Prediction of Lymph Node Metastasis in Patients with Bladder Cancer Using Whole Transcriptome Gene Expression Signatures

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Abbreviations and Acronyms

FFPE = formalin fixed, paraffin embedded

LN = lymph node

MIBC = muscle invasive bladder cancer

NAC = neoadjuvant chemotherapy

PLND = pelvic lymph node dissection

TURBT = transurethral resection of bladder tumor

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Purpose: Clinical staging in patients with muscle invasive bladder cancer misses up to 25% of lymph node metastasis. These patients are at high risk for disease recurrence and improved clinical staging is critical to guide management.

Materials and Methods: Whole transcriptome expression profiles were generated in 199 patients who underwent radical cystectomy and extended pelvic lymph node dissection. The cohort was divided randomly into a discovery set of 133 patients and a validation set of 66. In the discovery set features were identified and modeled in a KNN51 (K-nearest neighbor classifier 51) to predict pathological lymph node metastases. Two previously described bladder cancer gene signatures, including RF15 (15-gene cancer recurrence signature) and LN20 (20-gene lymph node signature), were also modeled in the discovery set for comparison. The AUC and the OR were used to compare the performance of these signatures.

Results: In the validation set KNN51 achieved an AUC of 0.82 (range 0.71–0.93) to predict lymph node positive cases. It significantly outperformed RF15 and LN20, which had an AUC of 0.62 (range 0.47–0.76) and 0.46 (range 0.32–0.60), respectively. Only KNN51 showed significant odds of predicting LN metastasis with an OR of 2.65 (range 1.68–4.67) for every 10% increase in score ($p < 0.001$). RF15 and LN20 had a nonsignificant OR of 1.21 (range 0.97–1.54) and 1.39 (range 0.52–3.77), respectively.

Conclusions: The new KNN51 signature was superior to previously described gene signatures for predicting lymph node metastasis. If validated prospectively in transurethral resection of bladder tumor samples, KNN51 could be used to guide patients at high risk to early multimodal therapy.

Key Words: urinary bladder neoplasms; neoplasm metastasis; lymph nodes; genomics; biomarkers, tumor

In MIBC optimal clinical staging misses up to 25% of LN metastases.[1] These patients with LN positive findings are at high risk for disease recurrence and death.[2,3] An improved patient outcome has been demonstrated by treatment intensification with NAC.[4,5] NAC in principle is designed to target micrometastatic disease,[6,7] especially including positive

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LNs missed by clinical staging. Therefore, more accurate clinical staging is warranted to better predict patient outcomes and guide optimal management.

Gene expression signatures of primary tumors have been successfully applied to predict LN metastasis in cancer of the breast,[8] prostate,[9] head and neck,[10] and bladder.[11–13] Smith et al described LN20, a promising signature to predict LN metastasis in MIBC.[13] This signature of 20 protein coding genes was robustly tested in frozen and FFPE tissue, and it significantly predicted LN involvement in an independent validation cohort with an AUC of 0.67. Perhaps due to this modest AUC this signature has not been adopted into clinical practice.

The majority of genomic signatures depend only on protein coding RNA transcripts. With increasing understanding of the broad scope of different forms of RNA and their biological importance[14] there is reason to believe that the performance of genomic signatures can be enhanced by including nonprotein coding transcripts, which make up the majority of the transcriptome.[15] Several examples of such whole transcriptome analysis for cancer risk stratification have been reported in the literature,[8,16,17] including our prior experience with prognostic signatures in bladder[18] and prostate[19,20] cancer.

The aim of this study was to develop a novel genomic classifier to predict lymph node involvement at radical cystectomy in patients with MIBC using a whole transcriptome microarray platform. We also sought to compare this novel signature to previously reported signatures based only on protein coding transcripts.

PATIENTS AND METHODS

Patients, Followup and Pathological Findings

Tumor tissue from cystectomy specimens was collected from 225 patients with pT2-4N0-3M0/pT1N1-3M0 urothelial cancer of the bladder who underwent radical cystectomy with extended pelvic lymphadenectomy at University of Southern California between 1998 and 2004.[18] As in the previous study,[18] we selected the 199 patients with followup longer than 2 years and without recurrence. No patient received neoadjuvant chemotherapy and all were free of nodal and distant metastasis on preoperative imaging (cN0M0). All tumor specimens underwent histopathological rereview and were staged according to the 2009 UICC 6th classification.[21] The University of Southern California institutional review board approved this study and all patients consented to analysis of tumor tissues.

Sample Processing, Microarray Analysis and Quality Control

Hematoxylin and eosin stained sections were used to identify representative areas of tumor from cystectomy specimens for sampling. Using the RNeasy® FFPE kit total RNA was extracted from a 1 mm diameter core punch of the tumor. The Ovation® WTA FFPE System and the Encore® Biotin Module were used for cDNA amplification and labeling before hybridization to GeneChip® Human Exon 1.0 ST oligonucleotide microarrays according to manufacturer recommendations. Quality control was assessed by Affymetrix® Power Tools packages and internally developed metrics.[22] All array files for these cases are available at the NCBI (National Center for Biotechnology Information) GEO (Gene Expression Omnibus) database (http://www.ncbi.nlm.nih.gov/geo/) under GEO accession code GSE57933. The same random assignment to a discovery set of 133 patients and a validation set of 66 was used as in our previous study.[18]

15-Feature Genomic Classifier

As previously described,[18] this genomic classifier was discovered and validated using the same microarray data used for this study. It was designed to predict postcystectomy bladder cancer recurrence and it outperformed not only clinical models but also previously developed signatures. Gene expression values were determined for all samples and RF15 prediction scores were calculated.

LN20 Signature Re-Creation in Human Exon Array

The LN20 signature based on Affymetrix U133 arrays was ported to the Human Exon Array and recapitulated using the modeling method described by Smith et al.[13] Probe sets on the GeneChip Human Exon Array corresponding to each of the 20 genes in the LN20 signature were gene summarized and then standardized using a weighed z-score. After training on the 133 discovery samples a weighted nearest neighbor model was created using the Epanechnikov kernel with the Spearman correlation as the distance metric. For each patient this model was used

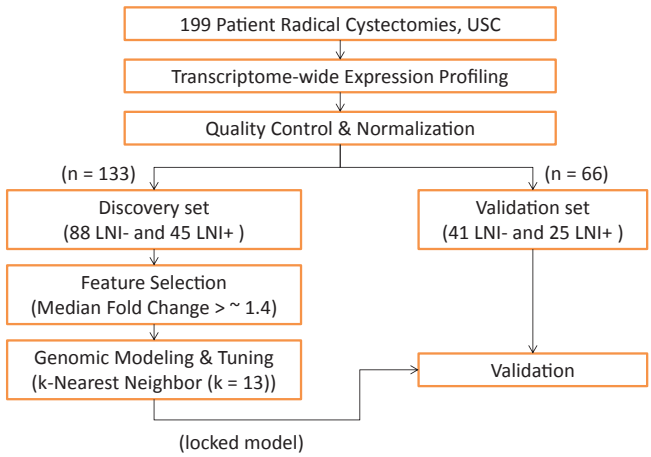

Figure 1. Discovery of KNN51. After whole transcriptome expression profiling, quality control and normalization cohort was divided into discovery and validation sets. For signature discovery features were selected according to median fold change and modeled as KNN classifier after tuning via tenfold cross validation. Signature was locked and validated in validation set. *USC*, University of Southern California. *LNI*, LN invasion.