Gene Panel Model Predictive of Outcome in Men at High-Risk of Systemic Progression and Death From Prostate Cancer After Radical Retropubic Prostatectomy

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ABSTRACT

Purpose

In men who are at high-risk of prostate cancer, progression and death from cancer after radical retropubic prostatectomy (RRP), limited prognostic information is provided by established prognostic features. The objective of this study was to develop a model predictive of outcome in this group of patients.

Methods

Candidate genes were identified from microarray expression data from 102 laser capture microdissected prostate tissue samples. Candidates were overexpressed in tumor compared with normal prostate and more frequently in Gleason patterns 4 and 5 than in 3. A case control study of 157 high-risk patients, matched on Gleason score and stage with systemic progression or death of prostate cancer as the end point, was used to evaluate the expression of candidate genes and build a multivariate model. Tumor was collected from the highest Gleason score in paraffin-embedded blocks and the gene expression was quantified by real-time reverse transcription polymerase chain reaction. Validation of the final model was performed on a separate case-control study of 57 high-risk patients who underwent RRP.

Results

A model incorporating gene expression of topoisomerase-2a, cadherin-10, the fusion status based on *ERG*, *ETV1*, and *ETV4* expression, and the aneuploidy status resulted in a 0.81 area under the curve (AUC) in receiver operating characteristic statistical analysis for the identification of men with systemic progression and death from high grade prostate cancer. The AUC was 0.79 in the independent validation study.

Conclusion

The model can identify men with high-risk prostate cancer who may benefit from more intensive postoperative follow-up and adjuvant therapies.

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INTRODUCTION

Current prognostic markers predictive of outcome in men with prostate cancer after radical retropubic prostatectomy (RRP) consist of Gleason score, TNM stage, surgical margin status, and preoperative serum prostate-specific antigen (PSA).¹⁻⁴ These clinicopathologic findings are used to identify patients who are at high-risk of systemic progression after surgery and may benefit from postoperative adjuvant therapy, such as androgen deprivation or radiation therapy. In men with high grade tumors after RRP, limited prognostic information is provided by established clinical and pathologic parameters.

Beyond the current clinicopathologic parameters, there have been other markers proposed to predict poor outcome of patients with prostate cancer. These markers include ploidy, tumor proliferative activity, alpha-methylacyl-CoA-racemase expression, and gene panel expression profiles.^{1,3-8} Recently, gene expression array technology applied to prostate cancer has resulted in the identification of a number of genes that have been associated with outcome. Many biomarkers reported to provide prognostic information show an association with outcome only in a univariate model and have limited to no prognostic value in a multivariate setting that includes Gleason score, stage, margin status, and preoperative serum PSA. In addition,

many studies use serum PSA progression or biochemical recurrence after RRP as the outcome end point rather than development of metastatic disease or death from prostate cancer. Depending on the definition of PSA progression, this end point may include patients that do not have life-threatening disease.

Recently, Tomlins et al⁹ reported that a majority of prostate cancers exhibit fusions between the control region of an androgen regulated gene, *TMPRSS2*, and the coding region of the ETS family of transcription factors, most frequently *ERG* and much less frequently *ETV1* and *ETV4*. The result of this fusion is the overexpression of the potential oncogenes, *ERG*, *ETV1*, and *ETV4*. The fusions are associated with an increased risk of cancer progression in patients treated surgically and those observed clinically without treatment.^{10,11} It is likely that the presence or absence of this fusion will be important in the clinical treatment of men with prostate cancer.

In this study, we sought to design a predictive model for men with surgically treated high-risk prostate cancer. An initial set of candidate genes were identified using microarray expression data from 102 samples collected by laser capture microdissection¹²; genes that could distinguish lower grade (Gleason score 6) from higher grade (Gleason score 7+) were selected using the methods given in Kosari et al.¹³ Variables for ETS-related fusion and DNA ploidy were also included in the modeling. The ETS fusion variable was added due its recent discovery, and a number of studies showing a significant association of fusion status with patient outcome. The variable for ploidy was added because studies have shown an association of ploidy with outcome, and it is routinely collected as part of the clinical practice on all patients with prostate cancer treated surgically at the Mayo Clinic.¹⁴ The candidate genes along with ETS fusion and ploidy were evaluated in a multivariate analysis using 157 tumor samples in a matched case-control study design. This case-control design was used to develop the model. The case group consisted of 76 men who developed systemic progression or died of prostate cancer within 5 years of surgery while the control group consisted of 81 men who did not develop systemic progression within 7 years after RRP. To maximize the practical utility of the candidate markers evaluated in this study, cases and controls were matched on all currently accepted clinical and pathologic prognostic features and were balanced for adjuvant therapy. The final

model was then validated using an independent set of 57 patient samples in a nested case-control design. Thus, discovery, model building, and validation used independent groups of patients. Herein, we report a four-variable model predictive of cancer-specific outcome in men with high-grade prostate cancer treated by RRP. All human investigations were performed after approval by the Mayo Clinic institutional review board and in accordance with an assurance filed with and approved by the Department of Health and Human Services.

METHODS

The methods for the generation of the microarray data used in this study are described elsewhere.^{12,13} The methods that describe patient information and patient material for the cohort study and the case control-studies presented here are described in the supplemental material.

RESULTS

Cohort Study for Current High-Risk Prostate Cancer Marker Status

Using patients treated from 1990 to 2004 in the Mayo Radical Prostatectomy database, the Cox proportional hazards model was used to identify significant clinical predictors of time to systemic progression. The strongest predictors were RRP Gleason score, pathologic stage components (extracapsular cancer, seminal vesicle involvement, lymph node metastases), and the use of adjuvant (within 90 days after RRP) hormone therapy (Table 1). The model had a receiver operating characteristic (ROC) area under the curve (as measured by the concordance statistic) of 0.82. Gleason score was the strongest predictor with 10-year rates of systemic progression for Gleason score 2 to 6, 7, and 8 to 10 of 1.9%, 10.6%, and 23.6%, respectively. When the patient cohort was restricted to Gleason score 7 to 10, the strongest significant clinical predictors of time to systemic progression were RRP Gleason score, seminal vesicle involvement, and lymph node involvement (Table 1), and the concordance statistic dropped to 0.69. Guided by these results, we concluded that a high-risk prostate cancer RRP population, with Gleason score ≥ 7 , would most strongly benefit

Table 1. Multivariate (Cox model) Analysis of Clinical and Pathologic Predictors of Systemic Progression After Radical Retropubic Prostatectomy From the Mayo Clinic Radical Retropubic Prostatectomy Database of Men (N = 10,626)

Variable	Gleason Score			
	All		7-10	
	Hazard Ratio	P	Hazard Ratio	P
Gleason				
7	4.0*	< .0001	NA	—
8-10	8.5*	< .0001	2.2†	< .0001
Extracapsular extension	1.7	< .0001	1.2	.26
Seminal vesicle involvement	2.9	< .0001	2.6	< .0001
Nodal involvement	1.6	.002	1.5	.030
Adjuvant hormone therapy	0.7	.005	0.8	.076
No. of patients	10,626		3,636	
No. of events	441		335	
Concordance statistic	0.82		0.69	

*v 2-6.

†v 7.

from molecular marker panels to improve prognostic predictions and designed this study to address that need.

Candidate Marker Selection

A set of 38 genes was selected from a microarray expression profiling experiment of normal and tumor cells from patients with prostate cancer.¹² Cells were collected by laser capture microdissection, from 29 non-neoplastic prostatic epithelia, five prostate intraepithelial neoplasias (PINs), 61 tumors with various degrees of differentiation (Gleason patterns 3, 4, and 5 prostate cancer), and seven node metastasis samples. Patient outcome was not known for these samples. We used a methodology described in Kosari et al¹³ to identify a list of variably overexpressed genes by comparing the expression of each gene in cancer samples with its expression in normal samples. Of those variably overexpressed genes, 38 were chosen as a candidate because they were overexpressed more frequently in high-grade cancers (Gleason patterns 4 or 5 and metastasis) than in intermediate cancers (Gleason pattern 3). We also defined a binary variable (pred_fusion) to indicate the ETS-fusion status of a patient's tumor and another binary variable to indicate aneuploidy (Appendix, online only). To evaluate the 40 variables and build a multivariable model, a case-control study was designed by using Gleason score ≥ 7 patients with and without systemic progression.

Model Building Case-Control Study

Cases were defined as men that developed systemic progression or died of prostate cancer within 5 years of RRP, and were matched for Gleason score, TNM stage, margin status, and preoperative serum PSA with controls that did not develop systemic progression or die of prostate cancer for at least 7 years. The cases and controls are very similar in their clinical and pathologic features with the exception of DNA ploidy (Appendix). The gene expression between the two groups was evaluated using univariate analysis (Table 2) for all 38 normalized genes, the predicted fusion variable, and the aneuploidy variable (see Methods in Appendix). As part of the Mayo Clinic standard practice, DNA ploidy¹⁴ is performed on all RRP cases. Based on this analysis, the best univariate predictors are *BIRC5*, *RRM2*, and *TOP2A*. *CDH10*, also a significant predictor, has a negative coefficient suggesting a protective role. The other genes with a significant ($P \leq .05$) prognostic univariate effect were *COL2A1*, *NRP1*, and *SSTR1*. Because aneuploidy status has been previously indicated^{3,4} as potentially prognostic, it was evaluated and found to be significantly associated with case/control status. When hierarchical clustering (Appendix) was performed for the normalized genes based on absolute correlation (online-only Figs A1 and A2), the genes *BIRC5*, *RRM2*, and *TOP2A*, which are the most important univariate genes, form a tight cluster and only one of them is retained in a multivariate model.

Multivariate analysis. We used a forward stepwise selection process to define an optimal model for prediction of prostate cancer outcome, yielding a four-variable model containing *TOP2A*, *CDH10*, and the fusion and aneuploidy status variables. At each iteration, all remaining variables were tested for their additional predictive power given the variables selected thus far (Appendix Tables A1, A2, and A3, online only). The first variable was *TOP2A*. With *TOP2A* in the model, *BIRC5* and *RRM2* were not significant predictors. The predictive performance of the model did not change significantly if either *BIRC5* or *RRM2* were selected as the first variable of the model. When any one of *TOP2A*, *BIRC5*, and *RRM2* was selected as the first variable

Table 2. Genes Examined by Quantitative Reverse Transcription Polymerase Chain Reaction in the Case/Control Study

Variable	Mean		IQR	Coefficient	P
	Case	Control			
<i>TOP2A</i>	-6.7383	-7.7932	1.79	0.58	.000
<i>RRM2</i>	-9.7550	-10.7669	2.11	0.40	.000
<i>BIRC5</i>	-8.0113	-9.1034	1.98	0.52	.000
Aneuploidy	0.250	0.0741		1.43	.004
<i>NRP1</i>	1.3674	-3.1159	-3.60	0.52	.010
<i>CDH10</i>	-8.2008	-7.4639	2.29	-0.25	.012
<i>SSTR1</i>	-8.2354	-8.8994	2.04	0.25	.015
<i>COL2A1</i>	-6.7203	-7.9509	5.43	0.11	.023
<i>KHDRBS3</i>	-5.3829	-5.7935	2.19	0.22	.065
Pred_fusion	0.3333	0.1026		0.27	.123
<i>DDC</i>	-9.9106	-10.8424	5.49	0.08	.127
<i>GRIN3A</i>	-6.4650	-6.8063	1.69	0.17	.137
<i>PTPRM</i>	-4.5718	-4.2459	1.85	-0.17	.174
<i>AMACR</i>	-0.7400	-0.3715	2.39	-0.13	.183
<i>TMEM45</i>	-7.7622	-8.0647	2.16	0.14	.216
<i>F5</i>	-6.9653	-7.3021	2.91	0.10	.257
<i>PKIB</i>	-5.5744	-5.7962	1.51	0.14	.284
<i>ADD2</i>	-13.9772	-13.6186	2.72	-0.08	.324
<i>RAB6B</i>	-6.8661	-7.0508	1.32	0.15	.324
<i>HS2ST1</i>	-6.6773	-6.8392	1.38	0.16	.327
<i>SDC2</i>	-5.1979	-5.0308	1.36	-0.13	.368
<i>C20orf22</i>	-6.4526	-6.2542	1.83	-0.09	.433
<i>HDAC9</i>	-10.4061	-10.6311	2.44	0.07	.447
<i>CDH7</i>	-8.3882	-8.6405	3.34	0.05	.482
<i>TDO2</i>	-11.8952	-12.237	4.27	0.03	.508
<i>B3GnT6</i>	-8.9959	-8.6781	5.14	-0.03	.523
<i>PCDHB10</i>	-6.9311	-7.0625	1.53	0.07	.559
<i>DCAMKL1</i>	-5.2343	-5.1299	1.73	-0.07	.611
<i>LTB</i>	-6.8683	-6.7466	1.83	-0.05	.629
<i>ERG</i>	-4.4620	-4.3092	4.73	-0.03	.698
<i>CADPS</i>	-7.6885	-7.8294	3.48	0.03	.702
<i>HOXC6</i>	-4.925	-4.8534	1.66	-0.04	.755
<i>PTPRT</i>	-5.7513	-5.8454	2.40	0.03	.755
<i>SERPINI</i>	-8.4649	-8.5329	1.75	0.04	.757
<i>Col10A1</i>	-7.5064	-7.5380	1.34	0.04	.831
<i>SLC3A1</i>	-4.9924	-5.0192	0.81	0.04	.831
<i>DSC54</i>	-6.2714	-6.3254	2.05	0.02	.835
<i>PPFIA2</i>	-6.9471	-7.0109	2.73	0.02	.840
<i>C20orf102</i>	-4.0823	-4.0578	1.80	-0.01	.917
<i>PHCA</i>	-5.9232	-5.9296	1.54	0.01	.970

Abbreviation: IQR, interquartile range (75% to 25%).

*Based on ERG, ETV1, and ETV4 (see Methods and Appendix). Fusion negative [-1], undetermined [0], positive [1].

in the model, the other two genes lost significance, demonstrating these genes are interchangeable and suggesting they are related. The next most significant variable added to the model was *CDH10*. After adding *CDH10* and *TOP2A* to the model, the significance of the fusion status increased and became the most significant remaining parameter (see Appendix for additional information). Adding fusion status to *TOP2A* and *CDH10* rendered the remaining genes insignificant. Each successive step of this modeling improved the corresponding area under the curve (AUC) in the ROC plot by at least 0.02 (Fig 1). The AUC with *TOP2A* alone was 0.71—it increased to 0.74 with *CDH10*, and increased to 0.79 with the predicted fusion parameter (Fig 1). Aneuploidy remained a significant variable in this analysis, so it was

Fig 1. (A) Receiver operating characteristic (ROC) analysis based on logistic regression models for predicting systemic progression. Predictors in each model are: 1 = *TOP2A*; 2 = *TOP2A*, *CDH10*; 3 = *TOP2A*, *CDH10*, predicted fusion; and 4 = *TOP2A*, *CDH10*, predicted fusion, aneuploidy. Models 1, 2, 3, and 4, ROC curves are shown as blue, yellow, red, and brown lines, respectively. The text shows the coefficients and the area under the curve for each model. Note that the coefficient of *CDH10* is negative. (B) Scatter plot of the final model score for cases (red points) and controls (yellow points) of the training set. The yellow bullets are patients with prostate-specific antigen progression but no sign of systemic progression.

added to the final model (Table 3) consisting of predicted fusion, *TOP2A*, *CDH10*, and aneuploidy which resulted to an AUC of 0.81 (95% CI, 0.74 to 0.88). The change in AUCs at each step was also tested for significance using the integrated discrimination improvement (IDI) method proposed by Pencina (Appendix) which agreed with the stepwise result. Further confirmation for the significance of the final model is provided by permutation tests and by the penalized fit to the original data, which also showed only minor changes in the coefficients. The score derived from the regression model is displayed in Figure 1B, showing the overall separation of the cases and the controls.

Independent validation using frozen tumor tissue from a separate cohort of 57 high-risk patients showed a slight decrease in the AUC to 0.79 (95% CI, 0.66 to 0.92; Fig 2A). The score derived from the regression model in this independent validation is displayed in Figure 2B.

It is important to note that patients were not matched for adjuvant postoperative therapy (hormone and radiation); however, nearly equal numbers of cases and controls received adjuvant therapy. In addition, stratified analysis indicated no association of adjuvant therapy with the final model, the treatment modalities were not significant predictors of outcome and the coefficients and *P* values of the other variables in the model changed only slightly.

DISCUSSION

Several candidate prognostic cancer models based on multiple gene panels or signatures have already been proposed.^{5,6,8,15-17} It is now important to construct models based on gene expression patterns that provide prognostic information in high-risk patients beyond that

Table 3. Final Logistic Regression Model Predicting Systemic Progression

Variable	Coefficient	SE	<i>P</i>	Odds Ratio	95% CI for Odds Ratio
<i>TOP2A</i>	0.71	0.17	< .0001	2.03	1.46 to 2.84
<i>CDH10</i>	−0.62	0.16	.0001	0.54	0.39 to 0.74
Pred_fusion*	0.91	0.28	.0010	2.48	1.44 to 4.30
Aneuploidy†	1.34	0.61	.0286	3.82	1.16 to 12.62

*Based on *ERG*, *ETV1*, and *ETV4* (see Methods): −1 = negative, 0 = undetermined, 1 = positive.

†0 = diploid or tetraploid; 1 = aneuploid.

Fig 2. (A) Receiver operating characteristic (ROC) analysis of model 4 ($0.71 \times TOP2A - 0.62 \times CDH10 + 0.91 \times \text{pred_fusion} + 1.34 \times \text{aneuploidy}$) for the independent validation set. (B) Scatter plot of the score for cases (red points) and controls (yellow points) for the independent validation set.

provided by established clinical and pathologic prognostic features. This need was demonstrated through a Cox model analysis of the Mayo Clinic Prostate Cancer Database cohort of more than 10,000 men with prostate cancer treated by RRP. In this study, we evaluated the prognostic utility of genes previously identified from gene expression profiling to be associated with high-grade (Gleason patterns 4 and 5) and metastatic prostate cancer.¹³ The expression of these genes was analyzed using real-time reverse transcription polymerase chain reaction in a prostate cancer tissue samples from patients treated by RRP. We compared expression in patients who developed systemic progression or died of prostate cancer with those who did not, where samples were matched for the established prognostic features of prostate cancer. This design allowed for the identification of prognostic biomarkers in men at high-risk of prostate cancer death, for whom established prognostic markers provide minimal or no additional prognostic information.

This study generated a model to distinguish men treated by RRP who develop systemic progression from men treated by RRP who do not develop systemic progression. The three-variable model, which includes *TOP2A* expression, *CDH10* expression, as well as predicted *TMPRSS2* (*ERG*, *ETV1*, or *ETV4*) fusion status, and aneuploidy had an AUC of 0.81 in training and 0.79 in validation. The slight reduction to the AUC could be attributed to the fact that the validation case control study was based in a risk-set study design (see Methods). Specific sensitivity and specificity are not reported as an optimal threshold would be arbitrary and not necessarily relevant based on the clinical situation.

The model was developed in a case-control study of men matched on Gleason score, TNM stage, margin status, and preoperative serum PSA who did and did not develop systemic progression or die of prostate cancer. Survival curves by score group could not be generated due to the case-control design, wherein the event rate is predetermined to be 50%. If the logistic score were applied prospectively, an increase in score of 0.7 (which is quite plausible) would imply a doubling in risk. For Gleason score 7, where the cumulative systemic progression rate at 10 years was 11%, this change in score could imply an overall increase in risk to around 20%.

It is important to note that the candidate genes were identified through association with Gleason pattern thus many of these genes were correlated with Gleason pattern or the degree of tumor differentiation but not necessarily patient outcome. Therefore, the majority of the candidate genes did not enter the model. Gleason pattern was used as a surrogate marker of tumor aggressiveness as there were inadequate numbers of frozen tissue samples from patients who had developed systemic progression or died of prostate cancer in the Mayo Clinic tissue bank. Indeed, among the 11,000 patients treated by RRP at the Mayo Clinic between 1990 and 2004, only 4% developed systemic progression or died of prostate cancer confirming the difficult nature of obtaining adequate patient samples to use systemic progression and prostate cancer death as an end point in analyses. Once Gleason grade was controlled for in the case-control design, many genes on the list no longer showed an association with outcome. Moreover, many of the variables in the initial list have an association with outcome; however, these genes frequently are strongly correlated with other genes on the list and thus dropout in subsequent steps in model building after the most significant gene in the cluster enters into the model (as demonstrated here with the proliferation markers *TOP2A*, *RRM2*, and *BIRC5*). Therefore, we believe that this methodology, and case-control study design resulted in an optimal predictive value that was validated in an independent set of samples.

TOP2A is an enzyme involved in segregation of newly replicated chromosome pairs, and has additional functions in the condensation and helix formation of chromosomal DNA and is part of the "cell proliferation cluster".¹⁸ Proliferation-related genes are the most differentially expressed genes when tumor and matched normal tissues are compared.¹⁹ Cell proliferation cluster markers have been shown to be associated with poor outcome in numerous human malignancies including prostate.^{1,3,4,20} Recently, Hughes et al²¹ identified that *TOP2A* was associated with Gleason grade and, *TOP2A* expression has been associated with hormone insensitivity. In regards to therapy, there are chemotherapeutic agents that target *TOP2A* including etoposide, doxorubicin, and mitoxanthrone.²²⁻²⁶ It is possible patients whose tumors express elevated levels of *TOP2A* may benefit from these

chemotherapeutic agents alone or in combination with other therapies, and we believe further studies are warranted. *TOP2A* overexpression may not only be prognostic but provide a target for chemotherapy.

CDH10 belongs to the large family of cell to cell adhesion proteins, classic cadherins type II.²⁷ The role of *CDH10* in the progression of human malignancies is unknown. However, it is known to play a role in cell to cell adhesion through stabilization of beta-catenin similar to type I cadherins such as E-cadherin.²⁸ In prostate cancer, decreased expression of E-cadherin is associated with aggressive tumor behavior.²⁹⁻³² Decreased E-cadherin expression in prostate cancer is associated with high Gleason score, advanced tumor stage, and cancer relapse.²⁹⁻³¹ Another study found that aberrant E-cadherin expression was associated with salvage radiation failure for PSA recurrence after prostatectomy lending support to our findings that the high-risk patients have a feature of systemic disease rather than local progression.³³ The loss of expression of adhesion molecules appears critical for tumor cells to develop an invasive phenotype that allows extension through prostatic stroma and access to vascular and lymphatic spaces for the systemic spread of tumor. In a manner analogous to E-cadherin, we demonstrate that decreased expression of another adhesion molecule, *CDH10*, is inversely correlated with prostate cancer progression suggesting its role in maintaining cell to cell adhesion and preventing tumor cell dissemination.

Recent studies support a role for the *TMPRSS2* to *ETS* gene fusions as a prognostic marker for men with prostate cancer. In a study of 106 tumors, the *TMPRSS2:ERG* was found to be associated with moderate to poorly differentiated tumors.³⁴ The fusion was not identified in any of five cases of benign prostatic hyperplasia, and in only one of 15 Gleason pattern 2 tumors. In contrast, approximately 40% of Gleason patterns 3, 4, or 5 tumors were found to contain fusions.³⁴ In a clinical watchful waiting cohort, there was a statistically significant association between the most common fusion (*TMPRSS2:ERG*) and prostate cancer-specific death. These findings suggest that the *TMPRSS2:ERG* cancers have a more aggressive phenotype, possibly associated with increased *ERG* expression.¹⁰ Furthermore, *TMPRSS2*

is an androgen-responsive gene, and may play a important role in the behavior of androgen-dependent cancers, while the requirement for androgen-regulated expression might be bypassed in late stage or androgen-independent tumors.³⁵ In this study, we show the prognostic significance of the *TMPRSS2* to *ETS* gene fusions in a multivariate but not univariate analysis in the high-risk prostate cancer population. Our findings suggest the need to determine the presence of these fusions in men who have the pathologic features that put them at high risk for systemic progression.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).