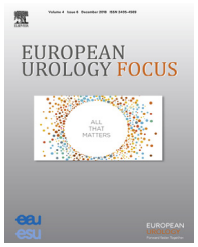
EUROPEAN UROLOGY FOCUS 4 (2018) 825–833

available at www.sciencedirect.com
journal homepage: www.europeanurology.com/eufocus



Prostate Cancer

Diagnostic and Prognostic MicroRNA Biomarkers for Prostate Cancer in Cell-free Urine

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Article info

Article history:
Accepted February 25, 2017

Associate Editor:
James Catto

Keywords:
Prostate cancer
Biomarker
Diagnosis
Prognosis
MicroRNA
Urine

Abstract

Background: Widespread use of prostate-specific antigen (PSA) testing for prostate cancer (PC) detection has led to extensive overdiagnosis and overtreatment. Urine-based microRNA (miRNA) biomarkers could be useful in PC diagnosis and prognosis.
Objective: To train and validate urine-based microRNA (miRNA) biomarkers that may assist in PC diagnosis and prognosis.
Design, setting, and participants: We profiled the expression levels of 92 miRNAs via reverse transcriptase–poymerase chain reaction in cell-free urine samples from 29 patients with benign prostatic hyperplasia (BPH) and 215 patients with clinically localized PC (cohort 1). Our findings were validated in an independent cohort of 29 BPH patients and 220 patients with clinically localized PC (cohort 2).
Results and limitations: We identified and validated several deregulated miRNAs in urine samples from PC patients. In addition, we trained a novel diagnostic three-miRNA model (miR-222-3p*miR-24-3p/miR-30c-5p) that distinguished BPH and PC patients with an area under the curve (AUC) of 0.95 in cohort 1, and was successfully validated in cohort 2 (AUC 0.89). Furthermore, we trained a novel prognostic three-miRNA model (miR-125b-5p*let-7a-5p/miR-151-5p) that predicted time to biochemical recurrence after radical prostatectomy independently of routine clinicopathological parameters in cohort 1, and was successfully validated in cohort 2.
Conclusions: Future clinical implementation of our novel diagnostic and prognostic three-miRNA signatures could help in primary diagnosis of PC and guide treatment decisions. Further validation studies are warranted.
Patient summary: Using two large patient cohorts, we searched for novel prostate cancer biomarkers in urine. We found two new sets of microRNA biomarkers in urine that could accurately predict the presence of prostate cancer and the likelihood of recurrence after prostatectomy. Further studies are needed before an actual clinical test can be developed.

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http://dx.doi.org/10.1016/j.euf.2017.02.018

1. Introduction

Prostate cancer (PC) is the second most commonly diagnosed malignancy and the fifth leading course of cancer death in males [1]. Early diagnosis is essential as localized PC may be cured by radical prostatectomy (RP) or radiation therapy, while metastatic PC is incurable. Currently, elevated serum levels of prostate-specific antigen (PSA) and/or a suspect digital rectal examination (DRE) will prompt a prostate biopsy for possible diagnosis via histopathological evaluation [2]. However, the low specificity of PSA for PC and a lack of accurate prognostic tools have resulted in many unnecessary prostate biopsies and overtreatment of many clinically insignificant PCs [3]. There is an urgent need for novel noninvasive molecular biomarkers that can accurately diagnose PC and predict aggressiveness to improve treatment decisions.

MicroRNAs (miRNAs) are a class of small noncoding RNAs and a crucial part of the gene regulatory network [4]. More than 2500 human mature miRNAs have been annotated [5], with each miRNA having the potential to regulate hundreds of genes [6]. It has been shown that miRNA expression is altered in PC tissue and in blood (serum/plasma) samples from PC patients [7–9]. miRNAs are attractive candidates for biomarker discovery in biofluids as their relatively small size and encapsulation into exosomes can make them remarkably stable [10].

Few studies have performed miRNA expression profiling in urine samples from PC patients, and results are limited by small sample size, low numbers of miRNAs assayed, and/or lack of independent clinical validation [11,12]. Here we report on the first comprehensive study of miRNA expression in urine from PC patients. By analyzing 92 miRNAs in two large independent cohorts, we have trained and independently validated a novel three-miRNA urinary diagnostic classifier that added diagnostic power to routinely used PSA. In addition, we identified and successfully validated a new prognostic three-miRNA urinary signature that predicted time to biochemical recurrence (BCR) after RP independent of routine clinicopathological parameters.

2. Patients and methods

2.1. Clinical samples and miRNA profiling

All samples were collected at the Department of Urology at Aarhus University Hospital, Denmark (2001–2009). The study was approved by the regional scientific ethics committee and by the Danish Data Protection Agency. Written informed consent was obtained from all patients. Cohort 1 (training) consisted of 29 urine samples from patients with benign prostatic hyperplasia (BPH) and 215 urine samples from patients with histologically verified clinically localized PC treated with radical prostatectomy (RP). Cohort 2 (validation) consisted of urine samples from 29 BPH and 220 PC patients. The clinicopathological characteristics are summarized in Supplementary Table 1. Midstream urine from PC and BPH patients was collected before RP and transurethral resection of the prostate (TURP), respectively, without a prior DRE. Expression levels of 92 miRNAs (Supplementary Table 2) were profiled using an miRNA reverse transcriptase–polymerase chain reaction platform from Exiqon (Vedbaek, Denmark; Supplementary material). After quality control (Supplementary Fig. 1), cohort 1 consisted of 20 BPH and 188 PC patients, and cohort 2 consisted of 20 BPH and 197 PC patients (Table 1).

Table 1 – Summary of patient characteristics.

	Cohort 1 (training)		Cohort 2 (validation)	
	BPH	PC	BPH	PC
Patients (*n*)	20	188	20	197
Median age, yr (range)	72 (54–85)	65 (36–73)	66 (46–79)	65 (47–77)
Serum PSA, *n* (%)				
≤10 ng/ml	15 (75.0)	63 (33.5)	18 (90.0)	78 (39.6)
>10 ng/ml	5 (25.0)	125 (66.5)	2 (10.0)	119 (60.4)
Unknown	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Median PSA, ng/ml (range)	2.8 (0.6–13.4)	11.7 (2.0–51.4)	2.6 (0.3–17.2)	11.5 (1.7–155)
Pathological T stage, *n* (%)				
pT2a–c	NA	119 (63.3)	NA	133 (67.5)
pT3a–b	NA	69 (36.7)	NA	64 (32.5)
pT4	NA	0 (0.0)	NA	1 (0.5)
Unknown	NA	0 (0.0)	NA	0 (0.0)
Gleason score, *n* (%)				
<7	NA	61 (32.4)	NA	68 (34.5)
7	NA	92 (48.9)	NA	97 (49.2)
>7	NA	35 (18.6)	NA	32 (16.3)
Unknown	NA	0 (0.0)	NA	0 (0.0)
Surgical margin status, *n* (%)				
Negative	NA	127 (67.5)	NA	129 (65.5)
Positive	NA	58 (30.9)	NA	67 (34.0)
Unknown	NA	3 (1.6)	NA	1 (0.5)
Recurrence status, *n* (%)				
No biochemical recurrence	NA	97 (51.6)	NA	102 (51.8)
Biochemical recurrence	NA	91 (48.4)	NA	95 (48.2)
Median follow-up, mo (range)	NA	99.0 (12–157)	NA	74 (3–143)

BPH = benign prostatic hyperplasia; PC = prostate cancer; PSA = prostate-specific antigen; NA = not applicable.

2.2. Statistical analyses

All statistical analyses were conducted in R (version 3.3.1) using R studio, version 0.99.893. Initially, miRNA expression levels were normalized to the mean of the three most stably expressed miRNAs (miR-200b-3p, miR-27b-3p, and miR-30b-5p), as identified by Normfinder [13]. Normalization was performed according to $\Delta Cq = \text{mean}(Cq_{miR\text{-}200b\text{-}3p}, Cq_{miR\text{-}27b\text{-}3p}, Cq_{miR\text{-}30b\text{-}5p}) - Cq_{miRNA}$.

Differences in miRNA levels between sample groups were assessed using the Wilcoxon rank-sum test and p values were adjusted for multiple testing using the Benjamini-Hochberg (BH) approach. Fold changes were calculated as $\Delta\Delta Cq = \text{mean}(\Delta Cq_{group1}) - \text{mean}(\Delta Cq_{group2})$ and converted on a $\log_2$ scale. The potential of each miRNA to distinguish two patient groups was investigated using ΔCq values in a receiver operating characteristic (ROC) curve analysis with the pROC package [14].

Diagnostic ratio models were generated on the basis of un-normalized expression data, using only miRNAs ($n = 40$) that were detected in at least 95% of all samples in cohort 1. This led to 780 unique two-miRNA ($miRNA_1/miRNA_2$) models, calculated as $Cq_{miRNA1} - Cq_{miRNA2}$, and assessed via ROC curve analysis and a Wilcoxon test (BH adjusted) for their ability to distinguish BPH and PC samples. The two-miRNA models were sorted by AUC value obtained in cohort 1, and the diagnostic performance of the top five pairs was assessed in cohort 2 using ROC curve analysis and a Wilcoxon test (BH adjusted). We also tested a set of top candidate three-miRNA diagnostic models, calculated as $Cq_{miRNA1} + Cq_{miRNA2} - 2 \times Cq_{miRNA3}$.

Similarly, prognostic miRNA ratio models were trained in cohort 1 and tested in cohort 2 (Supplementary material). A cutoff was trained using Youden's J statistic [14] in cohort 1. This numeric value was used to dichotomize PC patients in cohorts 1 and 2 into high- and low-risk groups to evaluate the prognostic performance of the ratio model in Kaplan-Meier and Cox regression analysis using the survival package [15]. For recurrence-free survival analysis, the endpoint was BCR (PSA $\geq$0.2 ng/ml). Patients who had not experienced BCR were censored at their last normal PSA test.

3. Results

3.1. Dysregulated miRNA in urine

We aimed to explore the potential of cell-free urine as a diagnostic tool for PC and profiled the expression levels of 92 unique miRNAs in urine samples collected from 20 patients with BPH and 188 patients with clinically localized PC (cohort 1, training; Table 1). We found that 14 miRNAs were significantly upregulated ($p < 0.05$, Wilcoxon, BH-adjusted for multiple testing) and 30 miRNAs were significantly downregulated in PC relative to BPH (Supplementary Tables 3 and 4). This indicates that dysregulated miRNA expression levels in urine can discriminate between BPH and PC.

For validation, we profiled the expression levels of the same 92 miRNAs in urine samples from an independent set of 20 BPH and 197 PC patients (cohort 2, validation; Table 1). We successfully validated six upregulated miRNAs and 22 downregulated miRNAs in cohort 2 ($p < 0.05$, BH-adjusted; Fig. 1, Table 2, Supplementary Tables 3 and 4). In addition to identifying dysregulation of several miRNAs in urine not previously identified for PC, we confirmed the previously reported downregulation of miR-191 and miR-205 in urine from PC patients [11] (Supplementary Table 5).

3.2. Diagnostic ratio models

To enhance biomarker performance and circumvent normalization, we constructed all possible two-miRNA ratio models from consistently detected miRNAs ($n_{pairs} = 780$). AUC was used to compare the diagnostic performance (PC vs BPH) of these models in cohort 1, and we successfully validated ($p < 0.05$, Wilcoxon, BH-adjusted) the top five two-miRNA models in the independent cohort 2 (Supplementary Table 6).

To further improve the classifier, we created all possible three-miRNA models ($n = 60$) from the miRNAs included in the top five two-miRNA models. In cohort 1, the two best three-miRNA models had comparable performance and contained the same miRNAs as numerators (miR-222-3p and miR-24-3p) but different denominators (miR-30a-5p or miR-30c-5p; Supplementary Table 6). We chose to proceed with miR-30c-5p, which had the highest stability using Normfinder [13]. The final model (miR-222-3p*miR-24-3p/miR-30c-5p) distinguished PC and BPH patients in

Table 2 – Top five validated miRNAs upregulated and downregulated in prostate cancer (ordered by *p* value in cohort 1 from a Wilcoxon test with Benjamin-Hochberg adjustment) and the performance of the three-miRNA diagnostic model in both cohorts.

	Cohort 1			Cohort 2		
	Fold change	*p* value	AUC	Fold change	*p* value	AUC
Upregulated in PC						
hsa-miR-30c-5p	1.46	1.28E−06	0.85	1.27	4.14E−04	0.76
hsa-miR-30b-5p	1.34	1.63E−06	0.84	1.23	4.14E−04	0.76
hsa-miR-30a-5p	1.49	2.41E−05	0.80	1.25	3.26E−03	0.72
hsa-miR-30a-3p	1.43	5.68E−04	0.75	1.24	2.47E−02	0.67
hsa-miR-125b-5p	1.43	7.09E−04	0.74	1.35	4.41E−04	0.76
Downregulated in PC						
hsa-miR-31-5p	−3.69	4.89E−09	0.92	−2.54	4.17E−05	0.81
hsa-miR-141-3p	−1.85	7.27E−09	0.91	−1.53	1.53E−05	0.82
hsa-miR-146a-5p	−13.42	1.09E−07	0.95	−17.24	9.83E−07	0.97
hsa-miR-24-3p	−1.84	1.25E−07	0.88	−1.56	1.27E−05	0.83
hsa-miR-222-3p	−1.88	3.11E−07	0.87	−1.66	1.01E−04	0.79
Three-miRNA model[a]	−7.37	3.00E−11	0.95	−4.37	7.37E−09	0.89

[a] The three-miRNA model is (hsa-miR-24-3p*hsa-miR-222-3p)/hsa-miR-30c-5p.

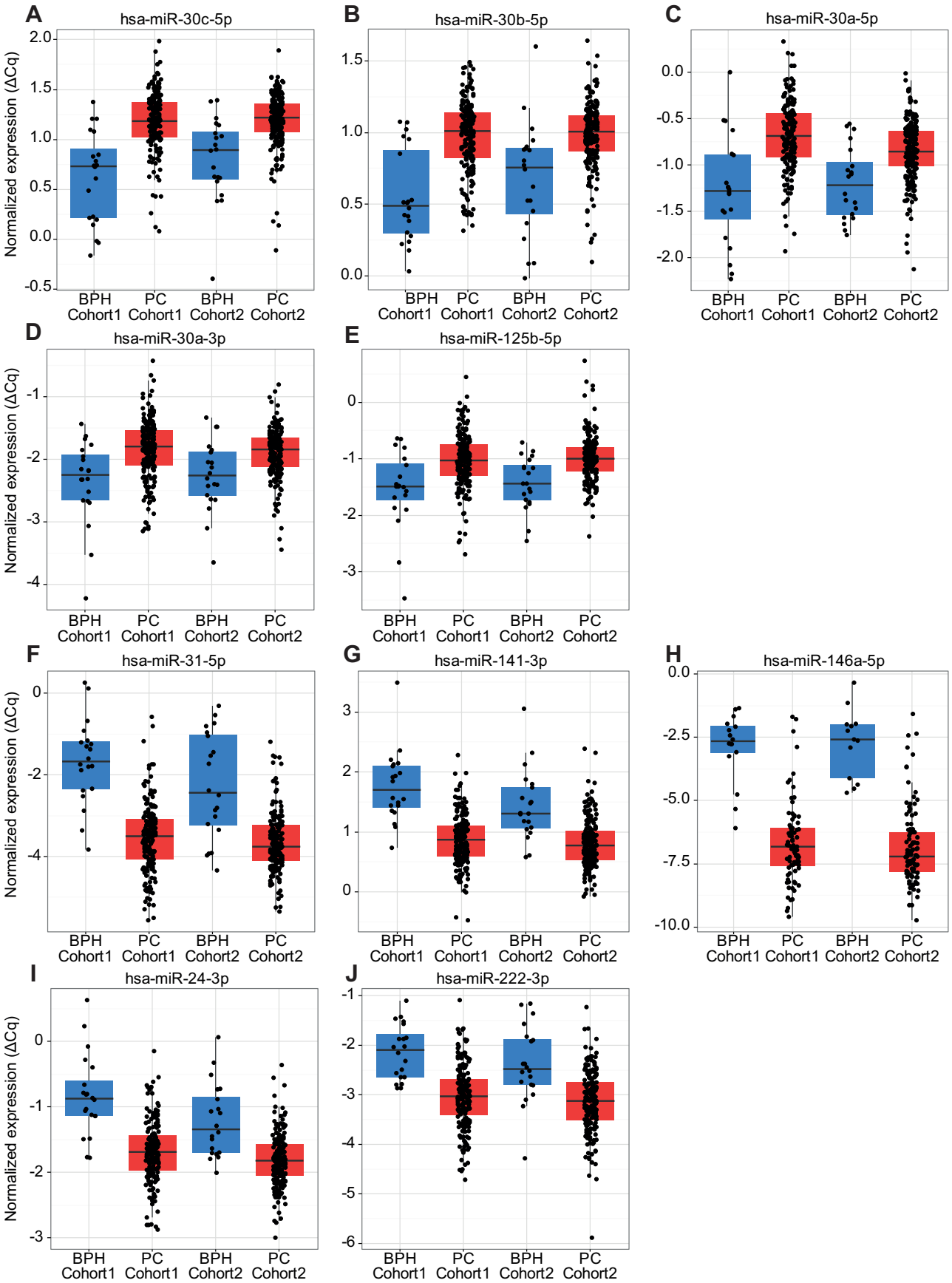

Fig. 1 – Top five microRNAs (A–E) upregulated and (F–J) downregulated in prostate cancer. Boxes represent the first and third quartiles, and the median is shown as a horizontal line. Dots indicate measurements for individual samples. BPH = benign prostatic hyperplasia; PC = prostate cancer.

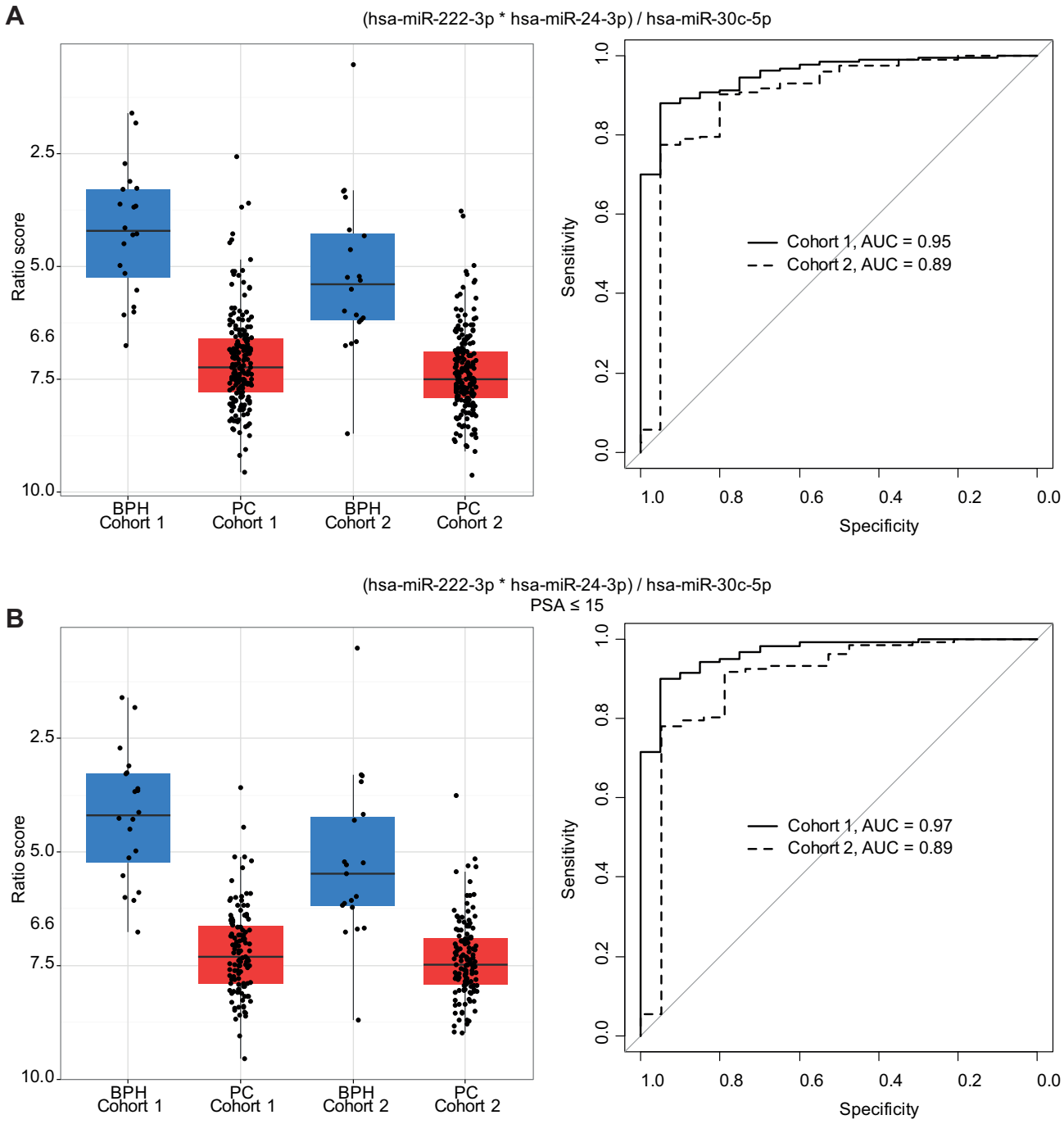

Fig. 2 – Box plot and receiver operating characteristic curve analysis for the three-miRNA diagnostic model (miR-222-3p*miR-24-3p/miR-30c-5p) scores in (A) the total population (cohort 1, 20 BPH and 188 PC; cohort 2, 20 BPH and 197 PC) and (B) the subgroup of patients with prostate-specific antigen PSA ≤15 ng/ml (cohort 1, 20 BPH and 122 PC; cohort 2, 19 BPH and 133 PC). BPH = benign prostatic hyperplasia; PC = prostate cancer; AUC = area under the curve.

cohort 1 with an AUC of 0.95, and was successfully validated in cohort 2 (AUC 0.89; Fig. 2A, Table 2). This strongly indicates that it is possible to differentiate BPH and PC patients with very high accuracy by measuring urinary levels of miR-222-3p, miR-24-3p, and miR-30c-5p. Inclusion of a fourth miRNA did not further improve the diagnostic accuracy (data not shown).

The three-miRNA diagnostic model (miR-222-3p*miR-24-3p/miR-30c-5p) also had high diagnostic accuracy (AUC 0.97 for cohort 1 and 0.89 for cohort 2) in the subgroup of patients with grey-zone PSA levels (≤15 ng/ml; 20 BPH vs 122 PC patients in cohort 1; 19 BPH vs 133 PC patients in cohort 2; Fig. 2B). Moreover, our three-miRNA model added diagnostic value to PSA in the full patient set as well as in the subset with PSA ≤15 ng/ml (Supplementary Fig. 2), further supporting the promising potential of our novel and noninvasive three-miRNA diagnostic model to aid in the primary diagnosis of PC.

3.3. *Prognostic classifier*

We defined several PC patient subgroups on the basis of clinicopathological variables associated with aggressiveness (PSA ≤10 vs >10 ng/ml, pathological T2 vs T3, Gleason score ≤7 vs >7, no recurrence vs BCR). However, no single miRNA could significantly (BH-adjusted $p < 0.05$) discriminate between these subgroups (data not shown).

This prompted us to train prognostic two-miRNA models instead (Supplementary material). We included only PC patients with PSA ≤15 ng/ml ($n = 122$ in cohort 1 and $n = 133$ in cohort 2) to develop new prognostic tools for this group of patients, in which overtreatment is the greatest. First, in cohort 1 we assessed the ability of two-miRNA ratios ($n_{pairs} = 325$) to differentiate PC patients with and without BCR via ROC curve analysis. We then excluded all pairs with AUC <0.6 (11 pairs remaining) and selected the top three two-miRNA models ranked by p value in univariate Cox regression analysis (Supplementary Table 7). As these two-miRNA models had moderate AUCs (0.602–0.632), we attempted to improve the performance by constructing all unique three-miRNA models ($n = 30$) from the top three two-miRNA prognostic models. One model (miR-125b-5p*let-7a-5p/miR151a-5p) had an improved AUC (0.639) and univariate Cox regression p value in cohort 1 (Supplementary Table 7). This three-miRNA model significantly predicted time to BCR in univariate and multivariate Cox regression analysis both as a continuous and dichotomized variable (Table 3 and Supplementary Table 8) as well as in Kaplan-Meier analysis in cohort 1 (Fig. 3). The prognostic value of this three-miRNA model was successfully validated in cohort 2 using univariate and multivariate Cox regression (Table 3 and Supplementary Table 8) as well as Kaplan-Meier analysis (Fig. 3). The estimated mean gain in recurrence-free survival for low-risk patients was 22 mo (95% confidence interval [CI] 3–40) in cohort 1 and 15 mo (95% CI 1–29) in cohort 2. Addition of the three-miRNA model to the multivariate Cox regression model improved Harrell's *C*-index from 0.754 to 0.767 in cohort 1 and from 0.740 to 0.774 in cohort 2 (Table 3). Importantly, in addition to providing prognostic value beyond postoperative clinical variables (Table 3), our three-miRNA prognostic signature also predicted time to BCR independently of preoperative parameters (PSA, clinical T stage, and biopsy Gleason score; Supplementary Table 9).

4. Discussion

Using two large independent patient cohorts, one for training and one for validation, we performed the most comprehensive urinary miRNA expression profiling study for PC to date. We identified and validated multiple new significantly deregulated miRNAs in urine samples from PC patients, as well as a novel three-miRNA diagnostic model that distinguished patients with and without PC with very high accuracy, and improved the diagnostic performance of the routinely used PSA test. Furthermore, we trained and validated a novel three-miRNA prognostic model that predicted BCR after RP, independently of routine clinicopathological variables. To the best of our knowledge, this is the first report of a validated urine-based prognostic miRNA signature for PC.

The miRNAs in our novel three-miRNA diagnostic model (miR-222-3p*miR-24-3p/miR-30c-5p) have previously been associated with PC. We found that miR-222-3p and miR-24-3p were significantly downregulated in urine samples from PC patients, and it has been found that these

Table 3 – Prognostic potential of the three-miRNA prognostic model (miR-125b-5p*let-7a-5p/miR151a-5p) evaluated in univariate and multivariate Cox regression analyses.

Variable	Univariate			Multivariate			
	HR (95% CI)	*p* value	*C*-index	HR (95% CI)	*p* value[a]	*C*-index[b]	
						(1)	(2)
RP cohort 1 (*n* = 122, 49 with recurrence)							
Age at diagnosis (continuous)	1.01 (0.96–1.06)	0.848	0.483	–	–		
Tumor stage (T2 vs T3)	3.26 (1.83–5.81)	<**0.001**	0.659	–	–		
Surgical margins (negative vs positive)	3.58 (2.00–6.39)	<**0.001**	0.662	3.52 (1.92–6.46)	<**0.001**		
Preoperative PSA (continuous)	1.12 (1.00–1.24)	**0.046**	0.593	1.13 (1.01–1.27)	**0.032**		
Gleason score (<7 vs 7)	3.50 (1.51–8.11)	**0.004**	0.648	3.94 (1.66–9.35)	**0.002**		0.754
Gleason score (<7 vs >7)	7.37 (2.94–18.5)	<**0.001**		6.02 (2.36–15.37)	<**0.001**	0.767	
Prognostic classifier (continuous)	0.63 (0.44–0.92)	**0.016**	0.619	0.61 (0.41–0.90)	**0.013**		
RP cohort 2 (*n* = 133, 54 with recurrence)							
Age at diagnosis (continuous	1.07 (1.02–1.13)	**0.010**	0.603	–	–		
Tumor stage (T2 vs T3	3.30 (1.91–5.69)	<**0.001**	0.632	–	–		
Surgical margins (negative vs positive)	2.82 (1.64–4.84)	<**0.001**	0.629	–	–		
Preoperative PSA (continuous)	1.13 (1.03–1.24)	**0.013**	0.621	1.15 (1.05–1.26)	**0.003**		
Gleason score (<7 vs 7)	4.33 (2.05–9.13)	<**0.001**	0.704	4.15 (1.97–8.77)	<**0.001**		0.740
Gleason score (<7 vs >7)	12.5 (5.29–29.6)	<**0.001**		14.15 (5.9–33.92)	<**0.001**	0.774	
Prognostic classifier (continuous)	0.52 (0.33–0.83)	<**0.001**	0.629	0.47 (0.28–0.77)	**0.003**		

RP = radical prostatectomy; HR = hazard ratio; CI = confidence interval; PSA = prostate-specific antigen; *C*-index = Harrell's concordance index. Bold indicates significant p values (<0.05).

[a] The three-miRNA prognostic model was analyzed in multivariate Cox regression analysis along with age at diagnosis, surgical margin status, pathological tumor stage, Gleason score, and preoperative serum PSA. In the final multivariate model, nonsignificant variables were excluded in stepwise backward selection.

[b] Harrell's *C*-index for (1) the multivariate model including all significant variables and (2) significant variables without the three-miRNA model.

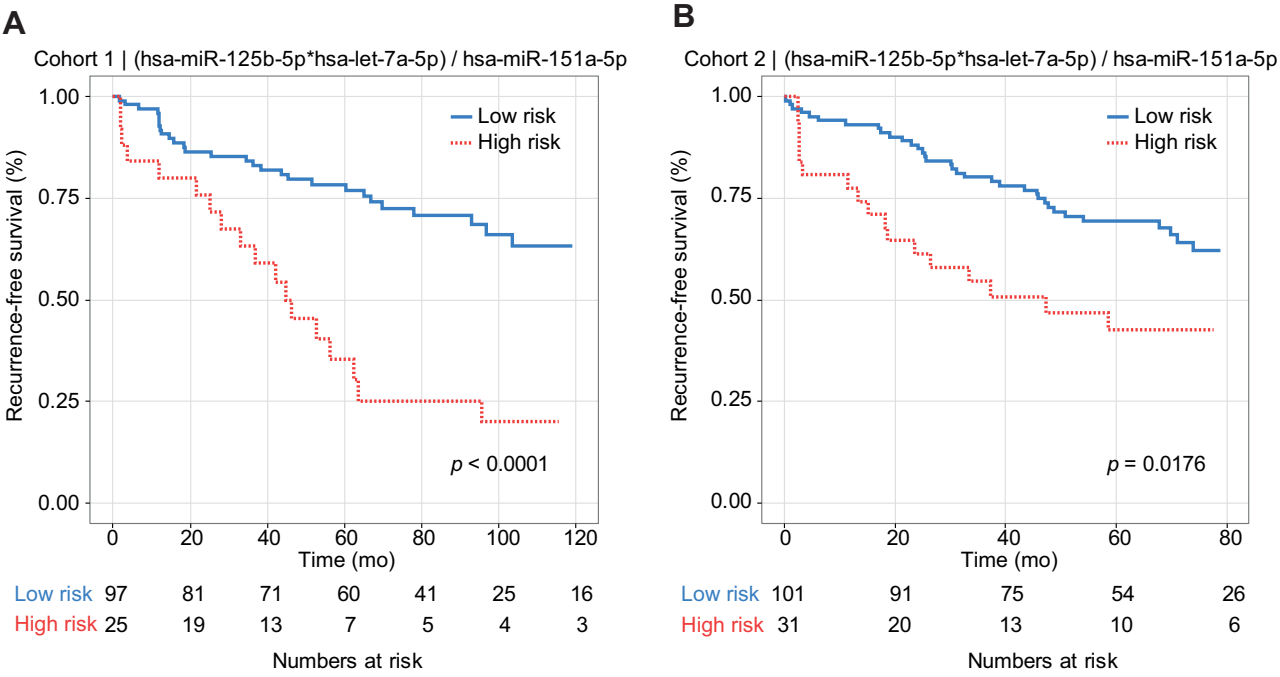

Fig. 3 – Prognostic potential of the three-miRNA model (miR-125b-5p*let-7a-5p/miR151a-5p) evaluated using Kaplan-Meier analysis of biochemical recurrence–free survival. (A) Patients in cohort 1 were dichotomized into low and high risk groups according to a cutoff obtained via receiver operating characteristic analysis. (B) The numeric cutoff trained in cohort 1 was used in cohort 2 to divide patients into high and low risk groups. The *p* value for a two-sided log-rank test is shown for each cohort.

miRNAs were downregulated in PC tissue [9,16]. Moreover, we found significant upregulation of miR-30c-5p in urine from PC patients, while miR-30c-5p overexpression in PC tissue has been associated with early BCR [17]. However, further studies are needed to determine the cellular origin of the miRNAs detected in cell-free urine samples.

There are a few urine-based tests available that aim to aid in early diagnosis of PC. Transcript levels of PCA3 in urine have been used to improve specificity for PC over PSA either alone (Progensa [18]) or combined with TMPRSS2: ERG (MiPS [19]). However, both tests require post-DRE urine, restricting clinical adoption. By contrast, our diagnostic three-miRNA model is measured in urine obtained without DRE, potentially allowing a simpler workflow. Other studies using non-DRE urine have reported that protein levels of EN2 or exosomal mRNA levels of ERG, PCA3, and SPDEF (ExoDx Prostate IntelliScore) may improve specificity for PC compared to PSA, yet both tests missed more than one third of all positive prostate biopsies [20–22].

We observed relatively high PSA levels in the PC group, which was also reflected by the high AUC for PSA in this study (>0.8 in both cohorts). However, our diagnostic classifier score did not correlate with serum PSA levels (Pearson's $r = 0.087$; data not shown) and is therefore unlikely to be a pseudo-PSA test, but rather a genuine classifier for PC. While we used urine samples from BPH and RP patients, future studies should investigate the diagnostic performance of our three-miRNA model (miR-222-3p*miR-24-3p/miR-30c-5p) in a more clinically relevant setting, such as patients referred for prostate biopsy. Nevertheless, the very high accuracy of our three-miRNA diagnostic model for patients with grey-zone PSA (AUC 0.89–0.97) suggests that it may be useful in the primary diagnosis of PC with the aim of reducing unnecessary biopsies while ensuring early detection of aggressive PC. Moreover, magnetic resonance imaging (MRI) and MRI-guided prostate biopsy (MRGB) have recently emerged as promising diagnostic tools for PC [23,24]. Thus, in future studies it will be important to assess the performance of our diagnostic model for prediction of MRI/MRGB results. It is conceivable that a urine-based miRNA test could be used for preselection of patients for MRI, for which capacity is often limited.

We also developed a novel urine-based three-miRNA prognostic model (miR-125b-5p*let-7a-5p/miR-151a-5p) that predicted BCR after RP independently of routine clinicopathological variables. It has been shown that miR-125b, let-7a, and mir-151a inhibit apoptosis, reduce proliferation, and promote cell migration and invasion of PC cells, respectively [25–27], suggesting that these miRNAs could play a functional role in PC progression. Although further studies are needed, our novel three-miRNA prognostic model could be used to guide treatment decisions at the time of diagnosis for patients with clinically localized PC. It is conceivable that patients classified as low risk using our three-miRNA prognostic classifier could be candidates for active surveillance, whereas patients scored as high risk should undergo immediate and more intensive treatment.

Our study has potential limitations. For diagnostic evaluation, in addition to having a relatively small control

group, we compared urine samples from BPH and PC patients treated by RP, which may not perfectly reflect the clinical situation, where PC is diagnosed via needle biopsies. Thus, future studies should include a larger control group and patients referred for prostate biopsy in multiple centers. However, the routinely used transrectal ultrasound-guided prostate biopsy procedure also has major shortcomings, including frequent understaging and numerous false-negative biopsies [28,29]. By contrast, the BPH patients included here had mean follow-up of 5.4 yr with no PC diagnosis (data not shown), making it highly unlikely they are false negatives. Finally, for our prognostic classifier we used BCR as clinical endpoint, whereas time to metastasis or PC-related mortality may have been a better gauge of PC aggressiveness. However, given the slowly progressing nature of PC [30], analysis of these additional endpoints would require >10–15 yr of follow-up.

5. Conclusions

We demonstrated that it is possible to accurately identify men with PC using a simple three-miRNA urine test. In addition, we trained and validated a novel three-miRNA prognostic model that was significantly associated with PC aggressiveness. Using two large independent cohorts, this is the first comprehensive study on miRNA in urine. Further validation studies in large independent patient cohorts are needed to assess the true clinical value of our novel urine-based miRNA signatures for PC diagnosis and prognosis.

Author contributions: Karina D. Sørensen had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Fredsøe, Rasmussen, Thomsen, Mouritzen, Ørntoft, Sørensen.
Acquisition of data: Rasmussen, Thomsen, Mouritzen, Høyer, Borre.
Analysis and interpretation of data: Fredsøe, Rasmussen, Thomsen, Mouritzen, Sørensen.
Drafting of the manuscript: Fredsøe, Sørensen.
Critical revision of the manuscript for important intellectual content: Rasmussen, Thomsen, Mouritzen, Høyer, Borre, Ørntoft.
Statistical analysis: Fredsøe, Sørensen.
Obtaining funding: Mouritzen, Ørntoft, Sørensen.
Administrative, technical, or material support: None.
Supervision: Sørensen, Mouritzen.
Other: None.

Financial disclosures: Karina D. Sørensen certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: Karina D. Sørensen has received consultancy fees from Exiqon A/S. Jacob Fredsøe, Anni R. Thomsen, Peter Mouritzen, Torben F. Ørntoft, and Karina D. Sørensen are co-inventors on pending patent applications regarding miRNAs as biomarkers for prostate cancer.

Funding/Support and role of the sponsor: This work was supported by Innovation Fund Denmark and by The Danish Cancer Society. The sponsors played no direct role in the study.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.euf.2017.02.018.

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