Gene Based Prediction of Clinically Localized Prostate Cancer Progression After Radical Prostatectomy

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Purpose: Accurate estimates of recurrence risk are needed for optimal treatment of patients with clinically localized prostate cancer. We combined an established nomogram and what to our knowledge are novel molecular predictors into a new prognostic model of prostate specific antigen recurrence.

Materials and Methods: We analyzed gene expression profiles from formalin fixed, paraffin embedded, localized prostate cancer tissues to identify genes associated with prostate specific antigen recurrence. Profiles of the identified markers were reproduced by reverse transcriptase-polymerase chain reaction. We used the profiles of 3 of these genes along with output from the Kattan postoperative nomogram to produce a predictive model of prostate specific antigen recurrence.

Results: After variable selection we built a model of prostate specific antigen recurrence combining expression values of 3 genes and the postoperative nomogram. The 3-gene plus nomogram model predicted 5-year prostate specific antigen recurrence with a concordance index of 0.77 in a validation set compared to a concordance index of 0.67 for the nomogram. This model identified a subgroup of patients at high risk for recurrence that was not identified by the nomogram.

Conclusions: This new gene based classifier has superior predictive power compared to that of the 5-year nomogram to assess the risk of prostate specific antigen recurrence in patients with organ confined prostate cancer. Our classifier should provide more accurate stratification of patients into high and low risk groups for treatment decisions and adjuvant clinical trials.

Key Words: prostate, prostatic neoplasms, prostate-specific antigen, nomograms, risk

Abbreviations and Acronyms

c-index = concordance index

FFPE = formalin fixed, paraffin embedded

PSA = prostate specific antigen

RP = radical prostatectomy

RT-PCR = reverse transcriptase-polymerase chain reaction

SAM = significance analysis of microarrays

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A recent review of cancer incidence rates in the United States indicated that 1/6 men will be diagnosed with prostate cancer during their lifetime.[1,2] PSA screening and advances in diagnostic technologies have resulted in the detection of prostate cancer at progressively earlier clinical stages. Approximately 84% of newly diagnosed patients with prostate cancer currently present with clinically localized disease.[3]

Identifying indolent cancer with a low risk of recurrence is critical for optimal treatment in patients diagnosed with clinically localized prostate cancer. The risk of morbidity from treatment is likely greater than the risk of death from prostate cancer in this group.[4,5] Conversely potential

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under treatment in patients at increased risk for recurrence remains an important issue in clinical management. Even with early presentation 15% to 20% of patients with localized prostate cancer experience biochemical (PSA) recurrence within 5 years of primary treatment and may benefit from adjuvant therapy.[6,7] Accurate risk estimates are also required for clinical trial design to ensure homogeneous patient groups.[8]

Of the currently available prediction tools to assess the risk of PSA recurrence in prostate cancer cases validated nomograms have gained wide acceptance as providing the greatest predictive accuracy.[8–11] These tools have generally been developed in prostate cancer cohorts including patients with seminal vesicle involvement and clinically advanced disease. However, classification tools developed specifically to accurately stratify newly diagnosed, clinically localized prostate cancer, potentially by adding novel biomarkers, are needed if treatment is to be better tailored to this patient subgroup. As in other cancers, a number of molecular markers and gene signatures of phenotype and prognosis have recently been developed for prostate cancer.[12–20] They provide some significant insight into the existence of distinct molecular classes of aggressive prostate cancer[18] and a number of potential candidate gene markers have been identified that predict progression to metastatic disease.[19,21] However, the specific application of these classifiers on management of clinically localized prostate cancer is not easily evaluated. The further considerations of practical use in the clinic and performance relative to established models, including nomograms, remain to be addressed in most instances.[8]

We identified genes correlating with PSA recurrence in patients with clinically localized prostate cancer with the goal of developing an accurate predictive classifier that can be readily applied in current routine clinical practice to manage organ confined disease. We report the development of a clinically viable test incorporating expression values of 3 novel gene markers, measurable by RT-PCR and the Kattan postoperative nomogram,[11] a widely used tool for clinical management of prostate cancer, to assess risk of PSA recurrence. Finally, we report that this new classifier provides improved accuracy compared to that of the Kattan nomogram to predict biochemical recurrence in this lower risk population.

MATERIALS AND METHODS

Patients and Tumor Samples

Patient information was obtained from the St. Vincent's Campus Prostate Cancer Group database with human research ethics committee approval. From January 1990 to December 2001, 960 patients were treated for prostate cancer with RP with no preoperative therapy at St. Vincent's Hospital, Sydney. The 316 consecutive patients with clinically localized disease assessed in the current study are those of the 960 in whom pathological stage was pT2A to pT3A. Minimum followup in censored patients was 5 years, RP was the primary treatment and tissue blocks were accessed from RP specimens for gene expression profiling experiments. The date of PSA recurrence was defined as the date of the first increase in serum PSA 0.2 ng/ml or greater after RP. These patients were randomly divided into a training and a test set. The test set was used only for validation purposes. Differences in the distribution of clinical variables between the training and test sets were evaluated by the t, log rank or chi-square test depending on whether the variable was continuous, time to event or categorical. All statistical tests were 2-sided with significance considered at $p < 0.05$.

Gene Expression Profiling

Sections (6 μm) from each FFPE tissue block were submitted for pathology review (JGK). They were macrodissected to ensure that 30% or greater malignant epithelium was used for total RNA extraction using the High Pure RNA Paraffin kit (Roche Diagnostics, Indianapolis, Indiana).

Gene expression profiling experiments were done in all total RNA specimens in the training series with a 1,200 gene custom designed DASL®.[22] Three control genes, including ALAS1, TUBA and ACTG1, were selected for the microarray and RT-PCR based on earlier prostate cancer studies.[12,23]

We used SAM with the survival mode to measure the prognostic significance of each probe on the array.[24–26] Probes were ranked by the absolute value of the test statistic. False discovery rates were calculated by data permutation.

RT-PCR was designed for the 30 top ranked prognostic marker candidates, including all genes under the lowest reported false discovery rates for increasing and decreasing probes. Genes with a poor Pearson correlation of <0.4 between the array and RT-PCR data among the training samples were excluded from further analysis.

Prognostic Model

Construction. Using the Memorial-Sloan Kettering Cancer Center online calculator (http://www.mskcc.org/mskcc/html/10088.cfm) we calculated the 5-year nomogram recurrence score in each patient using the postoperative historical model. To select variables for a multivariate model the δ CT values of candidate genes on RT-PCR and nomogram predicted probabilities were processed by the L1 regularization path algorithm in the training samples.[25] By cross-validating the training series using the path algorithm to set different limits on the potential for overfitting the Cox model we selected the signature with the least error. The final predictive model for deployment was built by fitting these select variables to the training set using a Cox proportional hazards model.

A cutoff for high and low model risk stratification, and a cutoff for the nomogram were chosen under the assumption that the costs of false-positive and false-negative results were equivalent. Under this assumption we chose a

cutoff providing the highest training set accuracy, as defined by the total number of correctly classified patients.

Validation. To evaluate the accuracy of predictive prognostic models with respect to actual freedom from recurrence in the test set we generated a calibration curve from the predicted 5-year recurrence-free probability estimated by Cox proportional hazards regression and the Kaplan-Meier estimates of the actual recurrence-free probability at 5 years.[24,27] We assessed the performance of the final prognostic model in the test set by Kaplan-Meier curves and HRs by stratifying test set patients into a low and a high risk group based on the preselected cutoff chosen to achieve the highest diagnostic accuracy in the training set. All statistical analysis was done with R, version 2.5.0.

RESULTS

Patient Characteristics

Total RNA was isolated from 316 prostatectomy FFPE tissues and 20 samples were excluded due to RNA degradation. Table 1 lists clinical and pathological characteristics in patients in the training and test sets. Median followup was 72 months and median time from RP to biochemical recurrence was 34 months in patients with recurrence. Recurrence developed in 98 of 296 patients, including 74 with recurrence within 5 years of surgery. The training series consisted of 138 patients with the remaining 158 set aside for the test set. There was no statistically significant difference in clinicopathological characteristics between the 2 patient sets (table 1).

Table 1. *Patient characteristics in test and training cohorts*

Characteristic	No. Training	No. Test	p Value
Age (continuous):			
Less than 60	58	56	0.50 (t test)
60 or Greater	80	102	
Gleason score:			
Less than 6	57	75	0.17 (chi-square test)
7	64	72	
8–10	17	10	
Unknown	0	1	
Clinical stage:			
T1	56	67	0.75 (chi-square test)
T2	80	90	
T3	2	1	
pT stage:			
pT2a	4	13	0.07 (chi-square test)
pT2b	13	14	
pT2c	61	81	
pT3a	60	50	
PSA ng/ml at diagnosis (continuous):			
10 or Less	97	107	0.55 (t test)
10–20 or Less	34	43	
Greater than 20	7	8	
Extracapsular extension:			
Capsular invasion	64	84	0.13 (chi-square test)
Focal	51	39	
Established	9	12	
None	14	23	
Margins:			
Pos	53	70	0.36 (chi-square test)
Neg	85	88	
Adjuvant treatment:			
Yes	12	13	1.00 (chi-square test)
No	126	145	
Outcome:			
Disease-free	89	109	0.40 (log rank test)
PSA recurrence	49	48	
Clinical (local/distant)	0	1	
5-Yr outcome:			
Disease-free	101	121	0.47 (log rank test)
PSA recurrence	37	36	
Clinical (local/distant)	0	1	

Gene Expression and Univariate Analysis

RNA samples from the training set of 138 patients were analyzed by DASL array. The permutation of the SAM algorithm revealed a 0% false discovery rate for 20 genes on the DASL array. As ranked by the SAM score, the top 30 genes had a 6.8% false discovery rate. These 30 genes were then assessed by RT-PCR using the same training set. Six of the 30 selected genes showed less than a 0.4 correlation between DASL and RT-PCR and, thus, they were removed from further analysis. The effect of each gene on recurrence-free probability was measured by Cox regression. The HR was used to quantify the relative risk of PSA recurrence for each increase of 1 normalized CT. HR and p values were recorded for the training and test sets (table 2). Of 24 markers 23 continued to have a significant association with recurrence in the test set. On the same analysis a 5-year postoperative nomogram was also a significant predictor of PSA recurrence in the training and test sets ($p = 0.001$ and 0.005, respectively).

Further variable selection was done in the RT-PCR training set to build a multivariate prognostic classifier. Four variables were selected by the L1 regularization algorithm, including the 3 genes DPT, SSBP1 and MYH11, and the 5-year nomogram. These 4 variables were then modeled in the training set using Cox regression analysis.

Classifier Validation and Survival Analysis

When testing the prognostic model in an independent test series of 157 patients, the classifier c-index was apparently higher than the nomogram c-index (0.77 vs 0.67) (fig. 1, *A*). Nomogram performance was consistent with that in published studies when tested in a consecutive prostate cancer cohort consisting of 960 patients from the same institution that was not limited to organ confined disease (c-index 0.72, fig. 1, *A*). We then used calibration curves to measure how close the 5-year predictive estimates in the test set were to actual recurrence probabilities. The classifier had good calibration across the spectrum of predictions for the test set

Table 2. *Cox regression of each tested RT-PCR marker in test and training cohorts*

Marker	Training		Test		Description
	HR*	Cox Regression p Value	HR*	Cox Regression p Value	
ACTG2	1.6	<0.001	1.35	<0.001	Actin, γ2
CALD1	1.37	0.007	1.44	0.003	Caldesmon 1
CBX3	0.53	0.05	0.64	0.02	Chromobox homolog 3
DCHS1	1.52	0.004	1.66	<0.001	Dachsous 1
DKK3	1.53	0.002	1.75	<0.001	Dickkopf homolog 3
DPT	1.48	<0.001	1.20	<0.001	Dermatopontin
FLNA	1.31	0.004	1.43	0.005	Filamin A, α
FLNC	1.65	<0.001	1.50	<0.001	Filamin C, γ
GAS1	1.43	<0.001	1.59	<0.001	Growth arrest-specific 1
GSN	1.63	0.003	2.02	0.001	Gelsolin
HIST1H3D	0.75	0.008	0.89	0.20	Histone 1, H3d
LIMS2	1.75	<0.001	1.76	<0.001	LIM and senescent cell antigen-like domains 2
LMOD1	1.80	<0.001	1.57	<0.001	Leiomodin 1
MT1X	1.56	0.001	2.06	<0.001	Metallothionein 1X
MYH11	1.68	<0.001	1.29	<0.001	Myosin, heavy polypeptide 11
MYLK	1.73	<0.001	1.56	<0.001	Myosin, light polypeptide kinase
PDLIM3	1.37	0.003	1.33	0.002	PDZ and LIM domain 3
PDLIM7	1.92	<0.001	1.47	0.01	PDZ and LIM domain 7
RASL12	1.84	<0.001	2.09	<0.001	RAS-like, family 12
SH3BGRL	1.32	0.04	1.81	<0.001	SH3 domain binding glutamic acid-rich protein like
SMTN	1.89	<0.001	1.78	<0.001	Smoothelin
SORBS1	1.68	<0.001	1.40	0.002	Sorbin and SH3 domain containing 1
SSBP1	0.34	0.02	0.32	<0.001	Single-stranded DNA binding protein 1
TNS1	1.88	<0.001	1.70	0.001	Tensin 1

* For each increase of 1 in normalized CT value.

compared to an ideal predictor while the 5-year nomogram showed less accuracy to detect more aggressive cases (fig. 1, *B*).

The training set cutoff was used to place test set patients into a high or a low risk group. Kaplan-Meier analysis for PSA recurrence-free probability showed a highly significant difference in time to PSA recurrence in the predicted low and high risk groups (HR 6.85, 95% CI 3.77 to 12.43, p <0.001, fig. 2, *A*). At 5 years the absolute difference in PSA recurrence

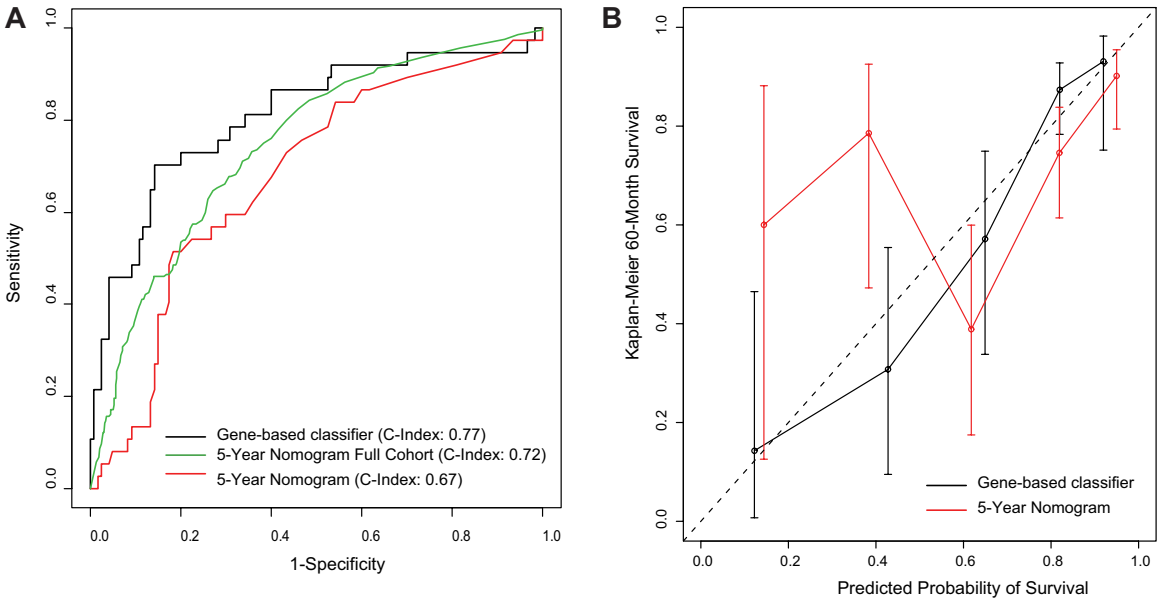

Figure 1. ROC curves (*A*) for gene based classifier (black curve), and 5-year nomogram in test cohort of 157 patients (red curve) and in full cohort of 960 (green curves) (c-index 0.77, 0.67 and 0.72, respectively), and calibration curves (*B*) for gene based classifier and 5-year nomogram (red curve) in test cohort.

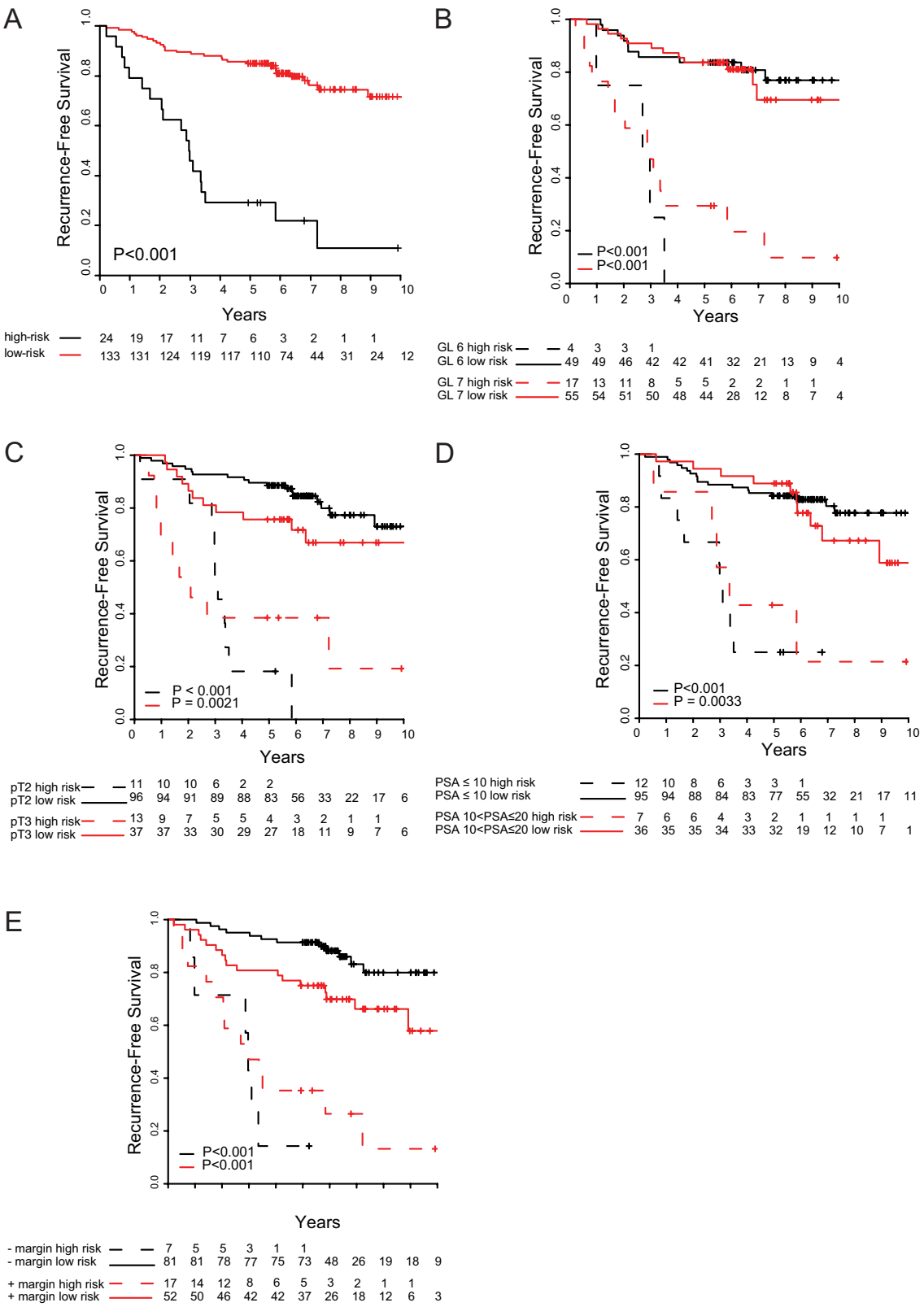

Figure 2. Estimated PSA recurrence-free probability in test set patients (*A*), and in those with Gleason score (*GL*) 6 or 7 (*B*), pT2 or pT3a (*C*), preoperative PSA 10 ng/ml or less, or 10 to 20 or less (*D*) and positive (+) or negative (–) surgical margins (*E*). Values indicate number of patients at risk.

between the 2 groups was 58% (85% vs 27%). The classifier also represented a strong prognostic factor for PSA recurrence in certain patient subgroups, including Gleason score 6 or 7, pathological stage pT2 or pT3a, preoperative PSA 10 ng/ml or less, or 10 to 20 ng/ml or less, and positive or negative surgical margins (fig. 2, *B* to *E*).

Improved Prognostic Model Application

To evaluate the potential impact of the model on disease management we compared the accuracy of prognostic stratification with the classifier with that of the 5-year postoperative nomogram in the test cohort. We used the cutoff based on the highest accuracy of each model and then applied these values to the test set (fig. 3). Of the 157 test set patients 136 predicted as at low risk by the nomogram had a 23.5% recurrence rate (32 of 136). In contrast, when applied to this group, the classifier identified 14 patients at high risk for recurrence, including 12 (86%) with documented recurrence. Of the 122 patients in whom the classifier conferred low risk status 20 (16.4%) experienced PSA recurrence. Conversely none of the 11 patients predicted to be at high risk by the nomogram but low risk by the classifier had a documented recurrence. Thus, the classifier conferred prognostic information in addition to that provided by the postoperative nomogram in this series of patients with prostate cancer.

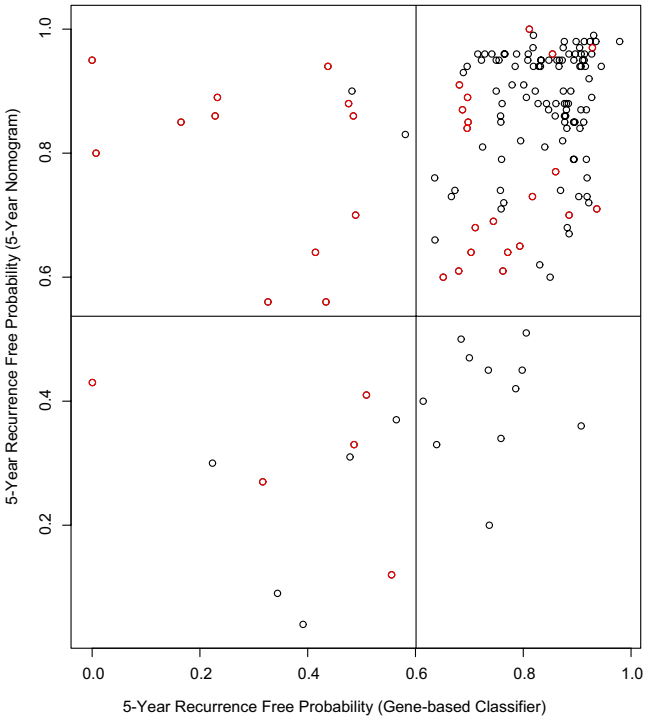

Figure 3. Prediction into low and high risk groups by gene based classifier vs 5-year nomogram in each test set patient, including 12 and 2 with recurrence (red circles) and no disease (black circles) in upper left, 20 and 102 in upper right, 5 each in lower left, and 0 and 11 in lower right quadrants, respectively.

DISCUSSION

We systematically assessed prostate cancer related gene expression correlates of PSA recurrence to develop a gene based recurrence classifier for clinically localized prostate cancer with potential broad clinical usefulness. We identified the novel gene predictors using a custom DASL array, a microarray platform that allows high throughput gene expression profiling of RNA derived from FFPE tissue.[22] A key component of this study was the design of the custom DASL microarray gene set, which is based on gene markers identified by reanalysis of published data sets,[12,13] unpublished in-house gene expression data and markers previously implicated in prostate cancer progression.[14,16,17] This provides a degree of independent validation for the 30 genes that were most significant in this study. The 24-gene markers that correlated with PSA recurrence on gene expression array analysis were further validated by RT-PCR to produce a 3-gene signature (*DPT, MYH11* and *SSBP1*) which, when combined with an established nomogram, resulted in a new classifier of PSA recurrence. This classifier was subsequently validated in an independent group of patients. DPT and MYH11 are novel prostate cancer prognostic markers while SSBP1 was previously associated with aggressive prostate cancer.[17]

Assessment of the value of this new classifier over that of a widely used nomogram for prostate cancer recurrence showed that the new classifier identified patients at low and high risk for recurrence with much greater accuracy than the postoperative nomogram alone.[9] The classifier presented also stratified patients in clinically relevant subgroups based on conventional clinicopathological parameters into high and low risk recurrence groups. The ability to stratify patients with Gleason 6 and 7 cancer represents a significant advance in predictive accuracy over current approaches. The use of PSA recurrence as a significant end point for prostate cancer has been disputed since only a proportion of patients who experience recurrence progress to clinically significant disease. These relationships will be more clearly defined as this cohort matures with data on metastasis and death from prostate cancer. However, the detection of increasing PSA after prostatectomy is an important decision point when most physicians and patients consider further treatment options.[5] In this context the 5-year nomogram to evaluate the potential impact of this classifier is valid with most biochemical recurrence after prostatectomy developing within 5 years.

Of significance is the impact of this new classifier as a decision tool when considered against other published signatures and gene markers of molecular phenotype and prognosis in prostate cancer cases.[12–20] To our knowledge this study is unique since it was developed specifically to aid in predicting the risk of recurrence in cases of clinically localized prostate cancer since they represent more than 80% of newly diagnosed prostate cancer cases in the United States. While published signatures and gene markers have been identified in patient cohorts representing the spectrum of pathological stages, the training and test cohorts in our study were restricted to organ confined prostate cancer. The importance of concordance between the patient group used to develop a predictive tool with the anticipated target group is reinforced by the relatively low performance of the 5-year nomogram in this cohort of patients with clinically localized disease compared with that in previous reports. On further analysis this may likely have been due to the limitation of assessing only organ confined cases in this study. When tested in our consecutive prostate cancer cohort from the same institution that was not limited to organ confined disease, nomogram performance was consistent with that in previous studies.[9,11]

The relatively low level of complexity of the classifier is also important. With the ability to measure expression of a small set of genes in FFPE tissue and the use of a platform that is approved for diagnostic testing in archival specimens it is a significant advance since it addresses some key factors affecting the likelihood of successfully implementing this predictive tool in a clinical diagnostic setting. The requirement for low RNA concentrations derived from FFPE tissue will also facilitate its potential long-term applicability to routine pathology specimens, including preoperative transrectal biopsy. Validation in external cohorts of surgical and preoperative biopsies, including replicating the gene selection in biopsies, is now required to confirm the wider applicability of this classifier in the preoperative setting.

The implementation of an accurate predictive classifier for localized prostate cancer has important implications for early prostate cancer management. Patients with localized disease and high risk features are likely to benefit from adjuvant therapy, including hormone, radiation and systemic treatment, and the benefits should be evaluated in treatment trials.[7,28,29] Early phase clinical trials of such agents are under way in the hormone refractory setting but may ultimately be tested in localized prostate cancer as adjuvant therapy.[30] Intrinsic to these studies is accurate identification of patients at high risk to ensure homogeneous patient groups.[8] Conversely the improved identification of patients at low risk for recurrence would decrease the number exposed to the morbidity of therapy as more indolent cancers are identified by PSA screening. In conclusion, the development of an improved prognostic model for localized prostate cancer has the potential to facilitate better treatment decisions, that is to forgo therapy for indolent disease or offer adjuvant chemotherapy in men at high risk for recurrence.

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