Development of a Multiplex Quantitative PCR Signature to Predict Progression in Non–Muscle-Invasive Bladder Cancer

Rou Wang, David S. Morris, Scott A. Tomlins, Robert J. Lonigro, Alexander Tsodikov, Rohit Mehra, Thomas J. Giordano, L. Priya Kunju, Cheryl T. Lee, Alon Z. Weizer, and Arul M. Chinnaiyan

Departments of Urology, Pathology, and Biostatistics, Michigan Center for Translational Pathology, Comprehensive Cancer Center, and Howard Hughes Medical Institute, University of Michigan, Ann Arbor, Michigan

Abstract

In bladder cancer, clinical grade and stage fail to capture outcome. We developed a clinically applicable quantitative PCR (QPCR) gene signature to predict progression in non–muscle-invasive bladder cancer. Comparative metaprofiling of 12 DNA microarray data sets (comprising 631 samples and 241,298 probe sets) identified 96 genes, which showed differential expression in seven clinical outcome categories, or were identified as outliers, historic markers, or housekeeping genes. QPCR was done to determine mRNA expression from 96 bladder tumors. Fifty-seven genes differentiated T2 from non-T2 tumors (P < 0.05). Principal components analysis and Cox regression models were used to predict probability of T2 progression for non-T2 patients, placing them into high- and low-risk groups based on their gene expression. At 2 years, high-risk patients exhibited greater T2 progression (45% for high-risk patients versus 12% for low-risk patients; P = 0.003, log-rank test). This difference remained significant within T1 tumors (61% for high-risk patients versus 22% for low-risk patients; P = 0.02) and Ta tumors (29% for high-risk patients versus 0% for low-risk patients; P = 0.03). The best multivariate Cox model included stage and gender, and this signature provided predictive improvement over both (P = 0.002, likelihood ratio test). Immunohistochemistry was done for two genes in the signature not previously described in bladder cancer, ACTN1 and CDC25B, corroborating their up-regulation at the protein level with disease progression. Thus, we identified a 57-gene QPCR panel to help predict progression of non–muscle-invasive bladder cancers and delineate a systematic, generalizable approach to converting microarray data into a multiplex assay for cancer progression. [Cancer Res 2009;69(9):3810–8]

Introduction

Approximately 75% of newly diagnosed patients with bladder cancer will have disease confined to the urothelium or lamina propria (stages Ta, Tis, and T1). These non–muscle-invasive tumors account for significant morbidity given recurrence rates of 50% to 70% (1) and the need for cystoscopic surveillance. Furthermore, 10% to 15% of these tumors will progress to muscle invasion or higher (T2-T4; ref. 2), with worsened prognosis and 5-year overall survival rates of 50% to 60% (3). To date, there has been no reliable means of predicting tumor progression other than clinical judgment, published risk estimates, or burgeoning clinical nomograms (2, 4).

In parallel to these clinical questions, there has been significant maturation of DNA microarray gene expression analysis over the last decade. Microarray analysis has become a high-throughput method of measuring the cancer transcriptome and can distinguish cancer from normal tissues, identify cancer subtypes, and predict recurrence or treatment response. For example, breast cancer has been studied extensively with microarray analysis, generating gene signatures to guide clinical management (5, 6). Other cancers, such as bladder cancer, have been investigated infrequently with microarray analysis. A recent query of the Affymetrix publication database and PubMed confirms the disparity in microarray attention between bladder and breast cancer; bladder cancer is linked to 102 Affymetrix and 223 PubMed publications, whereas breast cancer is linked to 757 Affymetrix and 1,677 PubMed citations. Even accounting for the increased incidence of breast cancer in 2008 (184,000 versus 69,000 for bladder; ref. 7), there are fewer bladder cancer microarray studies done.

Furthermore, clinical application of microarray gene signatures has been difficult given the lack of reproducibility. Small cohorts and variable microarray platforms may explain the minimal overlap between signatures. Ultimately, a gene signature that will be used for risk stratification must be well validated across various, independent patient populations. Previously, we sought to overcome the limitations of varied analyses through comparative metaprofiling of microarray data sets to characterize a common transcriptional profile across cancer types (8). Comparative metaprofiling generates gene signatures from the overlap of independent microarray data sets, limiting the noise of spuriously identified genes and accentuating true underlying signature patterns. Furthermore, quantitative PCR (QPCR), relative to microarrays, is more reproducible, possesses a larger dynamic range, and is a clinically more tractable platform for diagnostics and prognostics development.

The goal of this multiphase study was to use preexisting microarray data sets to develop a gene signature that would help predict progression for non–muscle-invasive bladder cancers. In phase I (Comparative Metaprofiling and Creation of Metasignature), we used comparative metaprofiling to analyze published bladder cancer microarray data sets and determine genes associated with cancer development, recurrence, progression, and outcome. We then sought to tailor the large number of genes to a smaller, robust metasignature of 96 genes associated with aggressive behavior in
bladder cancer. In phase II (Sample Selection and QPCR for Development of Gene Signature), these 96 genes were preconfigured onto a clinically applicable, high-throughput QPCR card. Gene expression values were quantified for 96 frozen tumor tissue specimens. Ultimately, 57 genes were selected, which differentiated between non–muscle-invasive and muscle-invasive tumors. In phase III (Evaluation of Gene Signature Predictive Ability and Biological Networking), we assessed the ability of a 57-gene signature to predict probability of progression of non–muscle-invasive bladder tumors to T2 disease and investigated the set’s overlap with biological networks. In phase IV (Immunohistochemical Confirmation of Sample Genes), we confirmed protein expression for two gene signature members, actinin (ACTN1) and cell division cycle 25B (CDC25B), using a bladder cancer tissue microarray.

Ultimately, this signature may aid in the identification of non–muscle-invasive bladder cancers that are more likely to progress and for which earlier definitive therapy, such as cystectomy, may be offered. More generally, we present a systematic approach to using publicly available cancer microarray data sets and converting them into a clinically applicable platform.

**Figure 1.** Development of a bladder cancer progression signature based on comparative metaprofiling. A, nine bladder cancer and three multicancer microarray data sets (representing 631 samples and 241,298 probe sets) were uploaded into Oncomine for bioinformatics analysis. Samples were reassigned into clinical category classes, and significant genes differentiating between classes were identified using Student’s t test. Significance thresholds were set for up-regulated and down-regulated genes, resulting in a meta-signature. This metasignature and bladder cancer historic markers, outliers, and housekeeping genes were used to create a 96-gene TLD card, which was used to analyze gene expression for bladder cancer tissue mRNA. B, detailed list of 96 genes whose assays were loaded onto the TLD QPCR card. Significantly up-regulated and down-regulated genes in the metasignature, with shaded boxes (red, up-regulated; blue, down-regulated) representing clinical categories. Bladder cancer historic markers are shown with accompanying up-regulation and down-regulation of gene or protein expression in literature review as well as bladder cancer outliers derived from Oncomine analysis and housekeeping genes.
Materials and Methods

Phase I: Comparative Metaprofiling and Creation of Metasignature

Comparative metaprofiling of existing microarray data. Nine previously published bladder cancer microarray profiling data sets and three multicancer microarray profiling data sets were identified, comprising 631 samples and 241,288 probe sets (Supplementary Table S1). These publicly available microarray data sets were uploaded into Oncomine (9), an online compendium and advanced analysis platform for gene expression data sets. The flow diagram of comparative metaprofiling leading to the creation of a TaqMan low-density array (TLDA) card is detailed in Fig. 1A.

For each of the microarray profiling studies, we reviewed clinical information for profiled samples, including cancer grade and stage, recurrence, local or distant progression, and patient death. Ultimately, six clinical categories were defined: cancer grade, muscle invasion, recurrence, progression to higher stage, positive lymph node status, and death from disease (Supplementary Table S2). A seventh clinical category for overall aggressiveness was devised, combining progression, positive lymph nodes, or death from disease. Individual samples were assigned to classes for each analysis, and in each study, genes were assessed in Oncomine for differential expression between these classes with Student’s t test to create metaprofiles for each clinical category (see Supplementary Methods). Genes were selected as candidates for the TLDA card if they were significantly overexpressed in at least four clinical category metaprofiles or underexpressed in at least three metaprofiles to increase the likelihood that they reflected significant processes in bladder cancer (Fig. 1B); from there, the list was further tailored by choosing genes with available TLDA primers, thus resulting in 50 overexpressed and 15 underexpressed genes. Six outlier genes in the data sets were also identified by Oncomine analysis and included in the metasignature as well as 6 housekeeping genes and 19 historic markers. This resulted in a metasignature of 96 genes of interest (Supplementary Table S3).

These 96 genes were then preloaded onto a 96-well format TLDA card (Applied Biosystems), which allows for multiplex high-throughput QPCR measurements. Five batches of 10 cards each were constructed.

Phase II: Sample Selection and QPCR for Development of Gene Signature

Sample selection and preparation. Cases with available frozen bladder cancer tissue from time of transurethral resection of the bladder tumor (TURBT) were selected from those patients enrolled in the bladder cancer database at the University of Michigan. All samples were collected with the informed consent of the patients and prior institutional review board approval. To be included in the bladder cancer tumor bank, samples had been previously pathologically reviewed to ensure adequate tissue and tumor representation as well as 6 housekeeping genes and 19 historic markers. This resulted in a metasignature of 96 genes of interest (Supplementary Table S3).

These samples were also run on the TLDA cards (see Supplementary Methods).

Gene expression was normalized relative to the average of four housekeeping genes (β-actin, cytochrome c, glyceraldehyde-3-phosphate dehydrogenase, and succinate dehydrogenase complex, subunit A); the values were then log2-transformed. 18S (18S rRNA gene) was imputed as zero, implying no expression of that gene relative to housekeeping genes. To reduce outlier influence, the distribution of each gene’s expression values was truncated at the third upward SD. Principal components analysis was used to reduce this gene set into a smaller number of variables explaining >75% of the data variance, and principal components were used as predictors in a multivariate Cox regression model for T2 progression. Patients who had already progressed to T2 at TURBT were coded as having time-to-event = 0. The best Cox model was chosen using a backwards selection algorithm incorporating the Akaike Information Criterion for model comparison.

Phase III: Evaluation of Gene Signature Predictive Ability and Biological Networking

Statistical analysis. To evaluate the signature’s predictive power, leave-one-out cross-validation was done, resulting in a predicted probability of T2 progression for each Ta and T1 patient at TURBT. These cross-validated predictions were used to stratify non-T2 patients into high- and low-risk groups for T2 progression using the median predicted probability as the cutoff. Differences in outcome were evaluated using Kaplan-Meier curves and log-rank tests. Additionally, Akaike Information Criterion was used to select a best multivariate Cox regression model for progression to T2 using age, gender, carcinoma in situ, stage, and grade as possible predictors, and the likelihood ratio test was used to evaluate the significance of the
signature when added to this clinical model. Associations between these clinical variables and T2 progression were assessed using univariate Cox models and likelihood ratio tests.

Molecular concepts map analysis. Molecular concepts map analysis computes pairwise associations between gene sets to create an “enrichment network” of associations across all available signatures, arising from a variety of cancer types, pathways, mechanisms, and drugs (11). This compendium of >14,000 “molecular concepts” or sets of biologically connected genes, is available at http://private.molecularconcepts.org. A gene set of interest can then be investigated for its functional overlap with other gene sets and biological concepts (see Supplementary Methods).

Phase IV: Immunohistochemical Confirmation of Sample Genes

Tissue microarray construction and immunohistochemical evaluation. Two genes, ACTN1 and CDC25B, were identified from the metasignature; these had available antibodies and were chosen for immunohistochemical analysis using a bladder cancer progression tissue microarray. This tissue microarray was constructed from 41 cases derived from 40 patients, representing benign bladder tissue, bladder carcinoma in situ, bladder cancer (noninvasive and invasive), and bladder cancer lymph node metastases. Three cores (0.6 mm in diameter) were taken from each tumor focus confirmed by two surgical pathologists (R.M. and L.P.K.). All tissues were derived from our institutional bladder cancer database with informed consent of the patients and prior institutional review board approval; there was minimal overlap of cases used for tissue microarray construction and mRNA extraction.

Immunohistochemistry was done on the tissue microarray using mouse monoclonal antibodies against CDC25B (LabVision; 1:50 dilution) and ACTN1 (Santa Cruz Biotechnology; 1:50 dilution) proteins and standard avidin-biotin complex techniques as described previously (12). Details of the tissue microarray construction and immunohistochemical staining are provided in Supplementary Methods.

Statistical analysis. For immunohistochemical analysis, one-way ANOVA was used to compare distributions of the median product scores by group. F tests were used to compare competing models, and comparisons between groups were made using Tukey’s honest significant difference procedure (for pairwise comparisons) and Scheffe’s method (other comparisons).

All statistical analyses were done using R version 2.7.0.7

Results

Characteristics of patients used for development of gene signatures. Frozen tumor sections were available for all 100 patients selected from the tissue bank and 12 benign bladder specimens (total n = 112). One benign and four tumor samples were eliminated from final analysis, secondary to low gene expression. The final cohort consisted of 107 samples-96 tumor samples, with 42 nonprogressing tumors, 54 progressing or T2 tumors, and 11 benign bladder samples. There was high QPCR reproducibility across batches (Supplementary Fig. S1). Patient demographics for the final 96 tumor samples are listed in Table 1. Median follow-up in non-T2 patients for whom predictions were done using R version 2.7.0.7

7 http://www.r-project.org
made was 2.4 years. Overall, 5 of 31 Ta tumors and 15 of 31 T1 tumors progressed to T2 during follow-up.

Univariate analysis revealed that pathologic stage (T1 versus Ta) was the only significant clinical predictor of T2 progression ($P = 0.01$). Grade (high versus low) approached significance as a predictor of T2 progression ($P = 0.08$), but a within-stage analysis revealed that grade was not predictive of progression (Supplementary Table S4).

**57-gene signature.** The 107 bladder samples were run on the preconfigured 96-element TLDNA cards. Fifty-seven genes showed differential expression between T2 and non-T2 tumors ($P < 0.05$), with an estimated false discovery rate of 1.1%. This set consisted of 37 overexpressed and 20 underexpressed genes in T2 tumors. Further gene signature details are available in Supplementary Results.

We sought to determine whether this gene signature was associated with progression of Ta and T1 tumors to muscle-invasive disease. Five-year outcomes for the Ta and T1 tumors are shown in Fig. 2. Using the 57-gene signature to divide this population into high- and low-risk groups, high-risk patients exhibited a higher rate of progression to T2 disease within 2 years (45% for high-risk patients versus 12% for low-risk patients; $P = 0.003$; Fig. 2A). As expected, stage alone was a significant predictor of T2 progression, with more T1 patients experiencing T2 progression than Ta patients ($P = 0.007$; Fig. 2B). Importantly, however, the gene signature prediction maintained significance within T1 tumors (61% progression for high-risk patients versus 22% progression for low-risk patients; $P = 0.02$; Fig. 2C) and Ta tumors (29% progression for high-risk patients versus 0% progression for low-risk patients; $P = 0.03$; Fig. 2D), showing that this gene signature provides additional risk stratification beyond stage alone. This difference in outcomes is most pronounced for T1 patients in the first year of follow-up, during which 7% of predicted low-risk T1 patients progressed to T2 disease versus 61% of predicted high-risk T1 patients.

Several clinical variables (age, gender, pathologic stage, histologic grade, and associated carcinoma in situ) were investigated with univariate analysis: the only significant predictor of progression was pathologic stage ($P = 0.01$; Supplementary Table S4). Although histologic grade was marginally associated with T2 progression ($P = 0.08$), this could be explained by a strong association between histologic grade and pathologic stage in this cohort ($P < 0.0001$, Fisher’s exact test). Indeed, in a multivariate Cox model using clinical parameters, the best model retained only stage and gender as significant predictors of T2 progression (Table 2). The gene signature, however, provided significant ability to predict progression independent of stage and gender ($P = 0.002$, likelihood ratio test).

A heat map comparing the 57 genes and all samples is shown in Fig. 3. Hierarchical clustering of genes showed the 37 overexpressed (cluster 1) and 20 underexpressed (cluster 2) genes in T2 disease. In the overexpressed gene set, two smaller gene subsets can be appreciated from hierarchical clustering (clusters 1A and 1B). The 20 underexpressed genes in T2 disease are also relatively overexpressed in Ta patients without progression. The benign samples show underexpression of clusters 1B and 2 genes. Cluster 2 genes are up-regulated with progression from benign to Ta disease but down-regulated again in transition to T2 disease.

**Molecular concepts map analysis.** To integrate the selected genes into a functional framework, the gene sets were investigated in the context of molecular concepts map analysis. This concept-based analysis of the 57-gene signature showed enrichment of cell adhesion and extracellular matrix invasion pathways as well as cell cycle regulation and mitosis in the up-regulated genes, confirming the importance of these programs in bladder cancer progression (13). For the underexpressed genes, these showed overlap with underexpressed genes in poorly differentiated lung and invasive breast carcinomas; also, included in the list of down-regulated genes are several well-known tumor suppressors, including $p53$ and $RB1$ (retinoblastoma 1). See Supplementary Results and Supplementary Figs. S2 and S3 for further details.

**Immunohistochemical analysis.** To further validate the components of the 57-gene signature with protein expression, we identified genes for which immunohistochemistry-compatible antibodies were available. Antibodies to ACTN1 and CDC25B were identified and immunohistochemistry was done to investigate protein expression in situ on a bladder cancer progression tissue microarray (Fig. 4). Both markers showed homogenous staining, with predominantly cytoplasmic expression for ACTN1 and nuclear expression for CDC25B. ACTN1 showed the most significant individual group comparison difference between the noninvasive and either invasive or metastatic groups ($P = 0.003$ for each, Tukey’s honest significant difference; Fig. 4B). CDC25B expression showed a more linear trend with disease severity ($P = 0.0002$; Fig. 4C). More detailed information is available in Supplementary Results.

**Discussion**

The accurate designation of Ta and T1 bladder cancers that will progress to muscle invasion has yet to be perfected and currently

**Table 2. Multivariable analysis of gender, pathologic stage, and gene signature score in relation to likelihood of progression to T2 disease**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio (95% confidence interval)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysis without signature*</td>
<td>4.12 (1.46-11.63)</td>
<td>0.007</td>
</tr>
<tr>
<td>Pathologic stage (Ta vs T1)</td>
<td>2.39 (0.67-8.49)</td>
<td>0.18</td>
</tr>
<tr>
<td>Gender (male vs female)</td>
<td>2.88 (0.98-8.47)</td>
<td>0.06</td>
</tr>
<tr>
<td>Analysis with signature†</td>
<td>1.29 (0.34-4.80)</td>
<td>0.71</td>
</tr>
<tr>
<td>Pathologic stage (Ta vs T1)</td>
<td>12.03 (2.40-60.2)</td>
<td>0.003</td>
</tr>
<tr>
<td>Gender (male vs female)</td>
<td>2.30 (0.67-8.49)</td>
<td>0.18</td>
</tr>
<tr>
<td>Gene signature (high vs low risk)</td>
<td>2.88 (0.98-8.47)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*Pathologic stage and gender (age, grade, and carcinoma in situ) removed during selection.
† $P = 0.002$, likelihood ratio test.
relies on pathologic review and surveillance with gold standards of cystoscopy and urine cytology. Urine cytology, however, lacks sensitivity for low-grade tumors (14, 15), and cystoscopic detection may occur months after muscle invasion depending on the interval. Earlier detection of progression to muscle-invasive disease may provide a survival benefit given decreased long-term survival of patients with muscle-invasive disease (likely due to the presence of concomitant micrometastasis). Additionally, patients with non–muscle-invasive cancers who progress to T2 on surveillance show similarly poor survival after cystectomy as patients presenting with T2 disease (16). Although urine-based tests exist to detect incipient or recurrent bladder cancer (14, 17), there are no widely used modalities to risk-stratify patients beyond initial detection. Nomograms exist to estimate risks of recurrence and progression but have not gained wide acceptance in the United States; these often require clinical information not readily available, such as

**Figure 3.** Heat map representation of the 57-gene signature for bladder cancer progression. Samples are separated by pathologic stage (benign, Ta, T1, and T2) and progression to T2 (gray, no progression; black, progression). By default, all benign samples are labeled gray and T2 samples are labeled black. Ta and T1 samples are ordered by predicted risk (low-risk to high-risk), and T2 samples are ordered via results of complete linkage hierarchical clustering with Spearman correlation used to measure distance. The genes were clustered using this hierarchical clustering, resulting in three smaller clusters (clusters 1A and 1B overexpressed in T2 samples and cluster 2 underexpressed in T2 samples). Color bar units correspond to SD.
tumor multiplicity and size (18), or incorporate single bladder tumor markers (4). A tumor-specific multigene signature can provide a more comprehensive picture of tumor aggressiveness. Microarray gene expression profiling is difficult to translate into a clinical prognostic tool given the large number of genes involved (19) and required time and expertise. QPCR is more clinically applicable, especially when working with a small group of highly selected genes. Our methodology is applicable across many cancer types: compiling microarray data for bioinformatics analysis, generating a larger list of robust genes involved in aggressive behavior, and deriving a smaller QPCR gene signature to predict an outcome of interest. In this study, we summarized the most essential transcripts from available bladder cancer microarray data into a prognostic gene set of 57 genes. This resulted in a clinically feasible test, using a small amount of frozen bladder tumor available from TURBT, to provide a gene signature that helps predict progression in non–muscle-invasive cancers. Specifically, patients who were designated as high risk by the gene signature were more likely to show progression to T2 disease than low-risk patients; this predictive ability surpassed information provided by pathologic stage alone. This provided evidence that a gene signature can provide additional risk stratification beyond pathology, particularly because interobserver variability exists in tumor staging and grading for bladder (20, 21) and other cancers.

Figure 4. Immunohistochemical staining of two metasignature candidates, ACTN1 and CDC25B. Representative immunostaining of ACTN1 (A1-A3) and CDC25B (A4-A6) across bladder cancer progression in noninvasive bladder cancer (A1 and A4), invasive bladder cancer (A2 and A5), and metastatic deposits in lymph nodes (A3 and A6). The higher-magnification insets represent expression levels of cytoplasmic staining for ACTN1 and nuclear staining for CDC25B. Bar, 100 μm, with all images at same magnification. Box plots of median product scores for ACTN1 (B) and CDC25B (C) show a clear trend of increasing product score by cancer progression. For ACTN1, the strongest differences appear when comparing noninvasive tumors with either invasive tumors or metastatic tumors (P = 0.003 for each). For CDC25B, there is an increasing trend with bladder cancer progression (P = 0.0002).
Two genes from the signature whose protein expression has not been described explicitly in bladder cancer were chosen for immunohistochemical analysis. ACTN1 has been shown to possess different splicing patterns in T2 versus Ta tumors (24), suggesting an ability to use ACTN1 in stage separation; in fact, ACTN1 protein expression was significantly between non–muscle-invasive and invasive or metastatic bladder cancers. CDC25B has been shown to be up-regulated in progressing bladder tumors in a previous microarray study (25), correlating with its gradually up-regulated protein expression on immunohistochemistry. Many of the other genes in the signature have also been studied in bladder cancer: TIMP2 in bladder cancer metastases (26) and p53 and RB1 in bladder cancer development and progression (27, 28). Furthermore, others have shown that cell cycle dysregulation is necessary for uroepithelial transformation and cell adhesion dysregulation is commonly found in uroepithelial tumor progression (13). This gene signature compiles the most essential genes from previous microarray studies and may prompt further investigation into their complex interactions necessary for progression.

Limitations of this study include the retrospective nature of sample collection, small sample size, and nonstandardized follow-up. A larger sample size was difficult to accrue given our institution’s tertiary referral pattern, although this issue plagues many single-institution bladder cancer microarray studies. Noninvasive tumors could have progressed later than the time of follow-up for some patients, thus creating false-negative predictions. However, poor outcomes in bladder cancer are more likely to manifest themselves early with a shorter natural history than prostate cancer, for example, and predicted low-risk patients would be maintained on standardized cystoscopic and imaging surveillance. We acknowledge that false-positive results could prompt more invasive treatment modalities earlier and emphasize the need to integrate this gene signature with clinical parameters.

Ideally, a prospective multi-institutional study with standardized follow-up and a larger sample size would be required. This type of study would include collection of tissue and urine, with the ultimate goal of creating a noninvasive test. Also, further studies may result in concentration of this gene set into a smaller essential set of genes. From a technical perspective, samples used in this study were grossly dissected, and the distinction between epithelial and stromal components was not made. Also, although reliance on a manufactured card streamlines QPCR, gene primers are limited to those commercially available and may not account for splicing or fusion variants. This list inevitably excluded other promising candidates in bladder cancer, such as the Ral family of GTPases (29), KiSS-1 (metastin; ref. 30), or PTEN (31, 32).

In conclusion, we used comparative metaprofiling of existing bladder cancer microarray data sets to define genes involved in aggressive behavior and refined this to a final 57-gene signature that was significantly associated with the risk of progression to muscle invasion. This signature can be preloaded onto a commercially available QPCR card and, with prospective validation, could become a clinically applicable point-of-care tool for risk stratification in non–muscle-invasive bladder cancers. The broader implications of this study are that we established a systematic “pipeline” for converting multiple independent microarray studies into a high-throughput QPCR platform more amenable to clinical translation.

Disclosure of Potential Conflicts of Interest

A.M. Chinnaiyan, R. Wang, D.S. Morris, and S.A. Tomlins have submitted a patent application for the gene signature described in this article. The other authors disclosed no potential conflicts of interest.

Acknowledgments

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References


www.aacrjournals.org 3817 Cancer Res 2009; 69: (9). May 1, 2009