d] Significant correlation was detected between investigated groups at $p < 0.05$ using chi-square test.