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Analysis of serum genome-wide microRNAs for breast cancer detection

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ABSTRACT

Objective: Among methods for profiling levels of miRNAs, next-generation sequencing (NGS) has an effective one for genome-wide profiles, which not only can accurately quantify known miRNAs expression, but also discovery novel miRNAs. In this paper, we investigated that whether specific miRNAs were co-expressed in the serum and tissue of breast cancer (BC) patients as novel biomarkers by SOLiD sequencing.
Methods: Different miRNA expression profiles of serum and tissue in breast cancer patients and control subjects were obtained by NGS -SOLiD sequencing. Real-time PCR was used to selected and validated candidate miRNA-biomarkers. Novel miRNAs were predicted by computational pipeline, and validated by Northern blot analysis.
Results: Of genome-wide miRNA analysis using SOLiD sequencing, 7 miRNAs were found to be co-upregulated (i.e., miR-103, miR-23a, miR-29a, miR-222, miR-23b, miR-24 and miR-25). miR-222 was significantly increased in the serum of BC patients by further validation ($P<0.05$), which may be a useful biomarker for differentiating BC patients from controls with receiver operating characteristic (ROC) curve area 0.67 of (95% CI = 0.5649 to 0.7775). A novel miRNA, named miR-BS1 was preliminarily identified and validated. Pre-miR-BS1 has a characteristic secondary structure. Mature miR-BS1 expression was detected in MCF-7 and MDA-MB-231 cells. Through gene ontology analysis, predicted target genes of miR-BS1, such as FOXO3 and KRAS, were involved in cancer-related signaling pathway.
Conclusions: This study presented a connection between serum- and tissue- based miRNA of breast cancer which suggested that serum-miRNAs may be potential biomarkers for BC detection. And next-generation sequencing will provide a robust platform for miRNA profilings.

1. Introduction

Breast cancer (BC) is the most common type of cancer in women. It is the second leading cause of death by cancer in women, following only lung cancer [1]. Death rates from breast cancer have been gradually declined in recent years, which are likely due to increased awareness, and screening and improved treatment methods [2]. It is suggested that application of molecular biomarkers combined with clinic-pathologic features could improve management of BC patients [3]. Recently, microRNAs(miRNAs), small noncoding RNAs of ~22 nucleotides (nt) in length, have been referred to as a new, promising biomarker for cancer detection and diagnosis[4–7]. In breast cancer, it has been shown that the signatures of miRNA expression are correlated with tumor characterization and prognosis [8–11]. In the past few years, studies have been focused on cancer-specific miRNAs in the circulation from cancer patients [12–15], which is suggested that serum- or plasma- miRNAs are novel noninvasive biomarkers for cancers. In this study, we hypothesize that there is a serum-miRNA signature that can classify breast cancer and be used as a novel biomarker. To address this hypothesis, we obtained the genome-wide miRNA expression profile of serum and tissue from BC patients by SOLiD sequencing followed by a validation using qRT-PCR. Meanwhile, preliminary study of discovering novel miRNAs will be carried on.

2. Samples and methods

2.1. Samples

In miRNA screening step: miRNA profiles were generated from serum from 13 BC patients *vs.* 10 healthy controls, and cancerous vs. adjacent non-cancerous breast tissue from 5 BC patients by SOLiD sequencing. Two differential miRNA expression patterns were established. By comparing the genome-wide miRNA expression with serum and tissue, co-upregulated miRNAs were identified for further analysis.

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In selection and validation of candidate miRNAs step: cancerous and adjacent noncancerous breast tissues were collected from an independent group of 20 BC patients. The selected miRNAs must be significantly elevated in 20 BC patients.

Serum was collected from a group of 50 BC patients before undergoing operation, 50 age-matched controls, 20 benign breast cancer (BBC) and 20 ovarian cancer patients (OVC), respectively. The selected miRNA were further validated on these samples.

Blood and tissue samples of BC patients and controls were collected from Nanjing General Hospital of PLA, Jiangsu Provincial People's Hospital and Zhongda Hospital. Informed consent was obtained from participants for the use of blood and tissue samples in this study. This project was approved by Southeast University and Nanjing Medical University Clinical Research Ethics Committee, Nanjing, China. No patients received chemotherapy or radiotherapy before blood and tissue samples collection.

2.2. Methods

The methodological details of sample processing, RNA extraction, library construction, SOLiD sequencing, qRT-PCR, target prediction and GO analysis were described in our pervious study [16]. The strategy for prediction and experimental validation of novel miRNAs was described in Figs. 1 and 2.

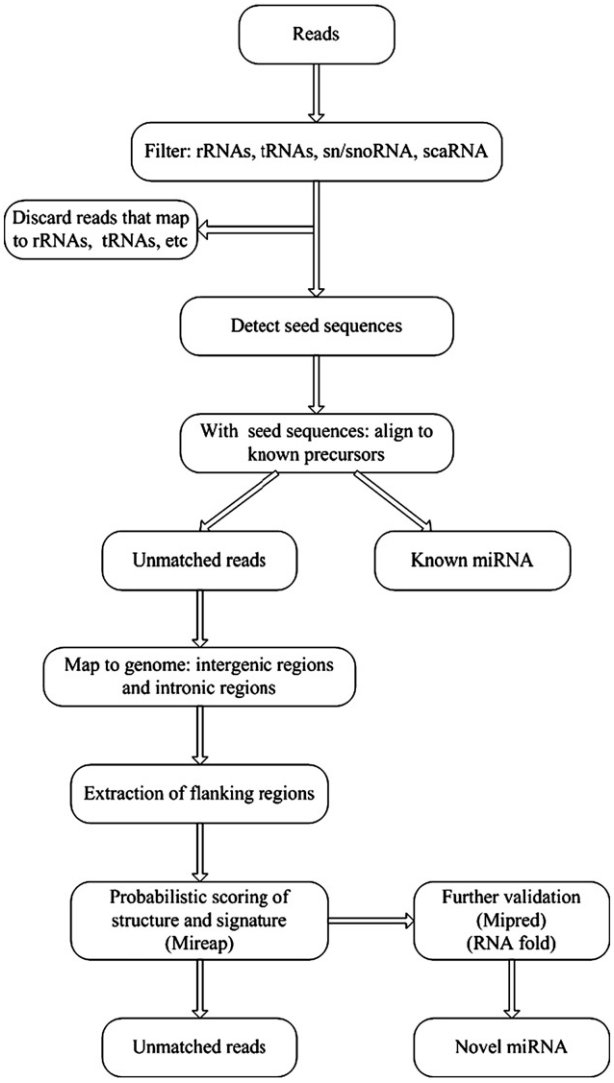

Fig. 1. Flowchart describing the computational pipeline for novel miRNAs prediction.

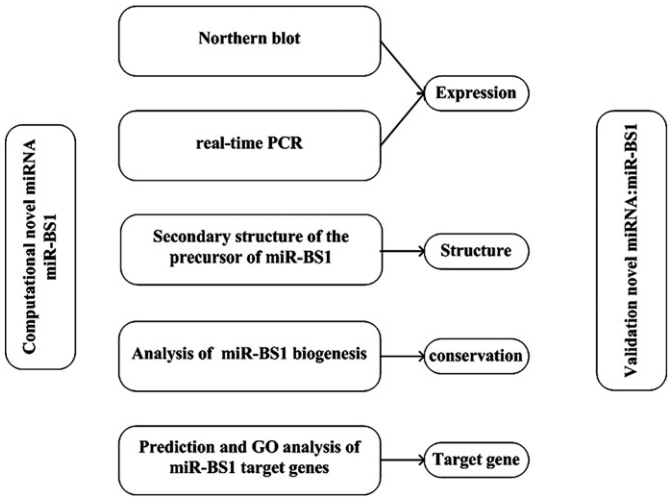

Fig. 2. Flowchart describing the experimental pipeline for novel miRNAs validation.

2.2.1. Cell lines

Human breast cell lines MCF-7 and MDA-MB-231 were gifts from Prof. LI Zhong (Nanjing Medical University). MCF-7 and MDA-MB-231 cells were grown in DMEM (Gibco) with 10% FBS (Hyclone), 100 μg/ml penicillin, and 100 U/ml streptomycin.

2.2.2. Northern blotting

40 μg of RNA was fractionated on a 15% polyacrylamide gel for an hour and transferred into Hybond-N + nylon transfer membrane (Amersham). Following transfer, RNA was fixed to the membrane using a UV-crosslinker. Oligo probes obtained from Invitrogen were labeled using gamma-^{32}P ATP (10 μCi/μl) and T4 polynucleotide kinase (10 U/μl, NEB). Membranes were hybridized with label probes using Ultrahyb-Oligo solution (BioDev-Tech). After hybridization, membrane were washed twice in 2 × SSC and exposed to film.

3. Results and discussions

3.1. Clinical characteristics of patients

A total of 188 participants including 88 BC patients, 60 healthy controls, 20 OVC and 20 BBC patients were recruited in the analysis. There were no significant differences of age between BC patients (55 ± 5.4 years), healthy controls (53 ± 6.4 years), OVC patients (56 ± 6.7 years) and BBC patients (50 ± 7.4 years) ($P > 0.05$, ANOVA). All of BC patients can be described as TNM stage I or II with no lymph node metastasis microscopically.

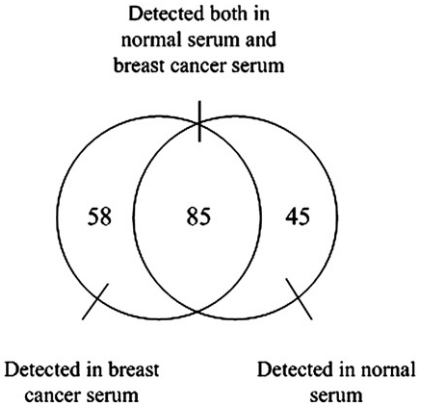

Fig. 3. Number and overlap of miRNAs between BC and C samples.