Identification and Validation of a Serum MicroRNA Panel for Detection of Early-Stage Breast Cancer

Ruiyang Zou[1,2], Lihan Zhou[1,2], Sau Yeen Loke[3], Patrick C.K Goo[4], Mikael Hartman[5,6], Heng-Phon Too[1,7]*, Ann Lee-Lim Siew Gek[3]*

1. Bioprocessing Technology Institute, A*STAR, Singapore; 2. MiRXES Laboratory, Singapore; 3. National Cancer Centre Singapore; 4. Diagnostics Development Hub, A*STAR, Singapore; 5. Department of Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; 6. Saw Swee Hock School of Public Health, National University of Singapore, Singapore; 7. Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore * Co-corresponding authors

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1. BACKGROUND

Survival outcomes of breast cancer patients can be significantly improved by early detection and treatment. Implementation of mammogram-based screening has significantly improved early detection of breast cancer in the west. However, the use of screening mammography is less prevalent in Asia partly due to social and cultural reasons. The aim of this study was to determine if a serum microRNA (miRNA) panel could be used as blood-based biomarkers to assist in the early detection of breast cancer.

We carried out a multi-center, multi-ethnic study to identify and validate miRNA biomarkers for the early detection of breast cancer. A total of 1042 subjects including 540 breast cancer cases (predominantly stage 1 and 2 cases) and 502 matched controls from 6 independent sources were included in this study. Among these, there were 750 American and European subjects recruited by biobanks and 292 Singaporean Asian Subjects recruited at the National Cancer Centre Singapore and the National University Hospital. The study was conducted in 3 phases in which sera of 289 European Caucasian serum samples (Discovery Cohort) were first interrogated to identify differentially expressed miRNAs between early-stage breast cancer cases and matched controls among 520 circulating miRNA candidates by quantitative RT-PCR using MiRXES assays. The remaining 753 subjects from 5 independent sources were assigned into two groups for biomarker optimization/algorithm development (Optimization Cohort, n=374) and validation (Validation Cohort, n=379).

2. STUDY DESIGN

578 candidate miRNAs
(miRbase annotated high confidence miRNA)

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1. Discovery Phase
Case-Control Cohort (Caucasian)
N=289 (183 cancers)

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33 candidate biomarker miRNAs
(Differentially expressed in cancer vs control)

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2. Optimization Phase
Case-Control Cohort
(Caucasian & Singaporean)
N=374 (177 cancers)

↓

8-miR multi-target miRNA panel
(With prespecified logistic-regression algorithm and cut-off)

↓

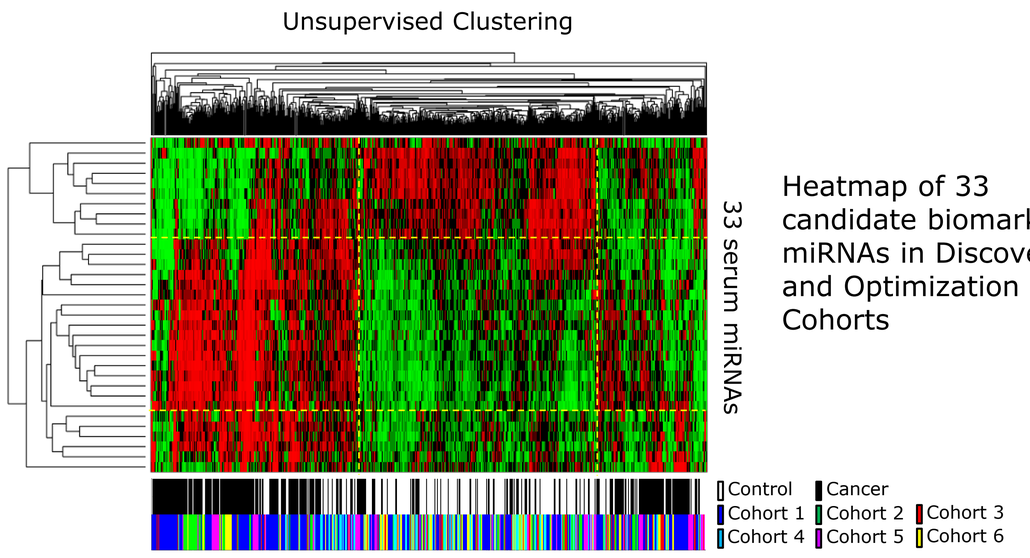
3. Validation Phase
Case-Control Cohort (Singaporean)
(Caucasian & Singaporean)
N=379 (180 cancers)

Cohort	Ethnicity	Enrolment Centre	Control	Breast Cancer
Discovery		**Total**	**106**	**183**
	Caucasian	Centre 1 (EU)	106	183
Optimi-zation		**Total**	**197**	**177**
	Caucasian	Centre 2 (US)	39	39
	Caucasian	Centre 3 (Ukraine)	33	23
	Caucasian	Centre 4 (Russia)	47	48
	Asian (Chinese, Malay, Indian)	Centre 5 (Singapore)	35	38
	Asian (Chinese, Malay, Indian)	Centre 6 (Singapore)	43	29
Validation		**Total**	**199**	**180**
	Caucasian	Centre 2 (US)	39	40
	Caucasian	Centre 3 (Ukraine)	34	24
	Caucasian	Centre 4 (Russia)	47	48
	Asian (Chinese, Malay, Indian)	Centre 5 (Singapore)	35	38
	Asian (Chinese, Malay, Indian)	Centre 6 (Singapore)	44	30

3. RESULTS

Identification of breast cancer associated miRNA biomarkers

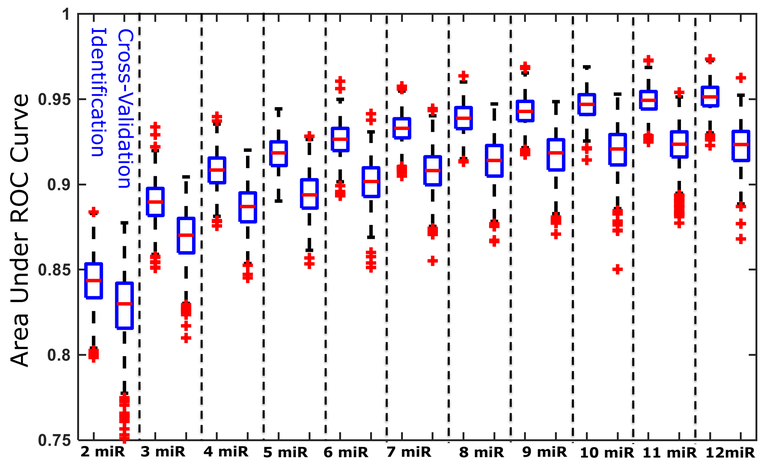
The absolute expression copy numbers of 520 candidate miRNAs were quantified in Discovery Cohort (n=289) using analytically validated miRNA-specific RT-qPCR assays (MiRXES, Singapore) via a highly-controlled workflow. Thirty-three miRNAs were found to be regulated between cancer and matched controls.



Heatmap of 33 candidate biomarker miRNAs in Discovery and Optimization Cohorts

Multi-miR biomarker panel optimization & cross validation

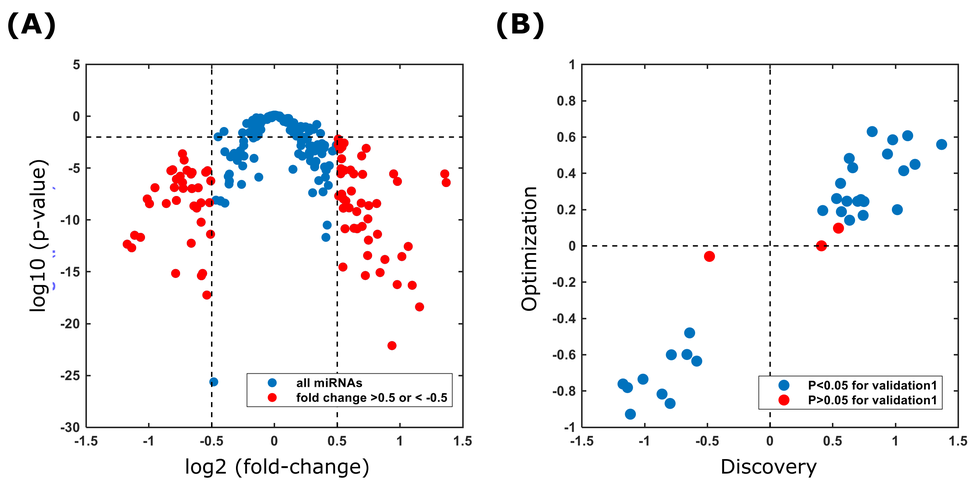
Combinations of distinctively-regulated miRNAs enhanced diagnostic accuracy. We identified and tested several miRNA panels (3-12 miRNAs) with high AUC in distinguishing breast cancer subjects from controls using four-fold cross-validation (matched by ethnicity, cancer subtype and stage). Improvements are not significant beyond 8 miRNAs.



miRNA Panel	Mean AUC	
	Identifi-cation	Cross-validation
2-miR	0.842	0.827
3-miR	0.890	0.870
4-miR	0.908	0.886
5-miR	0.919	0.893
6-miR	0.927	0.901
7-miR	0.933	0.907
8-miR	0.939	0.912
9-miR	0.943	0.917
10-miR	0.947	0.919
11-miR	0.950	0.922
12-miR	0.951	0.922

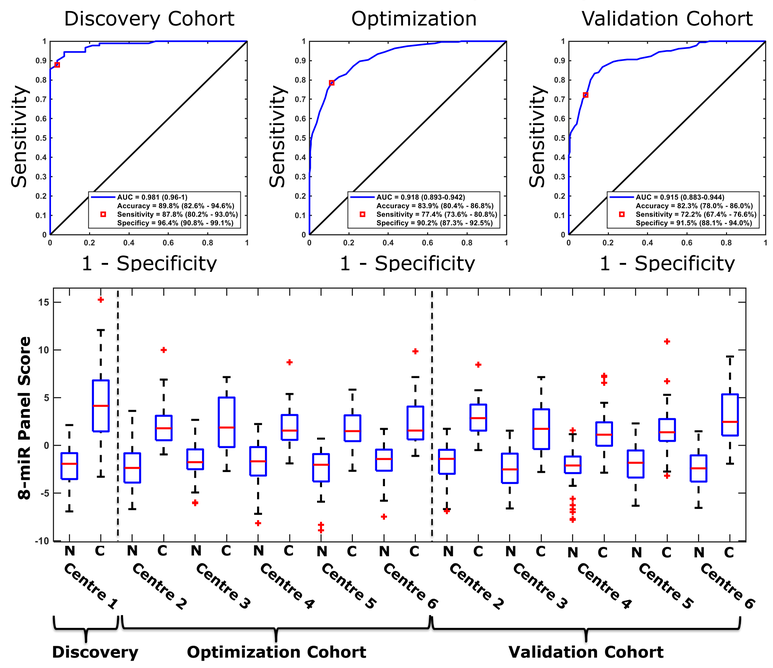
Verification of miRNA biomarker expression

(A) the volcano plot of the 324 detected in the Discovery Cohort. The x-axis is the log2 scaled fold-change of miR expression in breast cancer over matched control subjects. The y-axis is the p-value. Eighty-six miRNAs with p-value<0.01 and log2(fold-change)>0.5 were highlighted in red. (B) Correlation of the fold-change of 33 candidate miRNA biomarker between Discovery Cohort and Optimization Cohort. The miRNA biomarker candidates with p-value > 0.05 in the Optimization Cohort were highlighted in red.



Validation of 8-miR biomarker panel

The 8-miR panel, with a prespecified algorithm, was validated in independent sets of Caucasian and Singaporean Asian samples. Measured by AUC, the 8-miR biomarker panel could discriminate breast cancer from control subjects with AUC of 0.981, 0.918 and 0.915 respectively in the 3 Cohorts



Distribution of the 8-miR Panel Score across Cohorts and Centres. The breast cancer risk scores based on the 8-miR biomarker panel across different Cohorts and Centres are represented in box-plot.
N – Control Subjects,
C – Cancer Patients

Contact: lihan_zhou@mirxes.com

National Cancer Centre Singapore SingHealth · NUH National University Hospital · NUS National University of Singapore · A*STAR accelerate · A*STAR Bioprocessing Technology Institute