MicroRNA Expression Profiles in Upper Tract Urothelial Carcinoma Differentiate Tumor Grade, Stage, and Survival: Implications for Clinical Decision-Making

Brendan M. Browne, Kristian D. Stensland, Chintan K. Patel, Travis Sullivan, Eric J. Burks, David Canes, Jay D. Raman, Joshua Warrick, and Kimberly M. Reiger-Christ

OBJECTIVE	To evaluate microRNA (miRNA) biomarkers for upper tract urothelial carcinoma (UTUC) to improve risk stratification.
METHODS	miRNA was isolated from 157 radical nephroureterectomy specimens from 2 institutions. The relative expression of miRNA was examined for high grade vs low grade tumors as well as muscle invasive vs nonmuscle invasive tumors. Recurrence free survival (RFS) and overall survival (OS) were also stratified using relative expression of specific miRNA.
RESULTS	The optimized model to identify high grade UTUC included miR-29b-2-5p, miR-18a-5p, miR-223-3p, and miR-199a-5p, generating a sensitivity of 83%, specificity of 85%, and generated a receiver operating characteristic (ROC) curve with area-under-the-curve of 0.86. Similarly, the model classifier for predicting ≥ pT2 disease incorporated miR-10b-5p, miR-26a-5p-5p, miR-31-5p, and miR-146b-5p, producing a sensitivity of 64%, specificity of 96%, and area-under-the-curve of 0.90. RFS was best reflected by a combination of miR-10a-5p, miR-30c-5p, and miR-10b-5p, while OS was best predicted by miR-10a-5p, miR-199a-5p, miR-30c-5p, and miR-10b-5p.
CONCLUSION	High-grade vs low-grade as well as muscle invasive vs nonmuscle invasive UTUC can be reliable distinguished with unique miRNA signatures. Furthermore, differential expression of UTUC miRNA produces robust classifiers for predicting RFS and OS that may help identify patients who would most benefit from adjuvant therapies. UROLOGY 123: 93−100, 2019. © 2018 Elsevier Inc.

U pper tract urothelial carcinoma (UTUC) is an uncommon genitourinary malignancy comprising 5%-10% of all urothelial tumors with an annual incidence of 1-2 cases per 100,000 individuals.[1] These tumors often present with hematuria or renal obstruction and the diagnosis is confirmed by urine cytology, upper tract imaging, and/or ureteroscopic biopsy. Treatment options for UTUC are largely based on tumor grade and stage at time of biopsy. For high-risk cancers

the gold standard is radical nephroureterectomy (RNU) with an ipsilateral bladder cuff, whereas lower risk tumors may be approached with nephron-sparing segmental ureterectomy, percutaneous resection, or endoscopic ablation.[2,3] While nephron sparing procedures have traditionally been reserved for patients with a solitary kidney, renal insufficiency, or bilateral UTUC, recent EAU guidelines have included low-grade/low-volume UTUC as an additional indication even in patients with a normal contralateral kidney.[1]

Despite attempted definitive extirpative surgery, UTUC still demonstrates considerable rates of recurrence and mortality particularly for locally advanced and nonorgan confined disease.[4] Adjuvant chemotherapy appears to confer an overall survival benefit,[5,6] but the toxicity of platinum-based regimens is compounded in patients with post-RNU solitary kidney and thus would be ideally targeted only at higher risk tumors. Histologic findings of tumor grade and muscle invasion status are currently the primary metrics to predict RFS and OS,[1] but there remains room for

improvement. Risk stratification using biomarkers in addition to established clinicopathologic parameters could better identify those specific high-risk patients who would benefit most from adjuvant treatment.

MicroRNAs (miRNA) are a class of small noncoding nucleic acids that have demonstrated a role in oncogenesis and tumor suppression by regulating cell cycle checkpoints.[7] Aggressive tumors exhibit aberrant miRNA expression in many tissues, including kidney,[8] prostate,[9] colon,[10] breast,[11] and bladder.[12] To date, the miRNA expression profile in UTUC remains largely unexplored.[13-17] This study investigates the utility of UTUC miRNA profiles to stratify tumors by grade and muscle invasion, as well as predict survival.

MATERIALS AND METHODS

Tissue Samples
In this IRB approved study, samples were collected from patients undergoing RNU between 2005 and 2013 at our hospitals. All final pathology specimens were formalin fixed paraffin embedded. The specimen block with the greatest amount of tumor was selected for RNA isolation. TNM classification was defined using the seventh edition of the American Joint Committee on Cancer criteria. A single pathologist specializing in urologic malignancies (EJB) reviewed all samples from both institutions for diagnostic uniformity of tumor grade and stage.

Study Design
This is a retrospective study with the primary analysis aimed to identify miRNA capable of discriminating low-grade from high-grade tumors as well as < pT2 from ≥ pT2 tumors. Secondary analyses compared miRNA profiles prediction of overall survival (OS) and recurrence free survival (RFS). Testing was performed in 2 phases including a screening and a validation cohort (Supplemental Figure 1). For the initial screening cohort, 35 RNU samples were selected to include a spectrum of low-grade (n = 15) and high-grade (n = 20) as well as muscle invasive (n = 16) and noninvasive (n = 19) pathology. These specimens were screened using qRT-PCR arrays to identify miRNAs with higher or lower expression in one cohort vs its counterpart, ie, differentially expressed.

For the second phase, miRNA identified as differentially expressed in the screening cohort as well as control miRNA were tested on a separate cohort containing 123 additional RNU specimens from 2 institutions. Analysis of miRNA signatures was performed to discriminate low-grade (n = 47) and high-grade (n = 76) samples and then reanalyzed to compare < pT2 (n = 79) and ≥ pT2 (n =44) samples. In an effort to develop a diagnostic model, samples were randomly assigned to either a training set (70% of eligible samples, n = 86) to identify miRNA with differential expression, or validation sets (30% of eligible samples, n = 37) to assess the diagnostic capability of the training set miRNA signatures. Breakdown of grade and invasion for training and validation sets are shown in Supplemental Figure 1. OS and RFS were analyzed in all samples from the second cohort.

Recurrence and Survival Analysis
Post hoc analysis was done to evaluate the ability of the miRNA profile to predict UTUC RFS and OS. Evaluation for recurrence after RNU was not standardized across all surgeons, but generally was performed every 6 months for 2 years and annually thereafter. Assessments included history and physical, serum chemistry, urine cytology, cystoscopy, chest X-ray, and cross-sectional abdominal imaging with delayed contrast imaging of contralateral kidney. When clinically indicated, additional studies would be obtained, including magnetic resonance imaging, bone scan, and chest or head CT scan. Recurrence was defined as radiologic or pathologic recurrence in the kidney resection bed, regional lymph nodes, or distant metastasis through the entirety of patient's follow-up. Development of metachronous urothelial carcinoma of the bladder was not counted toward recurrence.

MiRNA Isolation and RT-PCR
Total RNA was isolated using the RecoverAll Total Nucleic Acid Isolation Kit (Ambion, Foster City, CA) according to the manufacturer's instructions using four sequential 20 μm formalin fixed paraffin embedded sections per sample. qRT-PCR reactions were performed using miRCURY LNA reagents following manufacturers' protocols (Exiqon, Vedbñk, Denmark). For each sample, 4 ng of total RNA was used in 20 μl universal reverse transcription reactions; subsequently 16pg of cDNA was used in each 10 μl qRT-PCR reaction. Screening analysis was performed using Human miRNome panels I and II version 4, analyzing 752 miRNA (Exiqon). Subsequently, a selection of differentially expressed miRNA identified by the screening array, as well as other miRNA reported in analogous UTUC miRNA studies, were validated using miRNA LNA primer sets with the second cohort of samples tested in triplicate.

Data Analysis and Statistical Analysis
For array analyses, expression levels of miRNA were normalized to the global mean of each sample for all miRNA with a Cq < 37 for all samples. Expression levels were quantified as x = $2^{-\Delta Ct}$ and log2 transformed. Two-sided Welch's t tests were performed on the transformed values. Significant differences were determined after adjusting for multiple comparisons using the Benjamini and Hochberg method with a false discovery rate of 0.1. Hierarchical clustering using one minus Pearson correlation was performed using GENE-E (Broad) with mean centered values. The expression levels for the subsequent analyses, with the second cohort of samples, were normalized to the average of the insignificant control miRNA detected in all samples: miR-125b-5p and miR-185-5p, which were selected from the screening array dataset using NormFinder (MOMA 2004, Aarhus, Denmark).

Univariate regressions were performed to identify miRNA associated with invasion or grade. The miRNA with significant associations were then assessed for clinical utility via sensitivity and specificity analyses. Multivariate models utilizing continuous miRNA expression values were created using stepwise selection to predict invasion and recurrence. The optimized equation was then utilized to predict the likelihood of high grade or invasive disease. For assessment of accuracy values including sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV), a numerical prediction of invasion or grade was defined as a probability greater than or equal to 0.5.

Recurrence-free and overall survival prediction was assessed via Cox proportional hazard models. All available miRNA and clinical variables were assessed for inclusion in the final model. ROC analysis was utilized for predicting death or recurrence for each miRNA, and Youden's index was used to identify cut-off

values for defining under or overexpression of each miRNA. Forward stepwise selection with requisite inclusion of clinical factors was performed to identify miRNA associated with OS or RFS.

RESULTS

Patient Characteristics
Tumor samples were collected from 158 patients. Mean age was 71 years at time of RNU and median follow-up time was 33 months (IQR 12-62 months). Patient and tumor characteristics are reported in Table 1.

Screening Array
The data from the screening array have been deposited in NCBI's Gene Expression Omnibus[18] and are accessible with GEO Series accession number GSE119601 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE119601). There were 215 miRNA detected (Cq < 37) across all samples of the screening cohort. For tumor grade, 52 miRNA were significantly differentially expressed, and for muscle invasion 52 miRNA were significantly differentially expressed. Of these, 20 miRNA were common to both analyses (Supplemental Table 3). Hierarchical cluster analysis of miRNA with a false discovery rate < 0.1, $P < .05$ and at least a ±1.75 fold difference in expression resulted in distinct clusters of samples discriminating for both grade and invasion (Fig. 1). The 2 clustered generated in the grade analysis contained 20 samples of which 18 were high-grade carcinomas and 15 samples of which 13 were low-grade carcinomas ($P < .001$). Similarly, the clusters generated by invasion depth contained 17 samples of which 14 were ≥ pT2 carcinomas and 18 samples of which 16 were < pT2 ($P < .001$).

Confirmation of Differential miRNA Expression
To confirm differential expression observed in our screening analyses, as well as previously identified miRNA from published UTUC studies, the relative expression of 26 miRNA was measured in the secondary cohort of RNU samples: 7 miRNA that were significantly differentially expressed both in our screening analyses for either grade or stage as well as in prior UTUC studies, 17 miRNA that were unique to our screening analysis, and 2 miRNA that were selected from prior UTUC publications but were not significant in our screening analysis. The data from this analysis have been deposited in NCBI's Gene Expression Omnibus[18] and are accessible via accession number GSE119899 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE119899). After applying a Bonferroni adjustment for multiple comparisons, 18 miRNA were confirmed to show differential expression in the secondary cohort

(Supplemental Table 1): 6 were common to both the grade and invasion analyses, 2 were unique to the grade analysis, and 10 were exclusive to the invasion analysis.

miRNA Classifier for Prediction of Grade and Invasion
Multivariate analysis was performed to generate statistical classifiers to predict UTUC grade and extent of invasion based on expression of specific miRNA relative to control values. For prediction of a high-grade carcinoma, the optimized model incorporating expression of miR-29b-2-5p, miR-18a-5p, miR-223-3p, and miR-199a-5p produced a sensitivity of 83%, specificity of 85%, PPV 91%, NPV 73%, and generated a ROC curve with area-under-the-curve (AUC) of 0.86 (95% confidence interval 0.73-0.99). The optimized model classifier for predicting ≥ pT2 disease incorporating expression of miR-10b-5p, miR-26a-5p-5p, miR-31-5p, and miR-146b-5p generated a sensitivity of 64%, specificity of 96%, PPV 90% and NPV 81% with an AUC of 0.90 (95% confidence interval 0.79-1). ROC curves are shown in Figure 2.

Recurrence Free and Overall Survival
Overall, 26/123 (21.1%) patients showed tumor recurrence at a median of 16 months (IQR 6.5-22). Additionally there were 41 deaths from all causes (33%) during the follow-up period. Post hoc multivariate analysis of the miRNAs tested in the secondary cohort was performed along with inclusion of clinical data points (smoking status, renal pelvis vs ureteral location, tumor grade, and muscle invasion status) to generate a classifier to predict RFS and OS, with results displayed in Supplemental Table 2. The most predictive classifier incorporating clinical factors and miRNA included miR-10a-5p, miR-30c-5p, and miR-10b-5p for RFS, while OS was best predicted by miR-10a-5p, miR-199a-5p, miR-30c-5p, and miR-10b-5p. Of these miRNAs, miR-10a-5p independently achieved statistical significance for predicting overall survival when expression was divided by the Youden's Index-defined cutoff (log rank $P < .001$). The Kaplan-Meier curve for OS based on miR-10a-5p expression is shown in Figure 3.

DISCUSSION
Primary tumor grade and pathological stage are the most frequently reported prognostic factors for UTUC,[1] but even muscle-invasive and nonmuscle invasive tumors that are nonmetastatic can recur with resultant cancer-specific mortality within 5 years of nephroureterectomy.[4] These unexpected early deaths point to malignant behavior beyond what histologic grade and stage can predict.

Table 1. Demographics and tumor characteristics

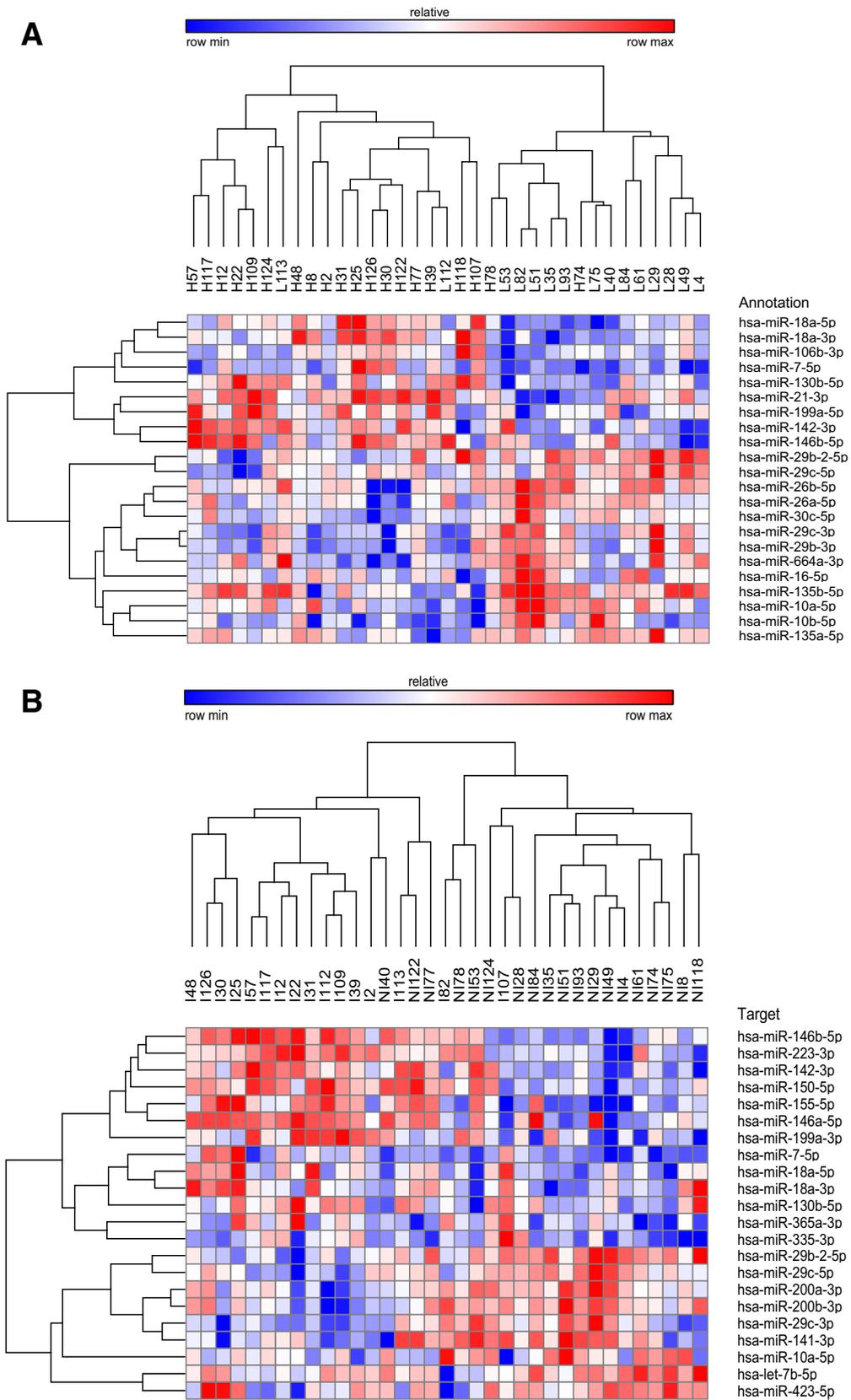|	Screening Cohort (n = 35)	Secondary Cohort (n = 123)	*P* value
Age (mean ± SD)	73.2 y ± 8.3	70.9 y ± 9.9	.23
Male	51%	67%	.46
Tumor size (mean ± SD)	3.4 cm ± 1.5 cm	3.7 cm ± 1.9 cm	.42
Low grade	42.9%	37%	.81
Tumor stage			.27
pTa	16 (44%)	45 (37%)	
pT1	3 (8%)	31 (25%)	
pT2	4 (11%)	15 (12%)	
pT3	10 (28%)	28 (23%)	
pT4	3 (8%)	4 (3%)	

Figure 1. Heat maps for miRNA cluster analysis, screening cohort. (**A**) Grade ("H" or "L" prefix denotes high grade or low grade, respectively). (**B**) Muscle invasion ("I" or "NI" prefix denotes invasive or noninvasive, respectively). (Color version available online.)
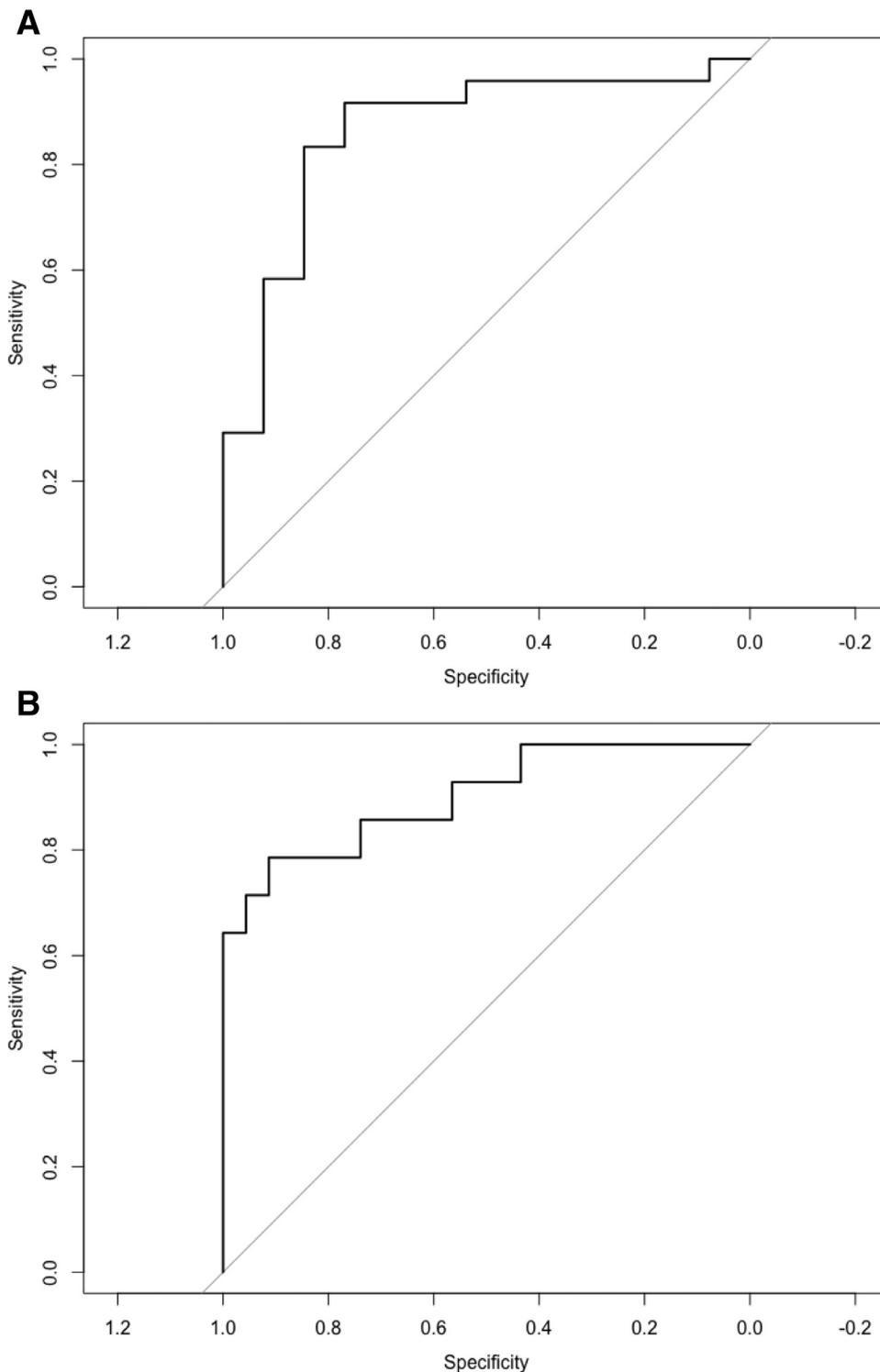
Figure 2. Receiver-operator curves for miRNA classifiers for **(A)** tumor grade and **(B)** muscle invasion.

Improved tumor risk stratification would better identify high-risk tumors to optimize postoperative surveillance and adjuvant therapy pathways.

Multiple biomarkers including cell surface proteins, transcription factors, and DNA microsatellites have been evaluated for diagnostic and prognostic ability for UTUC,[19] but large-scale validation and adoption is needed. MicroRNA can serve as high quality biomarkers, being both integrally involved in cellular regulatory pathways and sufficiently stable for measurement in tissue and body fluids without rapid degradation as seen with more complex biomolecules.[20] We therefore sought to characterize the miRNA profile of UTUC from RNU specimens in search of prognostic markers, but also to serve as a template for aberrant miRNA that may be detectable in serum and urine. To
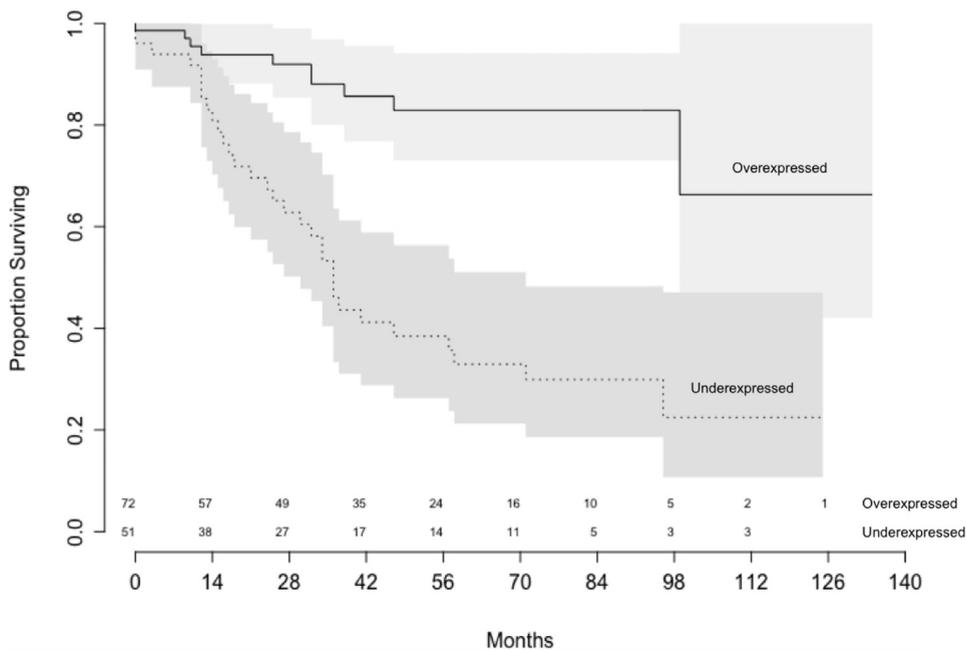
Figure 3. Kaplan-Meier survival estimates by miR 10a-5p expression.

that end, this multi-institutional study compiled the largest number of UTUC specimens to date for testing of miRNA profiles.

Recurrence and overall survival classifiers were generated based on clinical factors and miRNAs with consistent differential expression that effectively stratified patients with long vs short RFS and OS. Two miRNA appeared in both our RFS and OS models, with downregulation of miR-10a-5p and upregulation of miR-30c-5p in UTUC correlating with worse outcomes. Furthermore, miR-10a-5p showed significant capacity as an independent variable to differentiate OS. Decreased expression of miR-10a-5p has been associated with disease progression for papillary bladder tumors,[21] and neoplastic transformation, proliferation and invasion in other cancers including renal cell carcinoma,[8] gastric cancer,[22] and laryngeal cancer.[23] The antitumor effect of miR-10a-5p appears to be via targeting spindle and kinetochore-associated protein 1, and through knockdown of this protein reduce cancer cell migration and invasion.[24]

Differential expression of miR-30c-5p has not previously been reported in UTUC. As with many other miRNA, miR-30c has been reported in multiple intracellular regulatory pathways,[25] with different tumor types displaying tumor suppressor[26,27] vs oncogenic effects.[28,29] We are the first to show an oncogenic role in UTUC, impacting both RFS and OS.

Izquierdo et al identified miR-31-5p downregulation as an independent predictor of tumor progression,[17] similar to dysregulation seen in bladder cancer.[21] We found that downregulation of miR-31-5p correlates with muscle invasion; however, dysregulation of miR-31-5p was not a

predictor of DFS or OS in our analysis. Nevertheless this miRNA merits continued investigation as this field expands.

The concordance between these miRNA profiles and established histopathologic features is robust, as evidenced by the ROC curves with very high AUC values. The miRNA included in the classifiers are reproducibly aberrant for tumors with aggressive features and conceivably promote development of these characteristics. If miRNA dysregulation is driving this transformation, then the miRNA signature may be altered before any histologic changes are evident. Thus a biomarker profile may ultimately provide better clinical staging and prognostic information.

Identifying aggressive tumor biology with miRNA profiles would improve post-RNU risk stratification and selection of patients for additional treatment. Even following extirpative surgery, 5-year cancer specific mortality is 10% for patients with pTa/pT1 and up to 90% for pT4 patients.[4] Adjuvant chemotherapy with a cisplatin-based regimen appears to improve overall and disease-free survival in a recent meta-analysis,[5] but as evidenced by mixed results and wide confidence intervals, optimal patient selection for adjuvant chemotherapy vs surveillance is challenging. Furthermore, when considering high-risk UTUC disease (≥pT3 and/or LN+), a substantial number of patients fail to receive systemic adjuvant therapy.[30] Our miRNA profiles for OS and RFS readily differentiate tumors with long vs short survival and thus may indicate biochemical factors that promote recurrence, metastasis, and mortality. Adjuvant chemotherapy

could thus be targeted to patients with high-risk miRNA signatures and potentially spare the toxicity to low-risk patients.

In addition to targeting adjuvant chemotherapy, miRNA profile risk stratification could be incorporated into postoperative surveillance protocols. Current surveillance recommendations include a minimum of 5 years with rigorous radiologic and laboratory evaluation.[1] As shown in Figure 3, overexpression of miR-10a-5p showed much higher overall survival than tumors with low expression, with survival curves diverging as early as 2 years postoperatively. The opportunity to truncate postoperative surveillance based on low-risk miRNA profiles could reduce the patient's exposure to oxidizing radiation as well as the time and financial requirements to the patient and health system.

While we are encouraged by the results of this study, we do recognize its limitations. The tumor specimens were selected as the block that included the most quantity of tumor, but the blocks were not macrodissected. This could result in inclusion of attached tissues including normal urothelium, muscle or adipose that could alter the miRNA expression. Additionally the tumor specimens were not dissected to separate papillary from invasive components of individual tumors. Thus the miRNA profile may represent the tumors as a whole, but not necessarily discrete components (eg, the portion available for endoscopic biopsy). Last, the length of follow-up is relatively short in some cases and subsequent recurrence or mortality in these patients may alter the RFS and OS classifiers. Nevertheless, as this represents the largest cohort of UTUC specimens compiled for miRNA analysis, we believe that this study will help direct ongoing investigation in this area.

CONCLUSION
Differential expression of UTUC miRNA produces robust classifiers for predicting RFS and OS. Furthermore, high-grade from low-grade tumors as well as muscle invasive vs nonmuscle invasive cancers can be reliable distinguished with unique miRNA signatures. These miRNA profiles, captured from a multi-institutional cohort of tumors, may be useful for both determining UTUC prognosis and as an adjunct to tissue biopsy in order to determine which patients might be best served by nephron sparing procedures.

SUPPLEMENTARY MATERIALS
Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.urology.2018.10.004.

References
1. Rouprêt M, Babjuk M, Compérat E, et al. European Association of Urology Guidelines on upper urinary tract urothelial carcinoma: 2017 update. *Eur Urol.* 2018;73:111–122.
2. Seisen T, Peyronnet B, Dominguez-Escrig JL, et al. Oncologic outcomes of kidney-sparing surgery versus radical nephroureterectomy for upper tract urothelial carcinoma: a systematic review by the EAU non-muscle invasive bladder cancer guidelines panel. *Eur Urol.* 2016;70:1052–1068.
3. Browne BM, Stensland KD, Moynihan MJ, Canes D. An analysis of staging and treatment trends for upper tract urothelial carcinoma in the National Cancer Database. *Clin Genitourin Cancer.* 2018;16:e743–e750.
4. Lughezzani G, Burger M, Margulis V, et al. Prognostic factors in upper urinary tract urothelial carcinomas: a comprehensive review of the current literature. *Eur Urol.* 2012;62:100–114.
5. Leow JJ, Martin-Doyle W, Fay AP, Choueiri TK, Chang SL, Bellmunt J. A systematic review and meta-analysis of adjuvant and neoadjuvant chemotherapy for upper tract urothelial carcinoma. *Eur Urol.* 2014;66:529–541.
6. Margulis V, Shariat SF, Matin SF, et al. Outcomes of radical nephroureterectomy: a series from the UPPER TRACT UROTHELIAL CARCINOMA COLlaboration. *Cancer.* 2009;115:1224–1233.
7. Kent OA, Mendell JT. A small piece in the cancer puzzle: microRNAs as tumor suppressors and oncogenes. *Oncogene.* 2006;25:6188–6196.
8. Kowalik CG, Palmer DA, Sullivan TB, et al. Profiling microRNA from nephrectomy and biopsy specimens: predictors of progression and survival in clear cell renal cell carcinoma. *BJU Int.* 2017;120:428–440.
9. Doldi V, Pennati M, Forte B, Gandellini P, Zaffaroni N. Dissecting the role of microRNAs in prostate cancer metastasis: implications for the design of novel therapeutic approaches. *Cell Mol Life Sci.*. 2016;73:2531–2542.
10. Noguchi T, Toiyama Y, Kitajima T, et al. miRNA-503 promotes tumor progression and is associated with early recurrence and poor prognosis in human colorectal cancer. *Oncology.* 2016;90:221–231.
11. Lin Z, Li JW, Wang Y, et al. Abnormal miRNA-30e expression is associated with breast cancer progression. *Clin Lab.* 2016;62:121–128.
12. Wszolek MF, Rieger-Christ KM, Kenney PA, et al. A MicroRNA expression profile defining the invasive bladder tumor phenotype. *Urol Oncol.* 2011;29:794–801. e791.
13. Izquierdo L, Ingelmo-Torres M, Mallofré C, et al. Prognostic value of microRNA expression pattern in upper tract urothelial carcinoma. *BJU Int.* 2014;113:813–821.
14. Kriebel S, Schmidt D, Holdenrieder S, et al. Analysis of tissue and serum microRNA expression in patients with upper urinary tract urothelial cancer. *PLoS One.* 2015;10: e0117284.
15. Chung YH, Li SC, Kao YH, et al. MiR-30a-5p inhibits epithelial-to-mesenchymal transition and upregulates expression of tight junction protein Claudin-5 in human upper tract urothelial carcinoma cells. *Int J Mol Sci.* 2017;18:e1826.
16. Zaravinos A, Lambrou GI, Mourmouras N, et al. New miRNA profiles accurately distinguish renal cell carcinomas and upper tract urothelial carcinomas from the normal kidney. *PLoS One.* 2014;9: e91646.
17. Izquierdo L, Montalbo R, Ingelmo-Torres M, et al. Prognostic microRNAs in upper tract urothelial carcinoma: multicenter and international validation study. *Oncotarget.* 2017; 8:51522–51529.
18. Edgar R, Domrachev M, Lash AE. Gene expression omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res.* 2002;30:207–210.
19. Eltz S, Comperat E, Cussenot O, Rouprêt M. Molecular and histological markers in urothelial carcinomas of the upper urinary tract. *BJU Int.* 2008;102:532–535.
20. Armstrong DA, Green BB, Seigne JD, Schned AR, Marsit CJ. MicroRNA molecular profiling from matched tumor and bio-fluids in bladder cancer. *Mol Cancer.* 2015;14:194.
21. Segersten U, Spector Y, Goren Y, Tabak S, Malmström PU. The role of microRNA profiling in prognosticating progression in Ta and T1 urinary bladder cancer. *Urol Oncol.* 2014;32:613–618.
22. Jia H, Zhang Z, Zou D, et al. MicroRNA-10a is down-regulated by DNA methylation and functions as a tumor suppressor in gastric cancer cells. *PLoS One.* 2014;9:e88057.

23. Yao XD, Li P, Wang JS. MicroRNA differential expression spectrum and microRNA-125a-5p inhibition of laryngeal cancer cell proliferation. *Exp Ther Med*. 2017;14:1699–1705.
24. Arai T, Okato A, Kojima S, et al. Regulation of spindle and kinetochore-associated protein 1 by antitumor miR-10a-5p in renal cell carcinoma. *Cancer Sci*. 2017;108:2088–2101.
25. Ling XH, Han ZD, Xia D, et al. MicroRNA-30c serves as an independent biochemical recurrence predictor and potential tumor suppressor for prostate cancer. *Mol Biol Rep*. 2014;41:2779–2788.
26. Han X, Zhen S, Ye Z, et al. A feedback loop between miR-30a/c-5p and DNMT1 mediates cisplatin resistance in ovarian cancer cells. *Cell Physiol Biochem*. 2017;41:973–986.
27. Braun J, Hoang-Vu C, Dralle H, Hüttelmaier S. Downregulation of microRNAs directs the EMT and invasive potential of anaplastic thyroid carcinomas. *Oncogene*. 2010;29:4237–4244.
28. Duell EJ, Lujan-Barroso L, Sala N, et al. Plasma microRNAs as biomarkers of pancreatic cancer risk in a prospective cohort study. *Int J Cancer*. 2017;141:905–915.
29. Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci USA*. 2006;103:2257–2261.
30. Raman JD, Lin YK, Kaag M, et al. High rates of advanced disease, complications, and decline of renal function after radical nephroureterectomy. *Urol Oncol*. 2014;32. 47.e49–14.