Breast Cancer Res Treat (2010) 120:25–34
DOI 10.1007/s10549-009-0357-6

PRECLINICAL STUDY

Evaluation of malignancy-risk gene signature in breast cancer patients

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Received: 14 January 2009 / Accepted: 21 February 2009 / Published online: 22 March 2009

Abstract We recently developed a malignancy-risk gene signature that was shown to identify histologically-normal tissues with a cancer-like profile. Because the signature was rich with proliferative genes, we postulated it might also be prognostic for existing breast cancers. We evaluated the malignancy risk gene signature to see its clinical association with cancer relapse/progression, and cancer prognosis using six independent external datasets. Six independent external breast cancer datasets were collected and analyzed using the malignancy risk gene signature designed to assess normal breast tissues. Evaluation of the signature in external datasets suggested a strong clinical association with cancer relapse/progression, and prognosis with minimal overlap of signature gene sets. These results suggest a prognostic role for the malignancy risk gene signature in the assessment of existing cancer. Proliferative biology dominates not only the earliest stages of tumor development but also later stages of tumor progression and metastasis.

Keywords Gene signature · Malignancy risk · Histologically-normal tissue

Introduction

Predicting breast cancer risk in histologically-benign breast tissue has always been a challenge that has been relegated to the pathologist who must judge the risk of breast cancer based on the presence of histological abnormalities such as atypical ductal hyperplasia (ADH) and lobular carcinoma in situ (LCIS). Unfortunately, many breast cancers do not seem to be preceded by these characteristic lesions, and even when present, these are not uniformly predictive of cancer risk. For this reason, we developed a malignancy risk signature that identified histologically-normal, but molecularly-abnormal breast tissues with a invasive ductal cancer-like gene expression profile. The signature was found to show increased prevalence of expression from histologically-normal tissue to ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC). Moreover, the signature was composed of genes markedly enriched for cell cycle and proliferative gene functions. For this reason, we postulated that the signature might be prognostic for existing breast cancers.

In this study, we evaluated the malignancy risk gene signature to see its clinical association with cancer relapse/progression, and cancer prognosis using seven independent external datasets. To accomplish this goal, we had to identify microarray datasets with curated longitudinal clinical endpoints. We were able to then test the proficiency of the malignancy risk gene signature in predicting

Electronic supplementary material The online version of this article (doi:10.1007/s10549-009-0357-6) contains supplementary material, which is available to authorized users.

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outcomes such as recurrence and survival using these datasets. We were also able to determine the degree of overlap of the malignancy risk gene set with any published gene sets prognostic for similar clinical endpoints, finding surprisingly little overlap amongst genes.

There is a need to better stratify breast cancer patients in order to better apply potentially toxic therapies for best therapeutic outcome. It is rational to predict that the genes linked to identifying patients at risk for harboring breast cancer may be the same as those predicting the progression of cancer.

Materials and methods

Malignancy risk signature

We previously identified a malignancy risk gene signature that is capable of discerning molecularly-abnormal breast tissues that appear histologically-normal [1]. About 117 genes (140 probe sets: Table 1 and Supplementary Table 1) composed the signature that was principally proliferative in function, indicating a role in the earliest stages of breast tumor development. Its clinical association with cancer risk was validated by RT-PCR and evaluated in two independent datasets. This signature has a number of potential clinical applications such as judging risk of breast cancer development following routine breast biopsy, judging the need for adjuvant radiotherapy after lumpectomy, and determining the need for completion mastectomy following lumpectomy for the breast cancer patient.

Evaluation of clinical association

We assessed the prognostic potential of the malignancy-risk score on six external independent data sets characterized with carefully annotated clinical data and longitudinal endpoints. Because each data set had a different set of available genes based on a number of different microarray platforms, we used whatever genes were in common with the malignancy gene signature to evaluate each data set (essentially a subset of the original malignancy risk gene signature). For binary clinical outcome (e.g., cancer relapse or relapse-free), the malignancy risk score based on the 1st PCA was used for comparison in two ways: (1) the continuous risk score and (2) a dichotomized risk score using the median risk score as a cutoff to dichotomize patients into two risk groups: (high risk with score > median and low risk with score < median). Statistical analysis included logistic regression, response operating characteristic (ROC) curve, support vector machine (SVM), as well as the univariate analysis. For survival outcome (e.g., time to metastasis), we used the continuous malignancy risk score and the median-cutoff binary risk score for data analysis. The Cox proportional hazard model was used to analyze the continuous risk score and the univariate analysis. Log–rank test and KM survival curves were used to compare two risk groups from the binary risk score. Supervised principal components method (SuperPCA): [2] was used to compare performance of various PCAs. This method calculated a standardized Cox score for each gene to rank its relevance to survival. For a given threshold of the Cox score, a subset of genes was selected to generate the first three PCAs as covariates for survival analysis. For this study, cross-validation was performed to compare these three PCAs to examine if the 1st PCA is sufficient to represent the malignancy-risk score. For ordinal clinical variables (e.g., from ADH, DCIS, to IDC), the continuous malignancy-risk score was used to correlate with cancer severity using Pearson correlation to evaluate the trend of the malignancy-risk gene signature with cancer progression. Analysis of variance was used to test the differences among the groups with the Tukey method [3] to adjust for P value for pair-wise comparison. We also used SVM analysis to evaluate the prediction performance.

Results

We assessed the malignancy-risk score on six external independent datasets. Statistical procedures were described in "Methods and materials". These external datasets permitted the evaluation of a number of properties of the malignancy-risk signature including differentiation of normal versus IDC, cancer relapse, progression, and cancer prognosis. (Table 2) is the summary analysis results for these datasets.

Malignancy-risk gene signature association with IDC

Turashvili et al.'s IDC study [4]

This study examined five IDC samples with two paired normal samples for each IDC (a total of ten normal samples). Because this study used the same microarray platform [1], all malignancy-risk gene probe sets were available in this data set to calculate the malignancy risk score for the five IDCs and the associated ten normal breast tissues. Since this was a matched design (paired normal with IDC), conditional logistic regression was initially used, but failed to converge due to strong separation of the risk score between the normal and IDC groups. For this reason, the random effect model was used to test a difference of the risk score between IDC versus normal tissues while adjusting for pairing information (i.e., subject variation). Data analysis showed the malignancy risk score was higher in IDC than in normal tissue within the same

Table 1 A subset of malignancy-risk genes associated with cancer relapse/progression and metastasis

Affy probe set id	Gene symbol	Turashvili et al.	Chanrion et al.	Ma et al.	van 't Veer et al.	Wang et al.	Huang et al.	Gene title
222608_s_at	ANLN		Y	Y	Y			Anillin, actin binding protein (scraps homolog, Drosophila)
202095_s_at	BIRC5		Y	Y	Y	Y		Baculoviral IAP repeat-containing 5 (survivin)
209642_at	BUB1		Y	Y	Y	Y	Y	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)
203755_at	BUB1B		Y		Y	Y	Y	BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast)
214710_s_at	CCNB1		Y			Y	Y	Cyclin B1
202705_at	CCNB2	Y	Y		Y	Y	Y	Cyclin B2
205034_at	CCNE2		Y	Y	Y	Y		Cyclin E2
203213_at	CDC2		Y		Y	Y	Y	Cell division cycle 2, G1 to S and G2 to M
203214_x_at	CDC2		Y		Y	Y	Y	Cell division cycle 2, G1 to S and G2 to M
210559_s_at	CDC2		Y		Y	Y	Y	Cell division cycle 2, G1 to S and G2 to M
1555758_a_at	CDKN3		Y	Y	Y	Y	Y	Cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)
209714_s_at	CDKN3		Y	Y	Y	Y	Y	Cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)
204962_s_at	CENPA		Y	Y	Y	Y	Y	Centromere protein A, 17 kDa
207828_s_at	CENPF		Y		Y	Y	Y	Centromere protein F, 350/400 ka (mitosin)
218542_at	CEP55	Y			Y	Y		Chromosome 10 open reading frame 3
218252_at	CKAP2		Y			Y		Cytoskeleton associated protein 2
203764_at	DLG7				Y	Y		Discs, large homolog 7 (Drosophila)
203358_s_at	EZH2				Y	Y	Y	Enhancer of zeste homolog 2 (Drosophila)
213911_s_at	H2AFZ		Y		Y	Y		H2A histone family, member Z
202503_s_at	KIAA0101		Y		Y	Y	Y	KIAA0101
204709_s_at	KIF23				Y	Y	Y	Kinesin family member 23
202107_s_at	MCM2	Y	Y			Y		MCM2 minichromosome maintenance deficient 2, mitotin (S. cerevisiae)
204825_at	MELK				Y	Y		Maternal embryonic leucine zipper kinase
204641_at	NEK2	Y			Y	Y	Y	NIMA (never in mitosis gene a)-related kinase 2
201577_at	NME1		Y	Y		Y	Y	Non-metastatic cells 1, protein (NM23A) expressed in
218039_at	NUSAP1	Y			Y	Y		Nucleolar and spindle associated protein 1
219978_s_at	NUSAP1				Y	Y		Nucleolar and spindle associated protein 1
222077_s_at	RACGAP1	Y	Y	Y	Y	Y		Rac GTPase activating protein 1
201890_at	RRM2			Y	Y	Y		Ribonucleotide reductase M2 polypeptide
209773_s_at	RRM2			Y	Y	Y		Ribonucleotide reductase M2 polypeptide
209218_at	SQLE	Y	Y	Y		Y	Y	Squalene epoxidase
1554408_a_at	TK1		Y	Y	Y	Y		Thymidine kinase 1, soluble
202338_at	TK1		Y	Y	Y	Y		Thymidine kinase 1, soluble
201291_s_at	TOP2A		Y	Y		Y	Y	Topoisomerase (DNA) II alpha 170 kDa
201292_at	TOP2A		Y	Y		Y	Y	Topoisomerase (DNA) II alpha 170 kDa
204822_at	TTK		Y		Y	Y		TTK protein kinase
204026_s_at	ZWINT		Y		Y	Y	Y	ZW10 interactor

Y symbol was used to indicate the association of each malignancy-risk gene with cancer relapse/progression (Chanrion et al. or Ma et al.), or metastasis (van 't Veer et al., Wang et al., or Huang et al.)

patient (*P* value = 0.029; Fig. 1). Univariate analysis also yielded 25 malignancy-risk genes with *P* values < 0.05 (Supplementary Fig. 1). These data support a strong association of the malignancy risk score with IDC. Note that the use of other PCAs (the 2nd PCA and the 3rd PCA) to calculate the risk score did not show differentiation of

Table 2 Summary table of the six external datasets for the clinical association of the malignancy-risk gene signature

Dataset	Sample size (*n*)	Endpoint	Statistics method	Test statistics	*P* value
Cancer risk					
Turashvili et al.'s IDC study	10	IDC versus normal	Random effect model		0.029
Cancer relapse/progression					
Chanrion et al.'s tamoxifen-treated primary breast cancer	155	Relapse of primary breast cancer	Continuous risk score		
			Logistic regression	Coefficient = 0.137	<0.0001
			ROC	AUC = 0.81	<0.0001
			SVM	Accuracy rate = 74%	
			Two-sample *t*-test		<0.0001
			Binary risk score		
			Logistic regression	OR = 8.16	<0.0001
Ma et al.'s breast cancer study	61	Histological status (ADH, DCIS, IDC)	Correlation analysis	*r* = 0.50 (Pearson or Spearman)	<0.0001
			Logistic regression	OR (DCIS) = 2.28 (compared to ADH)	0.016
			Logistic regression	OR (IDC) = 3.31 (compared to ADH)	0.008
Prognosis					
van 't Veer et al.'s breast metastasis dataset	Training = 78; test = 263	Time to metastasis	Continuous risk score		
			Log–rank test	χ^2 = 11.8 (training set); χ^2 = 20.4 (test set)	0.0006 (training); <0.0001 (test)
			Binary risk score		
			Log–rank test	χ^2 = 12.2 (training set); χ^2 = 22.4 (test set)	0.0005 (training); <0.0001 (test)
Wang et al.'s breast cancer relapse free survival study	286	Metastasis-free survival	Continuous risk score		
			Log–rank test	χ^2 = 12.8	0.0004
			Binary risk score		
			Log–rank test	χ^2 = 12.6	0.0004
Huang et al.'s breast lymph node study	37	Lynph node (pos vs. neg)	Continuous risk score		
			Logistic regression	Coefficient = 0.2	0.0107
			ROC	AUC = 0.75	0.0041
			SVM	Accuracy rate = 73%	
			Two-sample *t*-test		0.004
			Binary risk score		
			Logistic regression	OR = 7.29	0.007

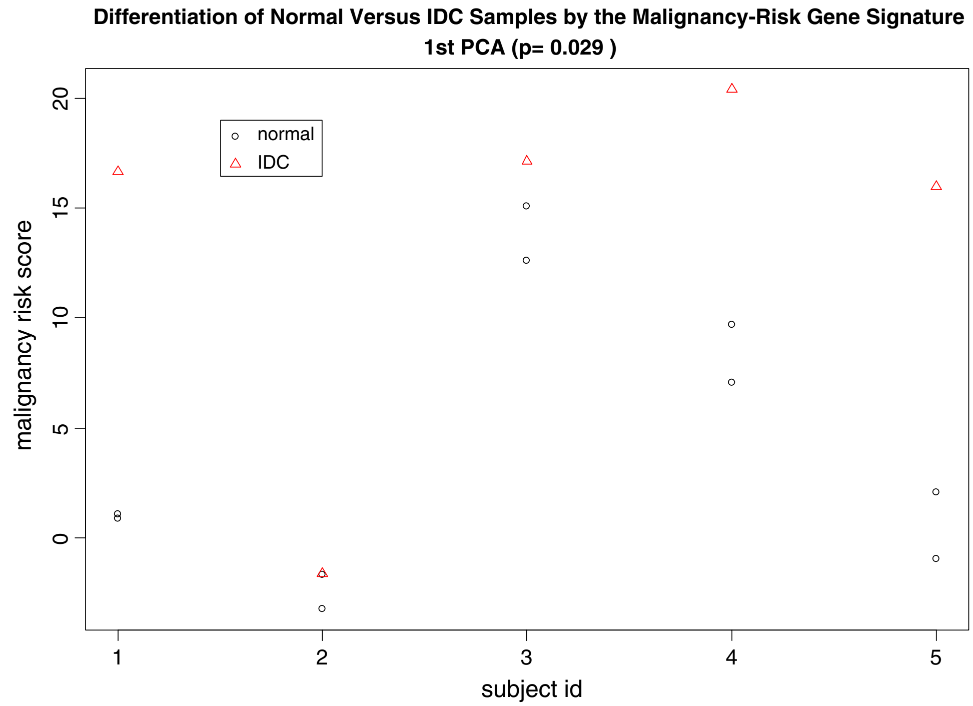


Fig. 1 Classification of normal and IDC tissues in Turashvili et al.'s study

the risk score between normal versus IDC samples (Supplementary Fig. 1).

Cancer relapse/progression

Chanrion et al.'s relapse study [5]

There were 155 patients (52 patients with relapse (R) and 103 patients who were relapse-free (RF)) who received adjuvant tamoxifen. The primary tumors from these patients were analyzed for expression profiles and a 36-gene signature was developed. In this study, we examined the malignancy-risk gene signature to see if it had comparable performance to the 36-gene signature to classify patients with relapse. The study used 70-mer oligonucleotide microarrays (22,656 genes) for expression profiles. There were 61 genes in common with the platform for the malignancy risk gene signature. Among these 61 malignancy-risk genes, there were only six genes in common with the Chanrion et al.'s 36-gene signature. We compared the malignancy-risk gene signature (61 genes in common), the top 36 malignancy-risk genes (based on univariate analysis), Chanrion et al.'s 36-gene signature, and the six malignancy-risk genes (in common with the Chanrion et al.'s 36-gene signature).

The four gene signatures showed a statistically significant association with the relapse of breast cancer in the logistic regression model (Table 2 and Supplementary Fig. 2). The continuous risk score yielded a statistically significant coefficient estimate (0.14–0.35 with $P < 0.0005$). The area under curve (AUC) for ROC curve ranged 0.60–0.83 with $P < 0.05$ (Fig. 2). The accuracy rate by SVM was similar, 72–74% (Supplementary Fig. 2). The two sample *t*-test also showed a statistically significant difference of risk score between relapse group versus relapse-free group ($P \leq 0.005$; Fig. 2). For the dichotomized risk score, the odds ratio (OR) ranged 2.99–11.67 with $P < 0.005$ for the first three gene signatures. Evaluation of other PCAs in the malignancy-risk gene signature showed that inclusion of the 2nd PCA and the 3rd PCA in the model showed little improvement in AUC and accuracy rate (Supplementary Fig. 2). In the univariate analysis based on two-sample *t*-test, there were 50 out of the 61 malignancy-risk genes (50/61 = 82%) with $P < 0.05$ (in contrast to 60% genes with $P < 0.05$ when using all the 22,656 genes; see Supplementary Fig. 2).

Ma et al.'s breast cancer study [6]

The study collected 8 ADH, 30 DCIS, and 23 IDC samples. There were 21 genes profiled in this study that were in common with the malignancy-risk gene signature, and were used to calculate the malignancy risk genes. Correlation analysis showed an increasing pattern of the risk score with cancer progression from ADH to IDC (Fig. 3). Pearson or Spearman correlation coefficient was 0.5, with a significant P value < 0.0001 by ranking the cancer status from 1 to 3 for ADH to IDC. Pair-wise comparison showed that the risk score was significantly different between IDC/DCIS and ADH (adjusted P value = 0.0001, and 0.0147

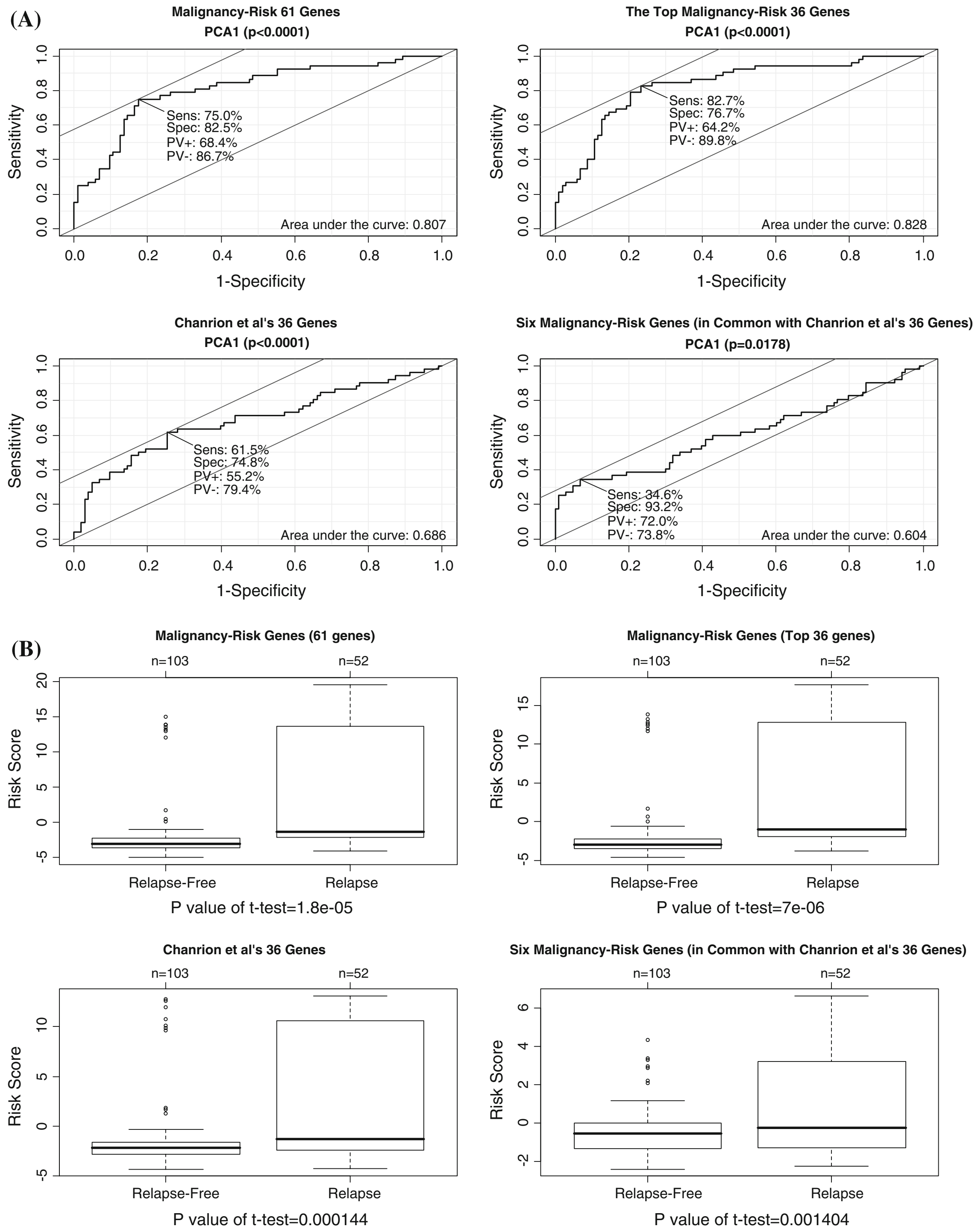


Fig. 2 Relapse classification of tamoxifen-treated primary breast cancers. (**A**) Comparison of ROC curve of the malignancy risk score among the four gene signatures. (**B**) Malignancy-risk score distribution among two groups, relapse and relapse-free

for IDC and DCIS, respectively; Fig. 3). Further analysis using logistics regression model (with the ADH group as the control group) demonstrated a strong association of the risk score with cancer status (OR = 2.28 and 3.31 for DCIS and IDC with *P* value = 0.016 and 0.008, respectively). In addition, we evaluated the prediction performance on the DCIS samples. A SVM classifier was built with an accuracy rate of 81% by leave-one-out-cross-validation. The classifier predicted most of the 30 DCIS samples to be in the IDC category (26/30) and a few DCIS cases favoring to ADH group (4/30; Supplementary Fig. 3). We also compared the malignancy risk score generated by PCA1 (1st

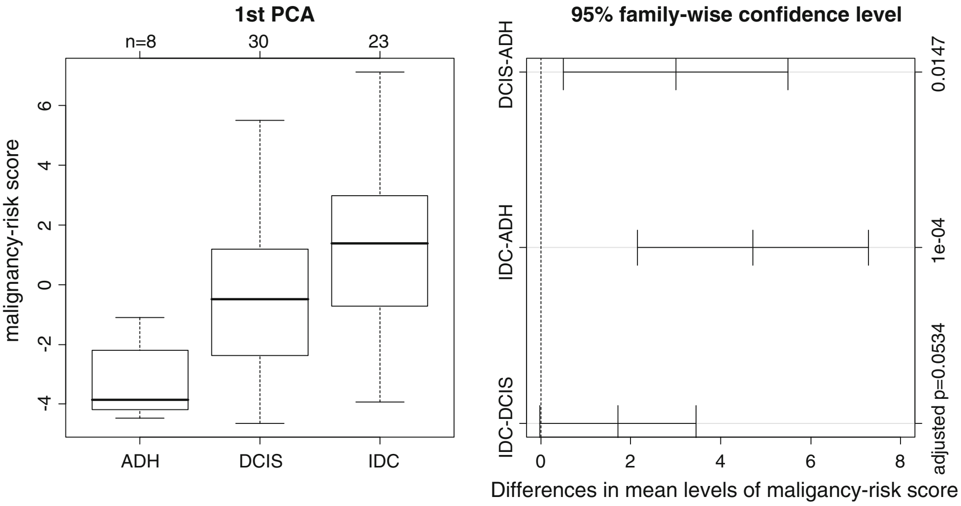


Fig. 3 Cancer progression in Ma et al.'s study by comparison of the malignancy-risk score among AHD, DCIS, and IDC. The 1st panel displayed distribution of malignancy-risk score among the three groups: ADH, DCIS, and IDC. The 2nd panel was 95% confidence interval of pair-wise comparison for the risk score among the three groups with adjusted *P* value in the right-hand side's y axis

PCA) versus other PCAs (2nd PCA and 3rd PCA). In contrast to PCA1 showing a cancer progression pattern, the 2nd PCA and the 3rd PCA did not demonstrate cancer progression from ADH to IDC (Supplementary Fig. 3). Univariate analysis by Pearson correlation yielded 16 malignancy-risk genes with a P value < 0.05 (Supplementary Fig. 3).

Cancer prognosis

van 't Veer et al.'s breast metastasis dataset [7]

This study collected one training set (a total of 78 breast cancer patient samples) and one test set ($n = 295$ patients, including 32 patients from the training set), with the time to metastasis as the clinical outcome, to develop a 70 gene signature. In our study, we used the training set ($n = 78$) and the test set which excluded the 32 patients from the training set ($n = 263$) to examine if the malignancy-risk genes could predict metastasis. There were 117 features that could be utilized to test with the malignancy-risk gene signature. Among them, there were seven genes in common (Supplementary Fig. 4) between the reported 70 gene signature and the malignancy risk gene signature.

We compared performance of survival analysis for the three gene signatures (malignancy-risk signature, 70 gene signature, and 7 genes in common) based on the malignancy risk score. For the dichotomized risk score, we used median of the risk score as cutoff to dichotomize the 78 patients (training set) into two risk groups. The median cutoff of the risk score from the training set was also used to dichotomize the patients into two risk groups for the test set ($n = 263$). Log–rank test and KM survival curves were used to compare the two risk groups for both datasets (training and test sets). The risk score was calculated in the same way for the 70 gene signature and 7 common genes, respectively.

The three gene signatures showed a statistical association with metastasis in both training and test sets (Table 2). For example, the three gene signatures performed well to separate survival curves of the two risk groups (Fig. 4; Supplementary Fig. 4) for both datasets (training and test sets). The 70 gene signature performed the best because the signature was derived from the dataset (Fig. 4). However, the performance for the malignancy-risk signature was comparable to the 70 gene signature, especially in the test set ($\chi^2 = 12.2$ with $P = 0.0005$ for the training data; and $\chi^2 = 22.4$ with $P < 0.0001$ for the test data). Even when limited to the seven genes in common, it also had a comparable performance (Supplementary Fig. 4). For comparison of various PCAs, analysis by SuperPCA showed that the model with the 1st PCA outperformed the other two models (2 PCAs and 3 PCAs), suggesting the 1st PCA was sufficient to represent the malignancy-risk score (Supplementary Fig. 4). Univariate analysis by the Cox proportional hazards model showed 48 malignancy-risk genes with a P value < 0.05 in both training and test sets (Supplementary Fig. 4).

Wang et al.'s breast cancer relapse free survival study [8]

The dataset includes 286 lymph-node negative breast patients with metastasis-free survival as clinical endpoint. A 76 gene signature was derived from this dataset [7] to predict distant metastasis. There were 102 probe sets (from the ~ 20 K probe sets) in common with the malignancy risk gene signature. There were only 4 genes in common (Supplementary Table 1; Supplementary Fig. 5) between the 76 gene signature and the malignancy risk gene signature. We compared the 3 gene signatures (malignancy-risk signature, 76 gene signature, and 4 genes in common) based on the malignancy risk score.

The three gene signatures performed well to show their statistically significant association with breast cancer

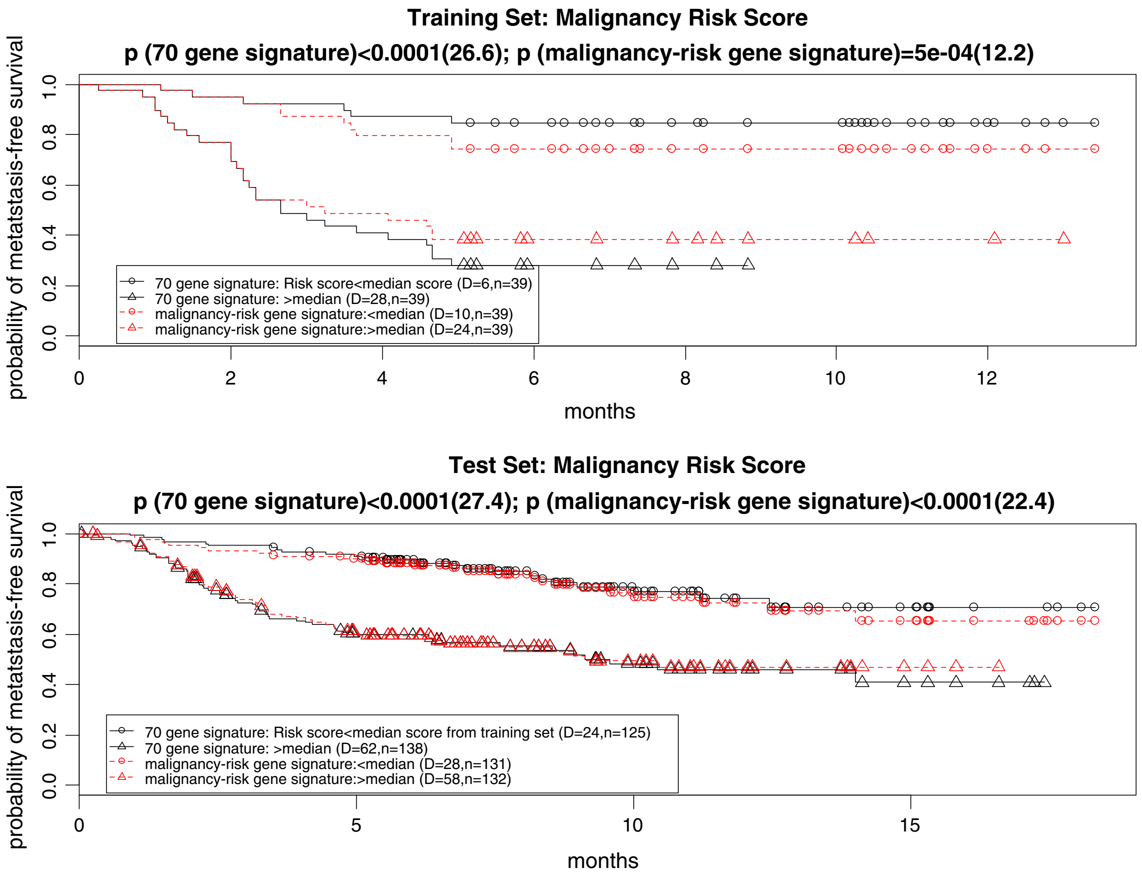
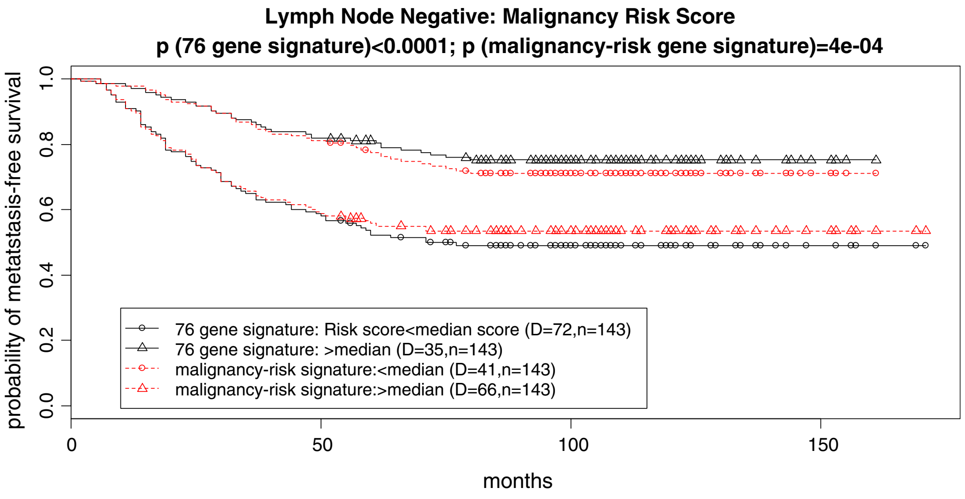


Fig. 4 Prognostic feature in van 't Veer et al.'s breast metastasis dataset

Fig. 5 Prognostic feature in breast cancer relapse free survival



relapse free survival either in the continuous risk score or in the dichotomized risk score (Table 2; Supplementary Fig. 5). For example, survival curves of the two risk groups were well separated (Fig. 5; Supplementary Fig. 5) for the dichotomized risk score. The 76 gene signature performed the best because the signature was derived from this dataset. However, the performance for the malignancy-risk signature ($\chi^2 = 12.6$; $P = 0.0004$) was almost comparable to the 76 gene signature. Even for the four genes in common, it also had a comparable performance (Supplementary Fig. 5). For comparison of various PCAs, analysis by SuperPCA showed that a cross-validation curve by the 1st PCA reached above the statistically significant level (Supplementary Fig. 5). While inclusion of the 2nd PCA and the 3rd PCA in the model also showed statistical significance, most was contributed by the 1st PCA (Supplementary Fig. 5). Univariate analysis yielded 64 malignancy-risk genes (of the 102 genes) with P value < 0.05 (Supplementary Fig. 5).

Huang et al.'s breast lymph node study [9]

This breast cancer microarray data included 18 patients with positive lymph node (LN) and 19 patients with negative LN. There were 112 probe sets from this dataset in common with the IDC-like normal gene signature. The malignancy risk score was generated using the 1st PCA from the 112 probe sets.

Logistic regression model showed both a statistically significant association of the malignancy risk score with the LN status (Table 2). The continuous risk score yielded a statistically significant coefficient estimate, 0.20, with $P = 0.0107$. The AUC for ROC curve was 0.75 (Fig. 6). For the dichotomized risk score, the odds ratio was 7.29 with $P = 0.007$ and an accuracy rate of 73%. SVM gave the same accuracy rate (Supplementary Fig. 6). Similarly, two sample t-test showed a statistically significant difference of risk score between positive LN versus negative LN ($P = 0.004$; Fig. 6). In addition, we included the 2nd PCA and the 3rd PCA in the model for analysis. Results showed little improvement in AUC, but had some improvement in accuracy rate for the first 3 PCAs (from 73 to 84%; Supplementary Fig. 6). Univariate analysis by two-sample t-test showed 34 probe sets (34/122 = 30%) with $P < 0.05$ (Note that three genes, CDC2, NME1, and TOP2A, had three probe sets per gene with $P < 0.05$; Supplementary Table 1; Supplementary Fig. 6). In contrast, there were only 7% genes (912 out of 12,625 probe sets) with $P < 0.05$ when using all probe sets. Fisher exact test showed a highly statistical significance ($P < 0.0001$), indicating that it is unlikely by chance to have such large proportion of significant genes (30%).

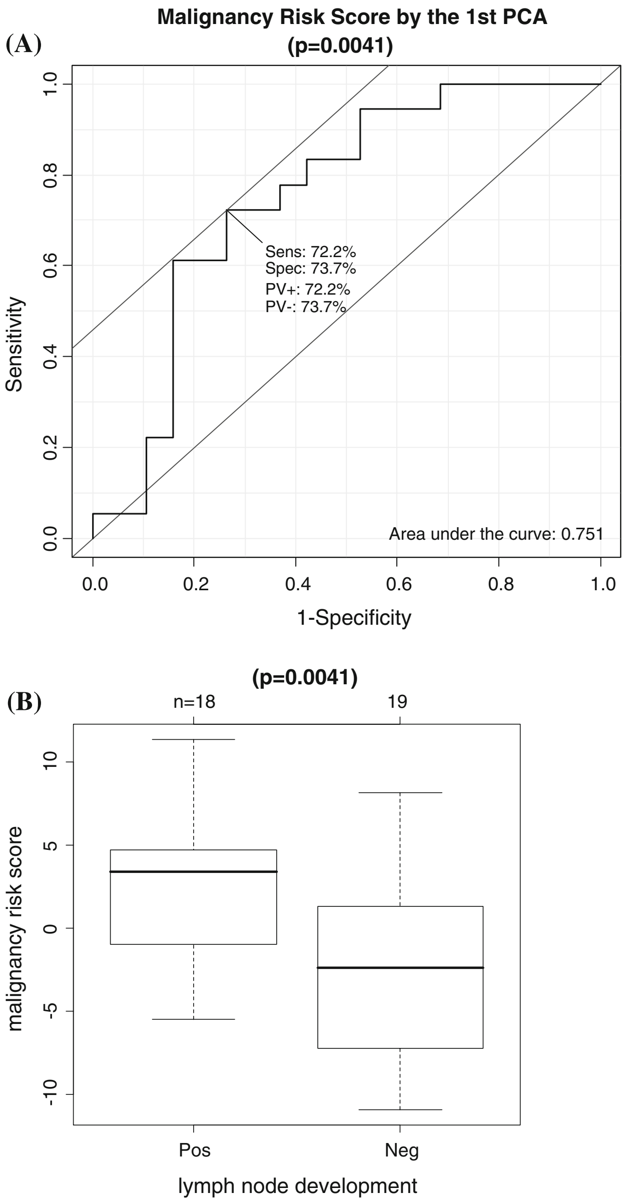


Fig. 6 Evaluation of malignancy-risk gene signature for breast lymph node development in Huang's breast study. (**A**) Area Under Curve (AUC) Calculation for Response Operating Characteristic Curve for the first PCA ($P = 0.0041$). (**B**) Difference of the malignancy risk score between positive LN versus negative LN ($P = 0.0041$)

Discussion

While the malignancy-risk gene signature may be principally useful to assess cancer risk, we explored its property in a broader scope. Evaluation on the six external independent datasets demonstrated the clinical relevance of the malignancy-risk gene signature not only to cancer risk, but also to cancer relapse/progression, and prognosis.

In the Turashvili et al.' study [3], we verified that the malignancy-risk genes identified in the 'IDC-like' normal tissues were highly associated with invasive ductal carcinomas (IDC). Our results showed that the malignancy-risk gene signature was able to differentiate the IDC and normal tissues *not linked* to cancer, confirming the malignancy-risk genes as a subset of IDC tumor associated genes.

In the Chanrion et al.'s study [5], we tested the malignancy-risk gene signature for its prediction ability on primary breast cancer relapse. Evaluation results showed that the malignancy-risk gene signature had comparable performance to the Chanrion et al.'s 36-gene signature to classify patients with cancer relapse. Interestingly, there were only six malignancy-risk genes in common with the 36-gene signature. In contrast, many significant malignancy-risk genes not in the 36-gene signature were proliferative genes (e.g., BUB1B, CCNB1, MCM2, and TOP2A).

We tested the malignancy risk gene signature on Ma et al.'s data [6] to evaluate its clinical relevance to cancer progression. We considered cancer risk as a continuous spectrum with normal tissue in the lower end and IDC tissue at the higher end. Since ADH and DCIS have been shown as precursors of IDC, we ascertained whether the malignancy risk gene signature exhibited a progressive trend from normal to IDC with ADH and DCIS as intermediate stages in the cancer risk spectrum. The existence of a strong trend with these features would provide a compelling evidence for the application of this signature on early prevention of cancer development. Results showed an increasing pattern of the malignancy-risk score with cancer progression from ADH to IDC. There were 16 malignancy-risk genes with an increasing expression pattern from ADH to IDC. Perhaps not surprisingly, the majority of these genes are known to be involved in the cell cycle. Since these genes were highly associated with cell proliferation and exhibited expression changes that were proportional to disease stage, these genes might be risk genes (precursor genes) useful in predicting cancer development and recurrence.

The last validation assessment of the malignancy-risk gene signature was to test its prognostic feature. Since patients with high cancer risk are likely to develop metastasis, the malignancy-risk genes may play a key role for cancer development. Validation results of the three datasets (van 't Veer et al.'s breast metastasis study [7], Wang et al.'s breast cancer relapse free survival study [8], Huang et al.'s breast lymph node study [9]) supported the hypothesis. The malignancy-risk gene signature performed well to show the statistically significant association with metastasis and lymph node development. The performance was comparable to the van 't Veer et al. and Wang et al.'s gene signatures. Again, there were only a few malignancy-risk genes in common with the two gene signatures.

In summary, we have developed a gene signature that has value in predicting both risk of cancer development as well as risk of cancer progression and metastasis. The signature hinges on genes with proliferative function, suggesting that the cell cycle plays a role both early and late in the spectrum of cancer.

Acknowledgments This work was supported by grants from the National Institutes of Health (RO1CA098522, R01CA112215, and P30CA76292). We thank Magaly Mendez for assistance in manuscript preparation. The authors gratefully acknowledge the helpful comments by anonymous referees.

References

1. Chen D-T, Nasir A, Culhane A, Venkataramu C, Fulp W, Rubio R et al (2009) Proliferative genes dominate malignancy-risk signature in histologically normal breast tissue. Breast Cancer Res Treat. doi:10.1007/s10549-009-0344-y
2. Bair E, Tibshirani R (2004) Semi-supervised methods to predict patient survival from gene expression data. PLoS Biol 2(4):E108. doi:10.1371/journal.pbio.0020108
3. Miller RG (1981) Simultaneous statistical inference. Springer, New York
4. Turashvili G, Bouchal J, Baumforth K, Wei W, Dziechciarkova M, Ehrmann J, Klein J, Fridman E, Skarda J, Srovnal J, Hajduch M, Murray P, Kolar Z (2007) Novel markers for differentiation of lobular and ductal invasive breast carcinomas by laser microdissection and microarray analysis. BMC Cancer 7:55
5. Chanrion M, Negre V, Fontaine H, Salvetat N, Bibeau F, Mac Grogan G et al (2008) A gene expression signature that can predict the recurrence of tamoxifen-treated primary breast cancer. Clin Cancer Res 14(6):1744–1752. doi:10.1158/1078-0432.CCR-07-1833
6. Ma XJ, Salunga R, Tuggle JT, Gaudet J, Enright E, McQuary P et al (2003) Gene expression profiles of human breast cancer progression. Proc Natl Acad Sci USA 100(10):5974–5979. doi:10.1073/pnas.0931261100
7. van de Vijver MJ, He YD, van 't Veer LJ, Dai H, Hart AAM, Voskuil DW et al (2002) A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 347(25):1999–2009. doi:10.1056/NEJMoa021967
8. Wang Y, Klijn JG, Zhang Y, Sieuwerts AM, Look MP, Yang F et al (2005) Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. Lancet 365(9460):671–679
9. Huang E, Cheng SH, Dressman H, Pittman J, Tsou MH, Horng CF et al (2003) Gene expression predictors of breast cancer outcomes. Lancet 361(9369):1590–1596. doi:10.1016/S0140-6736(03)13308-9