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ORIGINAL ARTICLE

Training and validation of a novel 4-miRNA ratio model (*MiCaP*) for prediction of postoperative outcome in prostate cancer patients

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Background: New molecular biomarkers for prostate cancer (PC) prognosis are urgently needed. Ratio-based models are attractive, as they require no additional normalization. Here, we train and independently validate a novel 4-miRNA prognostic ratio model for PC.

Patients and methods: By genome-wide miRNA expression profiling of PC tissue samples from 123 men who underwent radical prostatectomy (RP) (PCA123, training cohort), we identified six top candidate prognostic miRNAs and systematically tested their ability to predict postoperative biochemical recurrence (BCR). The best miRNA-based prognostic ratio model (*MiCaP*) was validated in two independent cohorts (PCA352 and PCA476) including >800 RP patients in total. Clinical end points were BCR and prostate cancer-specific survival (CSS). The prognostic potential of MiCaP was assessed by univariate and multivariate Cox-regression analyses and Kaplan–Meier analyses.

Results: We identified a 4-miRNA ratio model, *MiCaP* (miR-23a-3p×miR-10b-5p)/(miR-133a×miR-374b-5p), that predicted time to BCR independently of routine clinicopathologic variables in the training cohort (PCA123) and was successfully validated in two independent RP cohorts. In addition, *MiCaP* was a significant predictor of CSS in univariate analysis [HR 3.35 (95% CI 1.34 − 8.35), $P = 0.0096$] and in multivariate analysis [HR 2.43 (95% CI 1.45–4.07), $P = 0.0210$]. As proof-of-principle, we also analyzed *MiCaP* in plasma samples from 111 RP patients. A high *MiCaP* score in plasma was significantly associated with BCR ($P = 0.0036$, Kaplan–Meier analysis). Limitations include low mortality rates (CSS: 5.4%).

Conclusions: We identified a novel 4-miRNA ratio model (*MiCaP*) with significant independent prognostic value in three RP cohorts, indicating promising potential to improve PC risk stratification.

Key words: prostate cancer, prognosis, risk stratification, biomarkers, microRNA

Introduction

Prostate cancer (PC) is the most commonly diagnosed cancer in men in the Western world. Currently, serum prostate-specific antigen (PSA) is used for detection of PC and for monitoring of disease progression and treatment response [1, 2]. Over the past decades, extensive use of PSA testing has increased detection rates for early stage tumors, which may be cured by radical prostatectomy (RP) [3]. However, available prognostic indicators (PSA, tumor stage, and Gleason score) are inaccurate and overtreatment is common for these patients, who in reality may have either an aggressive tumor needing immediate intervention, or an indolent tumor that can be managed by active surveillance [4]. There is an urgent need for new molecular biomarkers that can improve risk stratifications for patients with early stage PC, in order to guide more personalized treatment decisions [5].

MicroRNAs (miRNAs) comprise a large class of regulatory noncoding RNAs (~22 nt) that control gene expression by binding to (partially) complementary sequences in target mRNAs, leading to gene silencing [6]. It has been estimated that 60% of all

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human mRNAs are regulated by miRNAs, which thereby influence key cellular processes, e.g. differentiation and proliferation [6, 7]. Furthermore, miRNAs constitute a particularly attractive source for biomarker discovery, as they are more stable than mRNAs and thus easier to extract and quantify from, e.g. formalin-fixed and paraffin-embedded patient tissue samples that have been stored for several years in pathology archives [6, 8]. Although single miRNAs have shown promising potential as diagnostic biomarkers for PC, current evidence suggests that single miRNAs have suboptimal prognostic potential for PC, when compared with multi-miRNA signatures [9, 10]. Ratio-based miRNA models could be particularly useful, as their design precludes the need for additional normalization factors [11].

In this study, we trained and successfully validated a new prognostic 4-miRNA ratio model for prediction of postoperative outcome in PC patients.

Patients and methods

Study design and participants

We used three independent patient cohorts [PCA123 (training set, $N = 123$), PCA352 (validation set, $N = 352$), and PCA476 (validation set, $N = 476$)] of men, who underwent RP for clinically localized PC (Table 1).

PC tissue samples from PCA123 and PCA352 were collected at the Department of Urology, Aarhus University Hospital, Aarhus, Denmark between 1997 and 2005. Clinical follow-up information, including time to BCR, was updated for all patients before this study. Inclusion and exclusion criteria for the cohorts are reported according to the REMARK guidelines (supplementary Figure S1, available at *Annals of Oncology* online). Written consent was obtained from all participants, and this study was approved by the regional scientific ethical committee and the Danish Data Protection Agency. Total RNA was extracted from archived PC tissue samples and analyzed for miRNA expression using the miRCURY LNA™ Universal RT microRNA PCR platform (Exiqon A/S) (supplementary methods, available at *Annals of Oncology* online). Assay linearity across a broad concentration range (five orders of magnitude) was confirmed for all 4-miRNA assays by dilution series experiments (supplementary Figure S2, available at *Annals of Oncology* online). Raw miRNA data for PCA123 can be found online (GEO, with accession number GSE115402).

The PCA476 cohort was collected by the TCGA consortium at multiple centers in the US and Europe [12, 13]. Normalized miRNA sequencing (small-RNAseq) data and clinical data were retrieved from the TCGA data portal [13] (see supplementary methods, available at *Annals of Oncology* online).

For *miRNA* analyses in plasma samples, see supplementary methods, available at *Annals of Oncology* online.

Ratio model training

A detailed description is found in supplementary methods, available at *Annals of Oncology* online. In brief, for model training (see flow chart, supplementary Figure S3, available at *Annals of Oncology* online), we used six top candidate prognostic miRNAs identified by genome-wide miRNA expression profiling of PC tissue samples from 123 RP patients (PCA123; Table 1). Ratio models were stringently trained in PCA123 (supplementary Tables S1–S3, available at *Annals of Oncology* online), and the prognostic potential of the top candidate 4-miRNA model (*MiCaP*) (supplementary Table S1, available at *Annals of Oncology* online) was subsequently tested in two independent validation cohorts: PCA352 and PCA476.

Outcome and statistical analysis

All statistical analyses were conducted in R [14] unless stated otherwise. *P* values <0.05 were considered significant. Associations between *MiCaP* score and clinicopathologic parameters were assessed using Wilcoxon rank-sum and Spearman correlation tests. For evaluation of prognostic potential, the primary clinical end point was BCR-free survival (RFS) after RP. BCR was defined as a postoperative PSA test ≥ 0.2 ng/ml. Patients not having experienced BCR were censored at their last PSA test. For survival analyses, we carried out uni- and multivariate Cox-regression as well as Kaplan–Meier analyses using the 'survival' package in R [11]. When relevant, *P* values were corrected for multiple testing using the Benjamini–Hochberg (BH) method [15]. Predictive accuracy was determined using Harrell's concordance index (C-index). Decision curve analyses and calibration plots are described in supplementary methods, available at *Annals of Oncology* online.

Results

MiCaP independently predicts postoperative BCR in three RP cohorts

A systematic procedure was used to train a new 4-miRNA prognostic ratio model *MiCaP* (miR-23a-3p×miR-10b-5p/miR-133a×miR-374b-5p). The model was strictly trained in the PCA123 cohort and subsequently tested in two independent validation cohorts, including 352 and 476 RP patients, respectively (Table 1; supplementary Tables S1–S3 and Figure S3, available at *Annals of Oncology* online). A high *MiCaP* score was significantly associated with advanced pathologic tumor stage, positive surgical margin status, high Gleason score and/or high preoperative PSA in at least one of these cohorts (supplementary Table S4, available at *Annals of Oncology* online). Furthermore, a significantly higher *MiCaP* score was observed in PC tissue compared with adjacent nonmalignant prostate tissue in PCA476 (supplementary Table S4, available at *Annals of Oncology* online).

To assess the prognostic potential of *MiCaP*, we stratified patients in the training cohort (PCA123) into a high- and a low-risk group based on *MiCaP* scores. A high *MiCaP* score was significantly associated with early BCR in univariate Cox-regression analysis, and remained significant in multivariate Cox-regression analysis after adjusting for the CAPRA-S clinical nomogram that includes clinicopathologic variables only (Table 2) [16]. Univariate analysis results for individual clinical variables can be found in supplementary Table S5, available at *Annals of Oncology* online. In the training cohort PCA123, *MiCaP* increased the predictive accuracy, as estimated by Harrell's C-index, from 0.718 to 0.750, compared with the CAPRA-S nomogram only (Table 2).

For independent testing, patients in each of the validation cohorts PCA352 and PCA476 were divided into high- and low-risk groups based on the cut-off (fraction) defined in PCA123. A high *MiCaP* score was significantly associated with short RFS in univariate Cox-regression analyses in both validation cohorts and remained significant after adjustment for routine clinical parameters using the CAPRA-S nomogram (Table 2). In addition, *MiCaP* increased the predictive accuracy (C-index) from 0.699 to 0.713 in PCA352, and from 0.661 to 0.687 in PCA476, suggesting improved prognostic power compared with a model based on the CAPRA-S clinical nomogram only (Table 2). Consistent with this, Kaplan–Meier curve analyses showed a

Table 1. Clinicopathologic characteristics for PC patient cohorts

	PCA123	PCA352	PCA476
Samples	RP (*N* =123)	RP (*N* =352)	RP (*N* =476)
Median age, years (IQR)	63.7 (59.4–68.9)	33.9 (60.2–67.6)	61 (56.0–66.0)
Preoperative PSA, median (IQR)	13.1 (9.9–28.1)	11 (7.7–16.9)	7.5 (5.1–11.4)
Pathologic T-stage			
pT2a-c	74 (60.1%)	238 (67.6%)	184 (38.7%)
pT3a	38 (31.0%)	74 (21.0%)	152 (31.9%)
pT3b	11 (8.9%)	33 (9.4%)	124 (26.0%)
Unknown	0	7 (2.0%)	16 (3.4%)
Gleason score (Grade according to ISUP)			
Grade I (GS=6)	47 (38.2%)	78 (22.1%)	45 (9.5%)
Grade II (GS=3+4)	48 (39.0%)	140 (39.9%)	144 (30.2%)
Grade III (GS=4+3)	4 (3.3%)	63 (17.9%)	94 (19.7%)
Grade IV (GS=8)	19 (15.4%)	45 (12.8%)	67 (14.1%)
Grade V (GS>8)	4 (3.3%)	6 (1.7%)	126 (26.5%)
Unknown	1 (0.8%)	20 (5.6%)	0
Surgical margin status			
Negative	85 (69.1%)	237 (67.3%)	304 (63.9%)
Positive	38 (30.9%)	98 (27.9%)	137 (28.8%)
Unknown	0	17 (4.8%)	35 (7.3%)
Recurrence status			
No recurrence	58 (47.2%)	199 (56.5%)	351 (73.7%)
Recurrence	65 (52.8%)	153 (43.5%)	58 (12.2%)
Unknown	0	0	67 (14.1%)
CAPRA-S			
Low	37 (30.1%)	87 (24.8%)	118 (24.8%)
Intermediate	51 (41.5%)	163 (46.3%)	166 (34.9%)
High	34 (27.6%)	79 (22.4%)	137 (28.8)
Unknown	1 (0.8%)	23 (6.5%)	55 (11.5%)
Median follow-up time, months (IQR)	136.6 (105.1–157.4)	99.5 (77.5–122.6)	15.1 (5.4–31.1)
Survival status			
Dead	15 (12.2%)	42 (12.0%)	NA
PC-specific deaths	4 (3.3%)	19 (5.4%)	NA
Alive	104 (84.5%)	310 (88.0%)	NA

Data are *N* (%) or median (IQR); PSA, prostate specific antigen, T-stage, tumor stage; IQR, interquartile range; NA, not available.

significant association between a high *MiCaP* score and short RFS in all three cohorts (Figure 1A–C). To further assess the prognostic potential of *MiCaP*, we carried out decision curve analysis and calibration plots (supplementary methods, available at *Annals of Oncology* online). The multivariate model including *MiCaP* (Table 2) added a modest net benefit for decision-making based on model predictions in all three cohorts (supplementary Figure S4, available at *Annals of Oncology* online). Furthermore, the multivariate model including *MiCaP*, divided into three risk-groups, showed strong agreement between observed (Kaplan–Meier estimates) and predicted (Cox-regression model based) outcomes using calibration plots (supplementary Figure S5, available at *Annals of Oncology* online).

As proof-of-principle, to test the prognostic potential of *MiCaP* in liquid biopsies, we analyzed plasma samples collected before RP from 111 PC patients. Patients with a high *MiCaP* score showed significantly shorter time to BCR in Kaplan–Meier analysis (supplementary Figure S6, available at *Annals of Oncology* online), thereby confirming and expanding on our findings from tissue-based analyses.

MiCaP predicts cancer-specific survival after RP

Prostate cancer-specific survival (CSS) analyses could only be carried out for PCA352 due to low event numbers/insufficient follow-up time in PCA123 and PCA476 (Table 1). Patients with a high *MiCaP* score in PC tissue had significantly shorter CSS in Kaplan–Meier analysis (Figure 1D) and in univariate Cox-regression analysis (HR 3.35, 95% CI 1.34–8.35, $P = 0.0096$, Table 3). *MiCaP* also remained a significant predictor of CSS after adjusting for the CAPRA-S nomogram (HR 2.43, 95% CI 1.45–4.07, $P = 0.0210$; Table 3).

Discussion

In this study, we systematically trained, tested and validated a novel 4-miRNA prognostic ratio model for PC, named *MiCaP*.

Table 2. Multivariate Cox-regression analysis of BCR using MiCaP in three RP cohorts

		Univariate			Multivariate			
Variable	**Characteristic**	**HR (95% CI)**	***P*-value**	**C-index**	**HR (95% CI)**	***P*-value**	**C-index[a]**	**C-index[b]**
PCA123, *N* = 123, 65 with recurrence								
CAPRA-S	Low	Ref	–	0.72	–	–	0.750	0.718
	Intermediate	4.54 (1.87–11.01)	**8.23E−04**		4.27 (1.76–10.38)	**1.36E−03**		
	High	13.42 (5.50–32.76)	**1.18E−08**		11.16 (4.52–27.54)	**1.69E−07**		
MiCaP	Low vs. high	3.23 (1.95–5.35)	**5.04E−06**	0.63	2.43 (1.45–4.07)	**7.66E−04**		
PCA352, *N* =352, 153 with recurrence								
CAPRA-S	Low	Ref	–	0.70	–	–	0.713	0.699
	Intermediate	3.30 (1.86–5.86)	**4.42E−05**		3.27 (1.85–5.81)	**5.08E−05**		
	High	9.43 (5.26–16.90)	**4.97E−14**		9.25 (5.16–16.59)	**8.30E−14**		
MiCaP	Low vs. high	1.54 (1.12–2.13)	**8.20E−03**	0.54	1.44 (1.04–2.00)	**2.90E−02**		
PCA476, *N* =405, 58 with recurrence								
CAPRA-S	Low	Ref	–	0.66	–	–	0.687	0.661
	Intermediate	2.04 (1.19–13.72)	**2.53E−02**		3.28 (0.95–11.37)	6.06E−02		
	High	9.00 (2.76–29.41)	**2.74E−04**		6.59 (1.94–22.39)	**2.51E−03**		
MiCaP	Low vs. high	2.45 (1.46–4.12)	**7.32E−04**	0.60	1.89 (1.08–3.32)	**2.69E−02**		

Significant *P* values ($P < 0.05$) are highlighted in bold.
[a]Harrell's C-index for final model including ratio model.
[b]Harrell's C-index for final model excluding the ratio model.

A high *MiCaP* score in PC tissue was a significant adverse predictor of BCR beyond routine clinicopathologic variables (as assessed by the CAPRA-S nomogram) in three RP cohorts, comprising more than 950 patients in total. Moreover, a high *MiCaP* score was significantly associated with shorter prostate CSS independently of the CAPRA-S nomogram [16]. These results suggest that *MiCaP* might be used in the future to improve risk stratification for patients with clinically localized PC and enable more personalized treatment decisions. To the best of our knowledge, this is the first report of a miRNA-based ratio model for PC with significant independent prognostic value in three distinct PC patient cohorts. Finally, we present proof-of-principle support for *MiCaP* as a promising minimally invasive biomarker in plasma.

For *MiCaP*, we found that relatively high expression of miR-10b-5p and miR-23a-3p and relatively low expression of miR-133a and miR-374b-5p in PC tissue samples was associated with poor outcome after RP. Consistent with this, high miR-10b expression has previously been associated with BCR in a small cohort of 52 PC patients [17]. There are no previous reports of a prognostic potential for miR-23a-3p and miR-133a as single markers in PC, while earlier small-scale studies on miR-374b showed contradictory results [18–20], highlighting the importance of using multiple independent and sufficiently sized PC patient cohorts for prognostic biomarker evaluation.

Of the four miRNAs in *MiCaP*, only miR-133a has previously been included in a multi-miRNA prognostic signature for PC (25-miRNA classifier associated with adverse clinicopathology), but the study lacked independent validation [21]. Other previously proposed models include a 16-miRNA signature [22], a 2-miRNA model [20], and a 3-miRNA classifier [9] for prediction of BCR after RP. However, apart from a single exception [9], all earlier studies lacked independent validation, multivariate analysis, and/or sufficient patient sample size. Furthermore, these proposed multi-miRNA models depend on additional normalization, which is circumvented using a ratio model such as *MiCaP* and which could potentially ease future translation into clinical practice. Before this study, miQ [(miR-96-5p×miR-183-5p)/(miR-145-5p×miR-221-5p)] was the only tissue-based prognostic miRNA ratio model proposed for PC, but it was not tested in multivariate analysis [10]. In contrast, we found that *MiCaP* predicted BCR in three distinct RP cohorts independent of routine clinical variables.

The currently used routine prognostic variables (i.e. clinicopathologic parameters and nomograms based on these) for early stage PC cannot accurately predict whether a tumor will progress or remain indolent [5]. Future clinical implementation of improved prognostic biomarkers, such as *MiCaP*, could help to solve this major challenge in primary PC management by enabling more accurate risk stratification at diagnosis, and thereby better treatment decisions, e.g. between active surveillance or surgery. Risk stratification based on *MiCaP* could potentially also be useful post-surgery, to assess the need for adjuvant treatment such as radiation or androgen deprivation therapy.

The function of miR-23a-3p, miR-10b-5p, and miR-133a in prostate cancer cells is not fully understood and miR-374b-5p remains to be investigated. MiR-23a and miR-10b promote DU145 PC cell migration [17, 23], while miR-10b was also shown to inhibit proliferation and invasion in PC cells [24]. Two studies report an inhibition of both proliferation and migration in PC3 and DU145 after miR-133a overexpression [25, 26]. Furthermore, overexpression of miR-374b has been shown to inhibit cell proliferation in T-cell lymphoblastic lymphoma [27], whereas a pro-invasive role has been seen in gastric cancer cells [28], together indicating that the function of miR-10b and

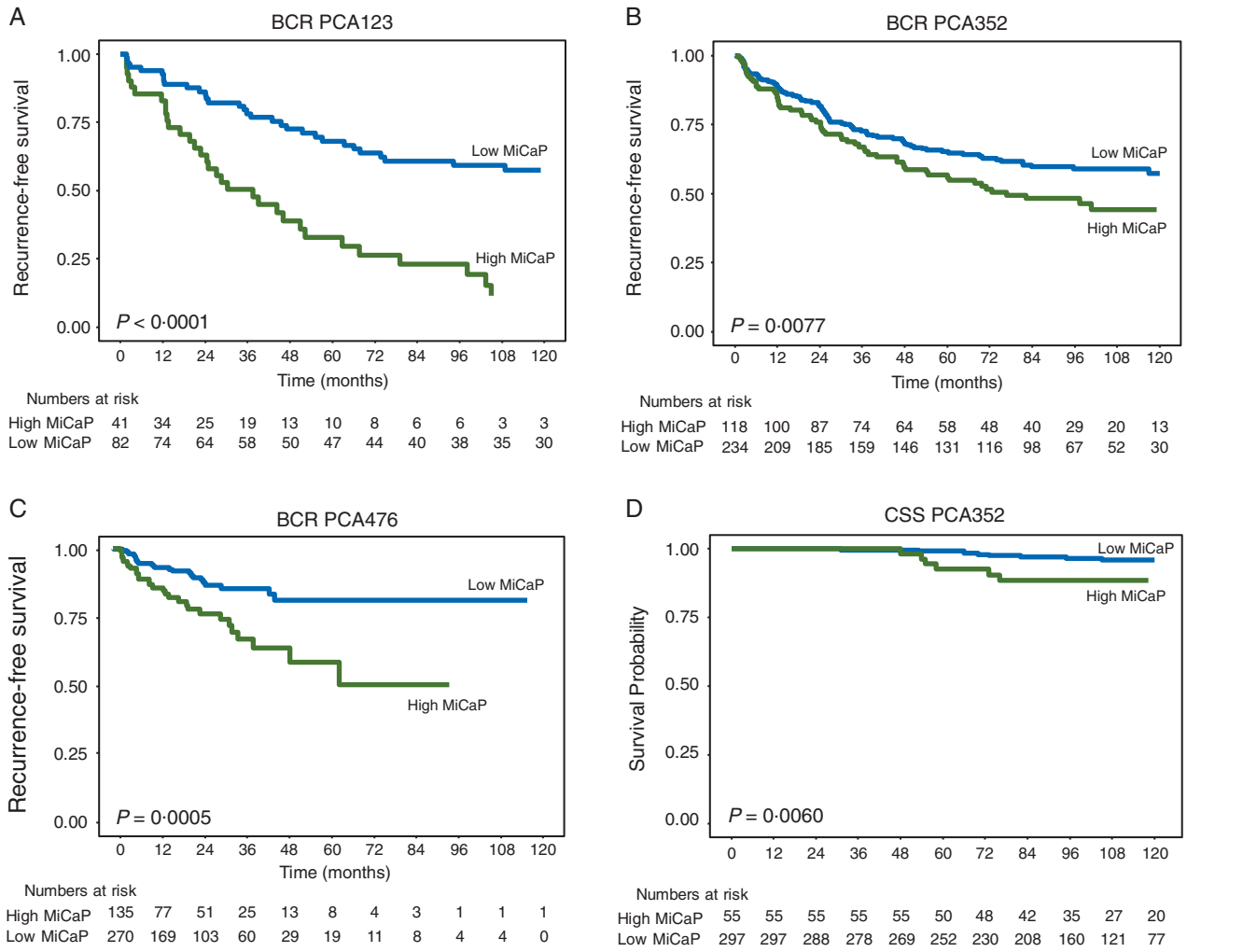

Figure 1. *MiCaP* score is associated with BCR and CSS. (A–C) Kaplan–Meier survival analysis of recurrence-free survival (RFS) based on *MiCaP* scores in three independent RP cohorts. Patients in the training cohort (PCA123) were divided in low- and high-risk groups based on their *MiCaP* scores. Patients in the validation cohorts (PCA352 and PCA476) were divided into high- and low-risk groups based on the cut-off (fraction) defined in PCA123. A high *MiCaP* score was significantly associated with shorter RFS in all three cohorts. *P* values for two-sided log-rank test are given. (D) Kaplan–Meier survival analysis prostate CSS based on *MiCaP* scores in the PCA352 cohort ($n = 352$, CSS events = 19). Patients were divided in high- and low-risk groups based on their *MiCaP* scores. A high *MiCaP* score was significantly associated with shorter CSS. *P* value for two-sided log-rank test is given.

Table 3. Uni- and multivariate Cox-regression analysis of CSS using MiCaP

		Univariate			Multivariate			
Variable	**Characteristic**	**HR (95% CI)**	***P*-value**	**C-index**	**HR (95% CI)**	***P*-value**	**C-index[a]**	**C-index[b]**
PCA352, $N = 352$, 19 dead								
CAPRA-S	Low	Ref	–	0.73	–	–	0.783	0.734
	Intermediate	2.85 (0.34–23.73	3.33E−01		2.74 (0.33–22.84)	3.51E−01		
	High	10.15 (1.30–79.37)	**2.72E−02**		8.90 (1.14–69.69)	**3.74E−02**		
MiCaP	Low vs. high	3.35 (1.34–8.35)	**9.60E−03**	0.631	2.43 (1.45–4.07)	**2.10E−02**		

Significant *P* values ($P < 0.05$) are highlighted in bold.
[a]Harrell's C-index for final model including ratio model.
[b]Harrell's C-index for final model excluding the ratio model.

miR-374b-5p is tissue-type dependent. Further studies are needed to unravel the function of miR-374b in nonmalignant and PC cells, as well as of the three other miRNAs included in *MiCaP*.

Limitations to the present study include different characteristics for the three RP patient cohorts. Clinical follow-up time was shorter in PCA476 (median 15 months) compared with PCA123 and PCA352 (137 and 100 months, respectively). Moreover, different miRNA expression profiling methods were used. PCA123 and PCA352 were profiled using RT-qPCR and PCA476 by small-RNA sequencing. Nevertheless, *MiCaP* showed significant independent prognostic potential in multivariate analysis in all three cohorts, suggesting it is robust. Furthermore, all PC tissue analyses were based on surgical specimens. Future studies should examine the prognostic potential of *MiCaP* in prostate needle biopsies to assess if *MiCaP* can improve risk stratification at the time of diagnosis. Moreover, our study did not address the multifocality/heterogeneity, as we analyzed miRNA expression only in one PC tissue sample (area with highest Gleason grade) from each patient. Thus, future PC tissue-based validation studies of *MiCaP* should account for possible multifocality/heterogeneity. However, our proof-of-principle study showed promising prognostic potential for *MiCaP* in plasma samples, although independent validation is needed. In addition, only one cohort was eligible for CSS analysis, in which a low CSS mortality rate was observed (5.4%). Further validation is warranted and should include large patient cohorts with PC-specific survival and >10 years follow-up, as early-stage PC is generally slow-growing [27].

In summary, the new 4-miRNA prognostic model *MiCaP* independently predicted BCR in three distinct RP cohorts, and was a significant predictor of prostate CSS in an RP cohort comprising 352 patients. Further studies are warranted to assess the true clinical utility of *MiCaP* for improving risk stratification of clinically localized PC.

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Disclosure

HK, PM, JF, TØ, and KDS are co-inventors on patent application(s) regarding miRNAs as biomarkers for prostate cancer. KDS has received consultancy fees from Exiqon A/S. All remaining authors have declared no conflicts of interest.

References

1. Ferlay J, Soerjomataram I, Dikshit R et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136(5): E359–E386.
2. Prensner JR, Rubin MA, Wei JT, Chinnaiyan AM. Beyond PSA: the next generation of prostate cancer biomarkers. Sci Transl Med 2012; 4(127): 127rv3.
3. Heidenreich A, Aus G, Bolla M et al. EAU guidelines on prostate cancer. Eur Urol 2008; 53(1): 68–80.
4. Welch HG, Albertsen PC. Prostate cancer diagnosis and treatment after the introduction of prostate-specific antigen screening: 1986-2005. J Natl Cancer Inst 2009; 101(19): 1325–1329.
5. Albertsen PC. Treatment of localized prostate cancer: when is active surveillance appropriate? Nat Rev Clin Oncol 2010; 7(7): 394–400.
6. Rüegger S, Großhans H. MicroRNA turnover: when, how, and why. Trends Biochem Sci 2012; 37(10): 436–446.
7. Di Leva G, Garofalo M, Croce CM. MicroRNAs in cancer. Annu Rev Pathol 2014; 9: 287–314.
8. Dijkstra JR, Mekenkamp LJ, Teerenstra S et al. MicroRNA expression in formalin-fixed paraffin embedded tissue using real time quantitative PCR: the strengths and pitfalls. J Cell Mol Med 2012; 16(4): 683–690.
9. Kristensen H, Thomsen AR, Haldrup C et al. Novel diagnostic and prognostic classifiers for prostate cancer identified by genome-wide microRNA profiling. Oncotarget 2016; 7(21): 30760–30771.
10. Larne O, Martens-Uzunova E, Hagman Z et al. miQ–a novel microRNA based diagnostic and prognostic tool for prostate cancer. Int J Cancer 2013; 132(12): 2867–2875.
11. Fredsøe J, Rasmussen AKI, Thomsen AR, et al. Diagnostic and prognostic microRNA biomarkers for prostate cancer in cell-free urine. Eur Urol Focus 2017 Mar 9 [Epub ahead of print], doi:10.1016/j.euf.2017.02.018.
12. Clark K, Vendt B, Smith K et al. The Cancer Imaging Archive (TCIA): maintaining and operating a public information repository. J Digit Imaging 2013; 26(6): 1045–1057.
13. Cancer Genome Atlas Research Network. The molecular taxonomy of primary prostate cancer. Cell 2015; 163(4): 1011–1025.
14. R Development Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, 2008, ISBN 3-900051-07-0.
15. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: a Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B (Methodological) 1995; 57: 289–380.
16. Cooperberg MR, Hilton JF, Carroll PR. The CAPRA-S score: a straightforward tool for improved prediction of outcomes after radical prostatectomy. Cancer 2011; 117(22): 5039–5046.
17. Fendler A, Jung M, Stephan C et al. miRNAs can predict prostate cancer biochemical relapse and are involved in tumor progression. Int J Oncol 2011; 39(5): 1183–1192.
18. Bell EH, Kirste S, Fleming JL et al. A novel miRNA-based predictive model for biochemical failure following post-prostatectomy salvage radiation therapy. PLoS One 2015; 10(3): e0118745.
19. He HC, Han ZD, Dai QS et al. Global analysis of the differentially expressed miRNAs of prostate cancer in Chinese patients. BMC Genomics 2013; 14(1): 757.
20. Lichner Z, Fendler A, Saleh C et al. MicroRNA signature helps distinguish early from late biochemical failure in prostate cancer. Clin Chem 2013; 59(11): 1595–1603.

21. Martens-Uzunova ES, Jalava SE, Dits NF et al. Diagnostic and prognostic signatures from the small non-coding RNA transcriptome in prostate cancer. Oncogene 2012; 31(8): 978–991.
22. Tong AW, Fulgham P, Jay C et al. MicroRNA profile analysis of human prostate cancers. Cancer Gene Ther 2009; 16(3): 206–216.
23. Wen YC, Lee WJ, Tan P et al. By inhibiting snail signaling and miR-23a-3p, osthole suppresses the EMT-mediated metastatic ability in prostate cancer. Oncotarget 2015; 6(25): 21120–21136.
24. Tang YZ, Zhang W, Li M, Yan L. miR-10b represses the proliferation and invasion of prostate cancer by targeting LRH1. Int J Clin Exp Pathol 2016; 9(2): 1424–1431.
25. Kojima S, Chiyomaru T, Kawakami K et al. Tumour suppressors miR-1 and miR-133a target the oncogenic function of purine nucleoside phosphorylase (PNP) in prostate cancer. Br J Cancer 2012; 106(2): 405–413.
26. Tao J, Wu D, Xu B et al. microRNA-133 inhibits cell proliferation, migration and invasion in prostate cancer cells by targeting the epidermal growth factor receptor. Oncol Rep 2012; 27(6): 1967–1975.
27. Albertsen PC, Hanley JA, Fine J. 20-year outcomes following conservative management of clinically localized prostate cancer. JAMA 2005; 293(17): 2095–2101.