Mok et al., Gynecol Obstet (Sunnyvale) 2017, 7:6
DOI: 10.4172/2161-0932.1000443

Gynecology & Obstetrics
ISSN: 2161-0932

Research Article Open Access

Use of Blood-based mRNA profiling to Identify Biomarkers for Ovarian Cancer Screening

Samuel C Mok[1]*, Jae-Hoon Kim[2], Steven J Skates[3], John O Schorge[3], Daniel W Cramer[4], Karen H Lu[1], Choong-Chin Liew[4]

[1]*The University of Texas M. D. Anderson Cancer Center, Houston, TX*
[2]*Yongdong Severance Hospital, Yonsei University College of Medicine, Youngdong, Seoul, Korea*
[3]*Massachusetts General Hospital, Harvard Medical School, Boston, MA*
[4]*Brigham and Women's Hospital, Harvard Medical School, Boston, MA*

Abstract

Purpose: To identify candidate genomic signatures for the early detection of ovarian cancer using whole blood-based gene expression profiles.

Experimental Design: We performed Affymetrix U133Plus 2.0 GeneChip microarray analyses on whole blood RNA samples obtained from 14 ovarian cancer patients and 15 age-matched, healthy women. Genes differentially expressed were identified using a parametric Welch t-test. Real-time qRT-PCR analyses were performed on RNA prepared from 96 ovarian cancer patients and 83 age-matched healthy women, using primer sets specific for 14 genes. A Mann Whitney U test assessed individual gene significance. CA125 levels were determined in the same set of samples. We used logistic regression analyses and cross validation to assess the ability of linear combinations of specific transcripts combined with CA125 to distinguish cancer from controls.

Results: Microarray analyses showed that 9583 probes were significantly different in blood gene expression profiles from healthy women as compared with those from ovarian cancer patients ($p<0.05$). Real-time RT-PCR analyses on the 96 cases and 83 controls validated 7 genes, which showed significantly different expression levels in cases and controls. Logistic regression analyses and cross validation identified an optimal panel of markers including CA125, BRCA1, and KIAA0562, that could improve the sensitivity of CA125 alone to over 90% at 98% specificity in the detection of early stage ovarian cancer.

Conclusion: Circulating blood gene expression profiles identified RNA markers that can improve the sensitivity of CA125 in the detection of early stage ovarian cancer. Further validation is warranted to confirm the clinical usefulness of these biomarkers.

Keywords: Blood; Biomarkers; Cancer

Introduction

Ovarian cancer is the fifth most common cancer in women, but is the leading cause of death of all gynecological cancers. This poor outcome is at least partly because ovarian cancer is hard to detect at an early stage. Currently, most ovarian cancers (75%) are found late in the course of disease, when prognosis is poor and five year survival rates are less than 20% [1]. However, when diagnosed early, the prognosis of ovarian cancer is usually excellent, with five-year survival exceeding 90% for well-differentiated disease [1].

The increase in survival when ovarian cancer is detected at early stages suggests the need for a screening test for the disease. The low incidence of ovarian cancer however 40-50 cases/year per 100,000 women over 50 years of age creates a significant hurdle to early detection. In addition, the morbidity associated with exploratory surgery for possible ovarian cancer has led to general agreement that an ovarian cancer screening program should refer to surgery at most ten women for each case of screen-detected ovarian cancer; that is, to be acceptable, a screening strategy must have a positive predictive value (PPV) of at least 10% [2]. To achieve a PPV exceeding 10%, a screening program requires a highly sensitive test (>5%) and an extremely high overall screening specificity (>99.7%) [3].

Large prospective screening trials have investigated two testing modalities: blood tests for biomarkers such as CA125, HE4 and ultrasound. As compared with ultrasound, the blood test has the advantage of lower cost. But ultrasound outperforms blood testing with respect to sensitivity for early stage disease (although a direct randomized comparison has not yet been carried out) [4,5]. Trials of the antigen CA125 (MUC16) blood test, followed by ultrasound when CA125 values are elevated, have resulted in a PPV in excess of 20%, with a pre-clinical sensitivity of more than 70% [6,7]. Although in a large proportion of cases this strategy detected disease prior to the appearance of clinical symptoms, sensitivity for early stage disease using CA125>30U/ml was only about 40% [6]. The combined CA125 and HE4 has only minimal increase in sensitivity and there is only a marginally significant mortality reduction as shown in the most recent UKCTOCS study [8]. Thus a low cost and high sensitivity and specificity test for early-stage disease have not yet been developed. There is a critical need to continue searching for informative serum/plasma biomarkers for preclinical, early stage ovarian cancer.

Circulating blood is a dynamic, highly complex system that communicates with every tissue and organ in the body. Blood plays essential roles in homeostasis, in response to injury or infection, and

***Corresponding author:** Dr. Samuel C Mok, Department of Gynecologic Oncology and Reproductive Medicine, University of Texas M. D. Anderson Cancer Center T4.3908, 1515 Holcombe Boulevard, Houston, TX 77030, Tel: 713-792-1442; Fax: 713-792-1459; E-mail: scmok@mdanderson.org

Received June 22, 2017; **Accepted** June 28, 2017; **Published** June 30, 2017

Citation: Mok CS, Kim JH, Skates SJ, Schorge JO, Cramer DW, et al. (2017) Use of Blood-based mRNA profiling to Identify Biomarkers for Ovarian Cancer Screening. Gynecol Obstet (Sunnyvale) 7: 443. doi: 10.4172/2161-0932.1000443

in hormonal communication. It has been hypothesized that circulating blood may act as a "sentinel tissue" that can reflect states of health or disease within the body [9]. This concept is supported by recent studies showing that RNA profiles generated from circulating blood can be used as defined liquid biopsy to identify patients with cardiovascular disease [10,11], early osteoarthritis [12], schizophrenia, bipolar disorder [13-15], kidney diseases [16,17], Crohn's disease [18], and liver cancer [19]. Thus, blood-derived RNA can be a novel alternative to proteins or peptides as a source of biomarkers.

In the present study, we describe mRNA expression analyses of circulating blood samples obtained from ovarian cancer patients and age-matched healthy control subjects, and we evaluate whether blood-derived RNA can be used for ovarian cancer detection.

Materials and Methods

Blood sample collection

Women of all ages and ethnic backgrounds were included in this study. Blood samples were collected in clinic from 96 patients with ovarian cancer (50 had serous tumors; 9, clear cell; 16, mucinous; 3, endometrioid; and 18 had mixed histological types of ovarian cancer) before surgery, and 83 age-matched (mean age=55) healthy women. These 179 samples include the 15 controls and 14 cancer patients whose samples were used in the initial microarray analyses. Among the 96 cases, 39 patients had early (stage I and II) ovarian cancer. Exclusion criteria include patients who received chemo or radiation therapy. The subjects were recruited equally from both Yongdong Severance Hospital, Korea, and Brigham and Women's Hospital. All patients gave informed consent under protocols approved by the participating institutions. Blood samples from both cases and controls were collected in EDTA Vacutainer TM tubes (Becton Dickinson, Franklin Lakes, N.J.) and processed within 3 hours. The samples were centrifuged at 2000 rpm at 4°C for 5 minutes. The separated plasma was removed, aliquoted and frozen at -80°C for future analysis.

RNA isolation protocol

After plasma removal, a hypotonic buffer (1.6 mM EDTA, 10 mM KHCO3, 153 mM NH4Cl, pH 7.4) was added at a 3:1 volume ratio to lyse the red blood cells. The mixture was centrifuged to yield a white blood cell pellet, which was resuspended into 1.0 mL of TRIzol® Reagent (Invitrogen Corp., Carlsbad, CA) and 0.2 mL of chloroform, according to the manufacture's instructions. RNA quality was assessed on an Agilent 2100 Bioanalyzer RNA 6000 Nano Chip, according to the manufacturer's instructions. RNA quantity was determined by absorbance at 260 nm in a Beckman-Coulter DU640 Spectrophotometer.

Microarray analysis

Five micrograms of white cell RNA from each patient were hybridized onto the Affymetrix U133Plus 2.0 Gene Chip oligonucleotide array (Affymetrix; Santa Clara, CA), according to the manufacturer's instructions. Hybridization signals were scaled in the Affymetrix GCOS software (version 1.1.1), using a scaling factor determined by adjusting the global trimmed mean signal intensity value to 500 for each array, and imported into GeneSpring version 7.2 (Silicon Genetics; Redwood City, CA). Signal intensities were then centered to the 50th percentile for each chip, and for each individual gene, to the median intensity of each specific subset, first to minimize the possible technical bias, then for the whole sample set. Each blood sample yielded a "gene expression profile" representing each of the approximately 20,500 genes on the array.

Data from the microarray was loaded into dChip version 1.3 [20,21] for normalization and quantification. Normalization was performed using the default settings in the software. Expression values were quantified using the Perfect Match-only model [22]. The expression levels estimated by dChip were exported and loaded into Gene Spring version 7.2 for further analysis.

Differentially expressed genes were identified using a parametric test (Welch t-test). A p-value of 0.05 or less was applied and was considered significant, but due to the relatively large number of significant ($p<0.05$) genes, we used more stringent thresholds to identify genes with even greater significance. Thus, a list was generated of potential biomarker genes significantly up- or down-regulated in disease as compared with controls. Fourteen genes were chosen for qRT-PCR validation, based on a number of criteria, including, p-value, fold change, biological relevance and on support from the literature.

Real-Time Quantitative Reverse Transcription-Polymerase Chain Reaction validation Forward and reverse primers were designed using "Primer Quest" (http://biotools.idtdna.com/primerquest, Integrated DNA Technologies, Coralville, IA) and "Primer3" (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) for the following genes: ADAM9, ADIPOR1, KIAA0562, BCL11A, RAB11A, CEACAM1, TAGLN, MRIP, HIST1H2AC, TP53, BRCA1, HIST1H2B, HSPH1, and HSPA. The primer sets for the 14 genes are given in Table 1 (published online only). Serial dilution measurements for target gene and housekeeping gene, beta-actin (ACTB), were used to ensure that the values were within the linear range and the amplification efficiency was approximately equal for the target and ACTB. ACTB was selected as a housekeeping gene because no statistically significant differences were observed between control and disease group in this study. An automatically calculated melting point dissociation curve and agarose gels were used to examine and ensure the specific PCR amplification and the lack of primer-dimer formation in each well.

Amplification efficiency and specificity of the primer pairs were determined using serial dilution of reference cDNA generated from a normal blood RNA pool, with confirmation on agarose gel to ensure that the values were within the linear range and that the amplification efficiency was approximately equal for each of the target genes tested. For real-time RT-PCR assay, 1st strand cDNA was synthesized from 2 μg of total RNA using the ABI High Capacity cDNA Archive Kit (Applied Biosystems) on a Perkin–Elmer DNA Thermal Cycler according to the manufacturer's protocol. Further amplification and quantitation of specific cDNA were performed by Qiagen Quatitect SYBR® Green PCR Kit using an ABI Prism 7500 real-time PCR system (Applied Biosystems, Foster City, CA). The confirmation of specific amplification and lack of primer dimer formation were determined by calculating melting dissociation curve. In each sample, the expression level of a target gene was quantified by its threshold cycle (Ct) value, which is the concentration-dependent PCR cycle number at which the amplicon becomes distinguishable over background. Relative fold change of each individual gene was calculated using the comparative Ct equation (User Bulletin #2, 2001, Applied Biosystems) as follows: $2^{-\Delta\Delta Ct}$ where $\Delta\Delta Ct=\Delta Ct$ (of a sample)- mean ΔCt (of the control samples). $\Delta Ct=Ct$ (target gene) - Ct (house-keeping gene) where Ct values of target genes were normalized to housekeeping gene (β-actin).

CA125 ELISA assay

An immunoradiometric assay was performed on CA 125, according to the manufacturer's instructions (Abbot Diagnostics). Results were expressed as the mean absorbance of triplicate wells after subtraction of background values.

Statistical Analysis

We employed logistic regression analyses using SAS (Statistical Analysis System Version 9.0, Cary, NC), with the branch and bound algorithm of Furnival and Wilson [23] to identify optimal panels of one to five markers with the highest (statistical) score. Following identification of the optimal panels, cross validation using "leave-one-out" prediction was performed to solve the overfitting bias problem. Sensitivities at 90%, 95%, and 98% specificity were estimated by ranking the predicted fit for each control subject, obtaining the cut-off points corresponding to these levels of specificity, and applying the cut-points to the ranked predictions for the ovarian cancer cases. Particular attention was paid to the results at 98% specificity, since a first line blood test at this level of specificity, followed by ultrasound, has been shown empirically to achieve an overall screening specificity of the required minimum of 99.7% [24]. Optimal panels were derived by, first, including CA125 in the selection set, and then, subsequently, by excluding CA125 from the selected marker set. This strategy was applied to cases of all stages of ovarian cancer cases (n=96) as compared with control subjects (n=83), and then to early stage cases (n=39) as compared with control subjects (n=83). Expression levels of single genes, as distinct from a panel, between cases and controls were compared by the non-parametric Mann Whitney U test.

Results

Expressed gene profile from blood

We profiled gene expression from blood samples obtained from 14 ovarian cancer patients and 15 controls. Parametric Welch t test analysis identified a set of 9,583 probes, with a $P<0.05$ that could differentiate controls from ovarian cancer samples. A hierarchical cluster analysis using a subset of 227 probes ($P<0.001$) of differentially expressed genes from these 29 blood samples is shown in Figure 1. The 14 ovarian cancer profiles clustered separately from the group of 15 healthy control profiles.

Real-time qRT-PCR validation

We first selected a list of 14 genes (ADAM9, ADIPOR1, KIAA0562, BCL11A, RAB11A, CEACAM1, TAGLN, MRIP, HIST1H2AC, TP53, BRCA1, HIST1H2B, HSPH1, and HSPA) for real time qRT-PCR

Symbol	Ref. ID 5	5' Primer		3' Primer		Product
		Primer sequence	Position	Primer sequence	Position	
ADAM9	NM 003816	TGCCACTGGGAATGCTTTGTGT	1767	CCAACATTTGGTGCCTCGACTA	1881	115
ADIPOR1	NM_015999	TCTGCTTGGTTTCGTGCTGT	616	TGAAAGAGCCAGGAGAAGCTGA	765	150
KIAA0562	BCO50721	TAATCAGGTTGCTTTGGTTG	760	CTGTTGTGCCCAAGGTAGT	873	114
BCL11A	NM 022893	CCGCAGGGTATTTGTAAAG	703	AATCCATGAGTGTTCTGTGC	812	110
RAB11A	NM 004663	TTTAATCTGGAAAGCAAGAGC	234	GCTCCACGATAATATGCTGA	379	146
CEACAM1	NM 001712	ATTGGAGTAGTGGCCCTGGTTG	1407	ATTGGAGTGGTCCTGAGTGTGGT	1550	143
TAGLN	NM 003186	TGAAGGCAAAGACATGGCAG	429	TTCCCTCTTATGCTCCTGCG	561	133
HIST1H2AC	NM 003512	CGCTATCAAACCCAAAGGC	463	TTGGCAGGCACTTAGGATC	612	150
HIST1H2BK	NM 080593	GCCAAGGAGGGACTTTCTCT	427	TGAATGAGATCAAGAGGCCA	599	173
BCL11A	NM 022893	CCGCAGGGTATTTGTAAAG	703	AATCCATGAGTGTTCTGTGC	812	110
TP53	NM 000546	ATTTCACCCTTCAGATCCGT	1230	CCCTTTTTGGACTTCAGGTG	1371	142
M-RIP	NM 201274	GCCGACTTGGATGGAGAAAT	1334	CGCCCTCCTTTGTATGTATCT	1433	100
HSPH1	NM 006644	AACCTCACAGTCTCCCCCTT	2047	ATAG G CAG CT CAACATTCACC	2190	144
HSPA8	NM 006597	GATGCTGGAACTATTGCTGGT	555	CCTCCCAGGTCAAAGATGAG	682	128

Table 1: Primers used for real-time RT-PCR analyses.

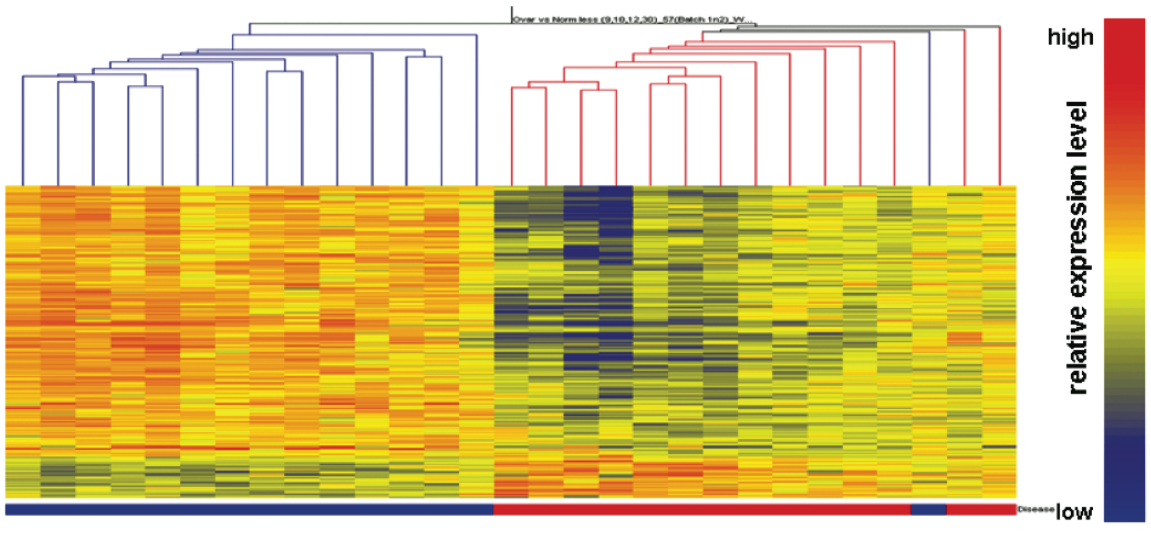

Figure 1: Hierarchical cluster analysis of 227 probes representing differentially expressed mRNA in blood obtained from healthy women and age-matched ovarian cancer patients. Healthy women (Blue): n=15, ovarian cancer patients (red): n=14.

validation. These 14 genes were selected based on their P value, fold change, number of appearances in the analysis, biological relevance and literature support. Primer sequences for the 14 candidate genes are listed in Table 1. These 14 candidate genes were tested against 179 samples (83 controls and 96 cases) using real-time qRT-PCR assay. Seven of the 14 gene transcripts were verified as significantly differentially expressed between cancer cases and controls (Mann Whitney U test P<0.05) with one gene down-regulated and six genes up-regulated in cancer cases. The P value and fold changes of the 4 of the genes are shown in Figure 2.

Cross validation analysis

We used logistic regression to identify optimal combinations of the 14 candidate biomarkers with panels of one to five biomarkers and estimated the cross-validated sensitivities at three high levels of specificity. Panels with more than five biomarkers did not significantly improve the sensitivity, and in some cases, decreased it (data not shown). Joint complementarity was observed by combining different markers such as BRCA1 and BRIP (Figure 3). We first evaluated the RNA markers alone and subsequently included CA125, which is the most widely researched ovarian cancer marker, to determine whether the RNA biomarkers can be used to complement CA125 in the detection of early stage ovarian cancer. The optimal panel including CA125 had five markers (CA125, BRCA1, MRIP, ADAM9, BCL11A or RAB11A) and gave a sensitivity of 95.8% at 98% specificity, as compared with 88.5% sensitivity with CA125 alone. Additional markers did not improve sensitivity. When CA125 was omitted from our selection, the optimal panel contained three markers (HIST1H2B, MRIP, and ADIPOR1) which gave a sensitivity of 62.5% at a specificity of 98% (Table 2A and 2B). Statistical analysis identified the optimal panel for early stage disease (CA125, BRCA1, and KIAA0562), which provided a sensitivity of 92.3% at 98% specificity, as compared with a sensitivity of 84.6% with CA125 alone. Without CA125 in the selection set, the optimal panel (BRCA1, MRIP, HIST1H2B, ADAM9, and KIAA0562) had a sensitivity of 53.8% at 98% specificity (Table 3A and 3B).

Discussion

Because ovarian cancer is usually discovered only at a late, difficult to manage stage, there is a need for better methods for early detection. The most widely researched ovarian cancer marker is CA125, and in pilot screening studies the marker has shown some value in postmenopausal women; sensitivity of this marker for early stage, pre-clinical disease detection however remains unclear [6]. A number of tumor markers for ovarian cancer, alone or as a panel, have also been identified and evaluated recently [25-28], but a useful screening marker or panel of markers has not been clearly established. Most of these markers are proteins or peptides in serum or plasma. Other sources, such as RNA from whole blood, have not been thoroughly explored.

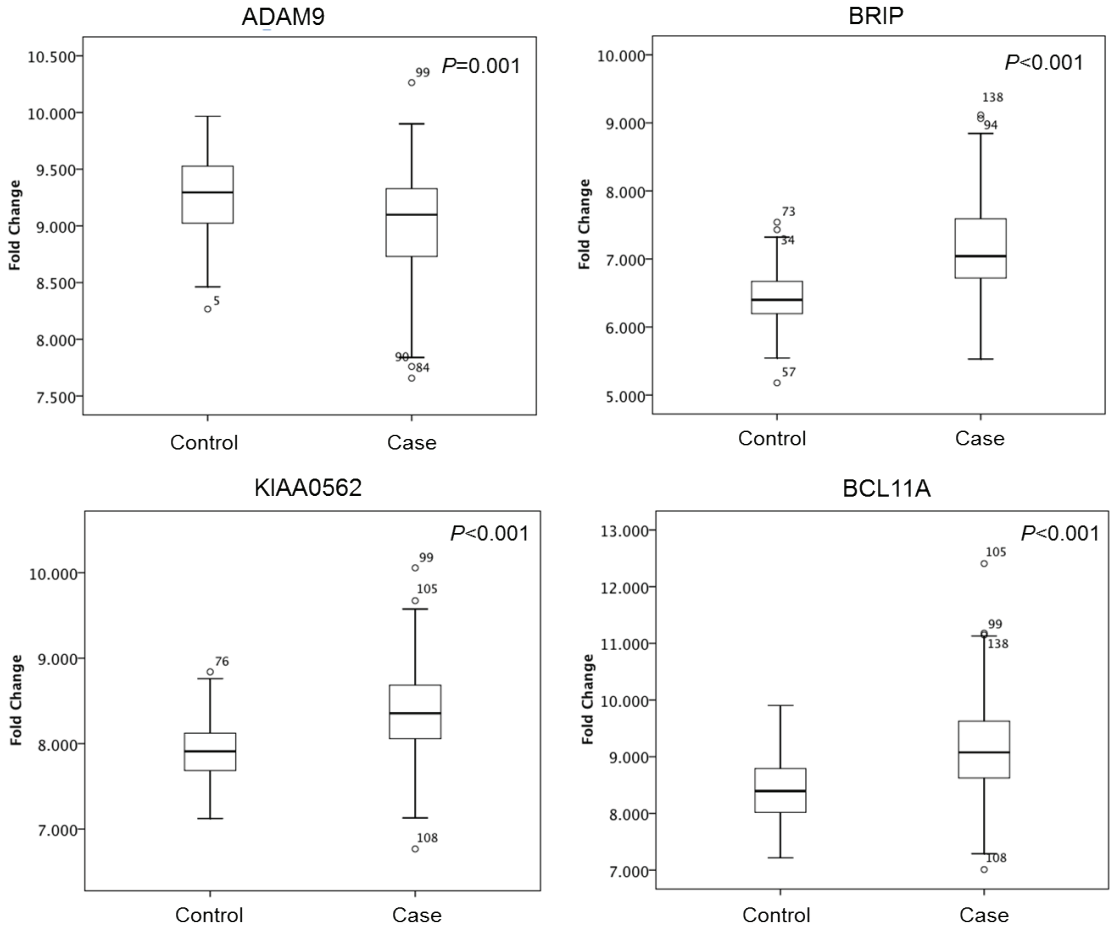

Figure 2: Expression levels of four candidate mRNAs in cases and healthy individuals. The box is bounded above and below by the 75th and 25th percentiles and the median is the line in the box. Whiskers are drawn to the nearest value not beyond a standard span from the quartiles; points beyond (out-liers) are drawn individually, where the standard span is 1.5 × (interquartile range). Statistical significance was determined by the Mann Whitney U test.

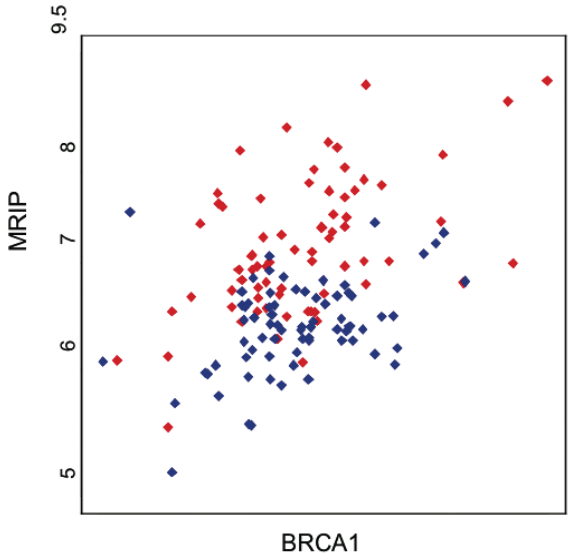

Figure 3: Plot of BRCA1 (horizontal axis) versus MRIP (vertical axis) showing the separation of cases (red, n=96) versus controls (blue, n=83) achieved through joint complementarity by the optimal panel of two markers at 98% specificity.

Marker Panel	Cross-validated Sensitivity (%)		
	90% Specificity	95% Specificity	98% Specificity
CA125	90.6	90.6	88.5
CA125 MRIP	95.8	91.7	91.7
CA125 MRIP BRCA1	95.8	95.8	94.8
CA125 MRIP BRCA1 ADAM9 CA125 MRIP BRCA1 ADIPOR1 CA125 MRIP BRCA1 BCL11A CA125 MRIP BRCA1 RAB11A CA125 MRIP BRCA1 HSPA8 CA125 MRIP BRCA1 HSPH1	95.8	95.8	94.8
CA125 MRIP BRCA1 ADAM9 BCL11A CA125 MRIP BRCA1 ADAM9 RAB11A	95.8	95.8	95.8

Early Stage Cases (n=39) and Late Stage Cases (n=57) Versus Controls (n=83)

Table 2A: Cross-validated sensitivity estimates of the best panels of 1-5 RNA markers including CA125 in early and late stage cases.

The first indication that RNA profiles generated from circulating blood could serve as diagnostic markers emerged in studies of cardiovascular disease [10,11]. Blood-derived RNA has since been shown to have discriminatory power in other conditions, such as schizophrenia, bipolar disorder [13-15], early osteoarthritis [12], kidney diseases [16,17] Crohn's disease [18], and liver cancer [19]. Using the RNA approach we have identified in circulating blood obtained from pre-operative ovarian cancer patients a gene expression signature that is distinct from the blood-based RNA signatures obtained from healthy women. Fourteen genes, which showed significant differences between cases and controls by parametric Welch t-test, were selected for further validation studies. Using the univariate non-parametric Mann-Whitney U test, expression levels of seven genes showed significant differences between cases and controls. Of interest, BRCA1, which did not show a significant difference between cases and controls by the non-parametric test, demonstrated complementarity with other markers using logistic regression analysis, in that it increased the sensitivity at a fixed specificity. These data suggest that, although a single marker may not show a significant difference between cases and controls, it may still be useful in the development of a marker panel as demonstrated by the regression model. With the limited number of patients available in any single clinical study and the increasing number of genes and gene products measurable with recent technologies, it will be difficult to determine which candidates are true signals of cancer and which are spurious. Resolving this difference will require new biological insights, as well as empirical replication across multiple geographical sites and clinical cohorts of patients. Nonetheless, our panel of biomarkers complementing ovarian cancer biomarker CA125 is a promising step towards a blood test for ovarian cancer screening. One component of our panel, BRCA1 RNA, is biologically related to ovarian carcinogenesis, and CA125 has empirically withstood the test of time through replication of positive results in many patient cohorts.

Our results demonstrate at a level of 98% specificity, the minimum required screening specificity for a first line test for ovarian cancer [3], our RNA marker panel including BRCA1, ADAM9, and BRIP increased the sensitivity of CA125 to more than 90% in detecting ovarian cancer in patients with early stage as well as all stages of the disease. These results suggest that these RNA markers together with CA125 may be used to develop a blood test for ovarian cancer screening.

Our data demonstrate the feasibility of using whole blood-based RNA biomarkers for ovarian cancer detection. The use of RNA biomarkers in screening is particularly attractive since it involves the use of multiplex quantitative RT-PCR analysis as a screening platform, which has a low coefficient of variation (CV) and does not require the generation of antibodies for protein biomarkers and the development of enzyme-linked immunosorbent assays (ELISA) for biomarker detection.

One limitation for this study is the relatively small size and they were collected from clinically identified patients. However, the pre-operative sensitivity estimates shown in this study are sufficiently high to encourage us to carry out future studies on a large collection of bio repository samples. These samples will be collected in prospective clinical screening studies, and include the panel of RNA markers alone or in combination with CA125 to obtain estimates of screening sensitivity and specificity. The crucial issue, which has yet to be addressed, is the sensitivity of a panel of biomarkers for detecting early stage ovarian cancer or screening subjects who would not have been

Marker Panel	Cross-validated Sensitivity (%)		
	90% Specificity	95% Specificity	98% Specificity
HSPA8	51.0	41.7	39.6
MRIP	62.3	43.8	33.3
MRIP HIST1H2B	67.7	60.4	57.3
MRIP BRCA1 HSPH1	75.0	54.2	47.9
MRIP BRCA1 HIST1H2B	71.9	65.6	50.0
MRIP BRCA1 KIAA0562	69.8	67.7	54.2
MRIP HIST1H2B ADIPOR1	66.7	64.5	62.5
MRIP BRCA1 KIAA0562 ADIPOR1	71.9	68.8	59.4
MRIP BRCA1 KIAA0562 ADAM9	70.8	67.7	58.3
MRIP BRCA1 CEACAM1 ADAM9 TAGLN	79.2	60.4	43.8
MRIP BRCA1 KIAA0562 HIST1H2B ADIPOR1	71.9	68.8	60.4
MRIP BRCA1 KIAA0562 ADAM9 BCL11A	69.8	63.5	62.5

Early Stage Cases (n=39) and Late Stage Cases (n=57) Versus Controls (n=83)

Table 2B: Cross-vali.dated sensitivity estimates of the best panels of 1-5 RNA markers excluding CA125 from selection process in early and late stage cases.

Marker Panel	Cross-validated Sensitivity (%)		
	90% Specificity	95% Specificity	98% Specificity
CA125	87.2	87.2	84.6
CA125 TP53 CA125 MRIP CA125 BRCA1	92.3 92.3 89.7	92.3 87.2 89.7	79.5 84.6 82.1
CA125 BRCA1 MRIP CA125 BRCA1 KIAA0562	94.9 92.3	94.9 92.3	89.7 92.3
CA125 BRCA1 MRIP ADAM9 CA125 BRCA1 MRIP HSPH1	94.9	94.9	92.3
CA125 BRCA1 MRIP ADAM9 BCL11A CA125 BRCA1 MRIP ADAM9 RAB11A CA125 BRCA1 MRIP ADAM9 CEACAM1 CA125 BRCA1 MRIP HSPH1 BCL11A	94.9	94.9	92.3

Early Stage Cases (n=39) Versus Controls (n=83)

Table 3A: Cross-validated sensitivity estimates of the best panels of 1-5 RNA markers including CA125 in early stage cases.

Marker Panel	Cross-validated Sensitivity (%)		
	90% Specificity	95% Specificity	98% Specificity
HSPH1 or HSPA8	46.2 51.3	38.5 33.3	20.5 25.6
BRCA1 MRIP	56.4	56.4	35.9
BRCA1 MRIP CEACAM1	71.8	51.3	41.0
BRCA1 MRIP CEACAM1 ADAM9	76.9	51.3	48.7
BRCA1 MRIP HIST1H2B ADAM9 KIAA0562 BRCA1 MRIP CEACAM1 ADAM9 TAGLN BRCA1 MRIP CEACAM1 ADAM9 KIAA0562	56.4 69.2 74.4	53.8 69.2 59.0	53.8 46.2 43.6

Early Stage Cases (n=39) Versus Controls (n=83)

Table 3B: Cross-validated sensitivity estimates of the best panels of 1-5 RNA markers excluding CA125 from selection process in early stage cases.

clinically detected until late stage disease. Sensitivity estimates based on pre-operative samples from clinically identified patients, either in early or late stage disease, are no substitute for estimates obtained from prospectively conducted clinical trials, and are at best a guide as to which markers to test in such a trial.

In conclusion, this study has demonstrated the potential value of using microarray analysis to identify differentially expressed mRNA in circulating blood. The findings suggest evidence for an association between a circulating blood mRNA signature and ovarian cancer, pointing to a rationale for further research to assess potential clinical utility. The identification of specific mRNAs in circulating blood that can improve sensitivity and specificity for early ovarian cancer detection will allow us to develop an ovarian cancer screening test for women which can detect the disease at early stages and thus improve patient survival rates.

Translational Relevance

It has been hypothesized that circulating blood may act as a "sentinel tissue" that can reflect states of health or disease within the body. Thus, blood-derived RNA can be a novel alternative to proteins or peptides as a source of biomarkers. In the present study, for the first time we describe using transcriptome profiling analysis on pre-operative circulating white cell RNA to identify a blood genomic signature (BGS) that is associated with ovarian cancer. Our results demonstrate that at a level of 98% specificity, our RNA marker panel increased the sensitivity of CA125 to more than 90% in detecting ovarian cancer in patients with early stage as well as all stages of the disease. These results suggest that these RNA markers together with CA125 may be used to develop a blood test for ovarian cancer screening.

Acknowledgements

This study was supported in part R01CA169200, and R01CA142832 from National Institute of Health, Department of Health and Human Services, Joanne Brookhart Ovarian Cancer Fund, National R&D Program for Cancer Control, Ministry of Health & Welfare, Republic of Korea (7-2006-0153) , and the CMB-YUHAN Research grant of Yonsei University College of Medicine.

References

1. Landis SH, Murray T, Bolden S, Wingo PA (1998) Cancer statistics. CA Cancer J Clin 48: 6-29.
2. Jacobs I, Bast RC, Jr. (1989) The CA 125 tumour-associated antigen: a review of the literature. Hum Reprod 4: 1-12.
3. Skates SJ, Xu FJ, Yu YH, Sjovall K, Einhorn N, et al. (1995) Toward an optimal algorithm for ovarian cancer screening with longitudinal tumor markers. Cancer 76: 2004-2010.
4. Van Nagell JR, De Priest PD, Reedy MB, Gallion HH, Ueland FR, et al. (2000) The efficacy of transvaginal sonographic screening in asymptomatic women at risk for ovarian cancer. Gynecol Oncol 77: 350-356.
5. Sato S, Yokoyama Y, Sakamoto T, Futagami M, Saito Y (2000) Usefulness of mass screening for ovarian carcinoma using transvaginal ultrasonography. Cancer 89: 582-588.
6. Jacobs IJ, Skates SJ, MacDonald N, Menon U, Rosenthal AN, et al. (1999) Screening for ovarian cancer: a pilot randomised controlled trial. Lancet 353: 1207-1210.
7. Einhorn N, Sjovall K, Knapp RC, Hall P, Scully RE, et al. (1992) Prospective evaluation of serum CA 125 levels for early detection of ovarian cancer. Obstetrics and Gynecology 80: 14-18.
8. Skates SJ (2016) EPIC Early Detection of Ovarian Cancer. Clin Cancer Res 22: 4542-4544.
9. Liew CC (1999) Methods for the detection of gene transcripts in blood and uses thereof. U.S. Patent Application No. 200200268730.
10. Ma J, Liew CC (2003) Gene profiling identifies secreted protein transcripts from peripheral blood cells in coronary artery disease. Journal of Molecular and Cellular Cardiology 35: 993-998.
11. Bull TM, Coldren CD, Moore M, Sotto-Santiago SM, Pham DV, et al. (2004) Gene microarray analysis of peripheral blood cells in pulmonary arterial hypertension. Am J Respir Crit Care Med 170: 911-919.
12. Lattanzio PJ, Marshall KW, Damyanovich AZ, Peemoeller H (2005) Characterization of proteoglycan depletion in articular cartilage using two-dimensional time domain nuclear magnetic resonance. Magnetic resonance in medicine: official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine 54: 1397-1402.
13. Tsuang MT, Nossova N, Yager T, Tsuang MM, Guo SC, et al. (2005) Assessing the validity of blood-based gene expression profiles for the classification of schizophrenia and bipolar disorder: a preliminary report. Am J Med Genet B Neuropsychiatr Genet 133: 1-5.
14. Glatt SJ, Everall IP, Kremen WS, Corbeil J, Sasik R, et al. (2005) Comparative gene expression analysis of blood and brain provides concurrent validation of SELENBP1 up-regulation in schizophrenia. Proc Natl Acad Sci USA 102: 15533-15538.
15. Vawter MP, Ferran E, Galke B, Cooper K, Bunney WE, et al. (2004) Microarray screening of lymphocyte gene expression differences in a multiplex schizophrenia pedigree. Schizophr Res 67: 41-52.
16. Alcorta D, Preston G, Munger W, Sullivan P, Yang JJ, et al. (2002) Microarray studies of gene expression in circulating leukocytes in kidney diseases. Exp Nephrol 10: 139-149.
17. Twine NC, Stover JA, Marshall B, Dukart G, Hidalgo M, et al. (2003) Disease-associated expression profiles in peripheral blood mononuclear cells from patients with advanced renal cell carcinoma. Cancer Res 63: 6069-6075.
18. Barnes EL, Liew CC, Chao S, Burakoff R (2015) Use of blood based biomarkers in the evaluation of Crohn's disease and ulcerative colitis. World J Gastrointest Endosc 7: 1233-1237.

19. Omar H, Lim CR, Chao S, Lee MM, Bong CW, et al. (2015) Blood gene signature for early hepatocellular carcinoma detection in patients with chronic hepatitis B. J Clin Gastroenterol 49: 150-157.

20. Li C, Wong WH (2001) Model-based analysis of oligonucleotide arrays: expression index computation and outlier detection. Proc Natl Acad Sci USA 98: 31-36.

21. Zhong S, Li C, Wong WH (2003) ChipInfo: Software for extracting gene annotation and gene ontology information for microarray analysis. Nucleic Acids Res 31: 3483-3486.

22. Zhang L, Miles MF, Aldape KD (2003) A model of molecular interactions on short oligonucleotide microarrays. Nat Biotechnol 21: 818-821.

23. Furnival GM, Wilson RW (1974) Regression by leaps and bounds. Technometrics 16: 499-511.

24. Skates SJ, Horick N, Yu Y, Xu FJ, Berchuck A, et al. (2004) Preoperative sensitivity and specificity for early-stage ovarian cancer when combining cancer antigen CA-125II, CA 15-3, CA 72-4, and macrophage colony-stimulating factor using mixtures of multivariate normal distributions. J Clin Oncol 22: 4059-4066.

25. Bandera CA, Ye B, Mok SC (2003) New technologies for the identification of markers for early detection of ovarian cancer. Current opinion in obstetrics & gynecology 15: 51-55.

26. Bast RC, Badgwell D, Lu Z, Marquez R, Rosen D, et al. (2005) New tumor markers: CA125 and beyond. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 15: 274-281.

27. Terry KL, Sluss PM, Skates SJ, Mok SC, Ye B, et al. (2004) Blood and urine markers for ovarian cancer: a comprehensive review. Disease markers 20: 53-70.

28. Petricoin EF, Ardekani AM, Hitt BA, Levine PJ, Fusaro VA, et al. (2002) Use of proteomic patterns in serum to identify ovarian cancer. Lancet 359: 572-577.