King's Research Portal

DOI:
10.1016/j.jmoldx.2022.05.005

Document Version
Peer reviewed version

Link to publication record in King's Research Portal

Citation for published version (APA):
Coradduzza, D., Solinas, T., Balzano, F., Culeddu, N., Rossi, N., Cruciani, S., Azara, E., Maioli, M., Zinellu, A., De Miglio, M. R., Madonia, M., Falchi, M., & Carru, C. (2022). miRNAs as molecular biomarkers for prostate cancer. *JOURNAL OF MOLECULAR DIAGNOSTICS*, *24*(11), 1171-1180. https://doi.org/10.1016/j.jmoldx.2022.05.005

Citing this paper
Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights
Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

•Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
•You may not further distribute the material or use it for any profit-making activity or commercial gain
•You may freely distribute the URL identifying the publication in the Research Portal

Take down policy
If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 14. Oct. 2023

MiRNAs as molecular biomarkers for prostate cancer

Donatella Coradduzza [1*], Tatiana Solinas [2], Francesca Balzano [1], Nicola Culeddu [3], Niccolò Rossi [4], Sara Cruciani [1], Emanuela Azara[3], Margherita Maioli [1], Angelo Zinellu[1], Maria Rosaria De Miglio[5], Massimo Madonia [2], Mario Falchi [4], Ciriaco Carru [1,6*]

1 Department of Biomedical Sciences, University of Sassari, Sassari, Italy

2 Urologic Clinic, Dep. of Clinical and Experimental Medicine, University of Sassari

3 Institute of Biomolecular Chemistry, National Research Council, Sassari, Italy

4 Department of Twin Research and Genetic Epidemiology, King's College London, London, UK

5 Department of Medical, Surgery and Experimental Sciences, University of Sassari, Italy

6 University Hospital of Sassari (AOU), Sassari, Italy

*Address to correspondence: Ciriaco Carru

Department of Biomedical Sciences, University of Sassari, Sassari, Italy

e-mail: carru@uniss.it

Received: date; Accepted: date; Published: date

Abstract:
MicroRNAs (miRNAs) are short noncoding RNA able to regulate specific mRNA stability, thus influencing target gene expression. Disrupted levels of several miRNA have been associated with prostate cancer, the leading cause of cancer death among men and the fifth leading cause of death worldwide. Here, we investigated whether miR-145, miR-148, and miR-185 circulating levels in plasma could be used as molecular biomarkers, to allow distinguishing between individuals with benign prostatic hyperplasia, precancerous lesion, and prostate cancer. In this study, we recruited 170 urological clinic patients with suspected prostate cancer who underwent prostate biopsy. Total RNA was isolated from plasma, and TaqMan MicroRNA assays were used to analyze miR-145, miR-185, and miR-148 expression. First, differential miRNA expression among patient groups was evaluated. Then, miRNA levels were combined with clinical assessment outcomes, including results from invasive tests, using multivariate analysis to examine their ability in discriminating among the three patient groups. Our results suggest that miRNA is a promising molecular tool for clinical management of at-risk patients.

Keywords: miRNA expression; molecular biomarkers; molecular diagnostics; biomolecular targets; prostate cancer

1. Introduction

Micro-RNAs (miRNAs) are a class of small non-coding ribonucleic acids that play important roles in regulating gene expression [1]. Recent expression profiling studies suggest miRNAs may serve as potential biomarkers for disease progression. MiRNAs appear to act in highly complex regulatory networks targeting batteries of genes [2 3] and seem to be involved in almost all key cellular processes, such as proliferation, differentiation, migration, apoptosis, stem maintenance and metabolism [4]. MiRNAs also circulate in cell-free form in body fluids, including serum and plasma [5 6 7 8], and altered expression of circulating miRNAs has been associated with age-related diseases, including cancer [9 10]. Particularly, altered miRNAs expression has been associated with the initiation and progression of prostate cancer [11 12 13 14], so they are emerging as promising biomarkers [15 16 17 18 19]. Prostate cancer (PCa) is the leading cause of cancer death among men, and the fifth leading cause of death worldwide [20]. Risk factors for this disease remain largely unknown and only 15% of cases are linked to hereditary factors [21 22 23]. Screening tests for prostate cancer include digital rectal examination (DRE) [24], prostate-specific antigen (PSA) blood test [25], transrectal ultrasound (TRUS) guided biopsy [26], and prostate biopsy [27] , the latter being considered as the gold standard. Serum and plasma tests are less invasive and faster than tissue biopsies and are, therefore, to be preferred [28]. Recently, the implementation of diagnostic assays based either on biomarkers found in urine, as PCA3, T2-ERG, exosome, and others, or blood, as four kallikrein proteins, have been studied [29 30 31 32 33 34 35 36 37 38 39 40]. Some authors proposed a multi-analytical blood test (CancerSEEK) to assess the type of cancer based on multiple analytes [41]. As inflammation appears to play a key role in the evolution of cancer diseases [42], the role of different inflammation biomarkers has been studied [43], derived from blood counts: neutrophil/lymphocyte ratio (NLR), NLR derivative [dNLR = neutrophils/(white blood cells - neutrophils)], platelet/lymphocyte ratio (PLR), monocyte/lymphocyte ratio (MLR), (neutrophil × monocyte)/Lymphocyte ratio (SIRI), and (neutrophil × monocyte × platelet)/lymphocyte ratio (AISI). Recently, researchers and physicians have expressed the need for biomarkers able to early discriminate between patients with prostate cancer (PC), from those with benign prostatic hyperplasia (BPH), or precancerous lesions (PL) [44]. Circulating biomarkers, as miRNAs, capable of distinguishing among the different forms of the disease (BPH, PL, PC), could enable early therapeutic approaches aimed at a more effective clinical response. MiRNA-145, 148, and 185 were selected on the basis of a careful review of the relevant literature as they play crucial roles in the pathogenesis and progression of prostate cancer [45]. In this study, we examined whether plasma-circulating miR-145, miR-148, and miR-185, which have been previously associated with prostate cancer [46 47 48 49 50 51] , were differentially expressed and were also able to distinguish among patients with PC, PL and BPH, recruited through the Urological Clinic of the University Hospital of Sassari. The PL category includes patients with histopathological diagnosis of atypical small acinar proliferation (ASAP) and prostatic intraepithelial neoplasia (PIN). These are the categories at increased risk of developing prostate adenocarcinoma, therefore patients must repeat needle biopsies within a period of three to six months [52 53 54].

2. Materials and Methods

2.1 Study population

170 patients, of which 92 were diagnosed with prostate cancer, 26 with precancerous lesion, and 49 with benign prostatic hypertrophy, with an average age of 58-79 years, were recruited for the differential diagnosis and divided in groups according to biopsy at the Urology Department of the University Hospital of Sassari, between September 2018 and September 2019. The number of samples included in this experiment has been selected to maximise cost-effectiveness of the experiment aimed at the discovery of clinically relevant biomarkers – therefore showing an effect size that support their utility in clinical setting. Indeed, with 92 cases and 78 controls we have >80% power to detect a difference of 0.5 of a standard deviation (SD) – thus a medium effect size of Cohen's d=0.5 - between cases and controls at alpha level=0.05/3 (Bonferroni multiple testing correction for the three tested miRNA). Exclusion from the study was based on factors that may influence miRNA expression: a previous diagnosis of prostate cancer, other diagnosis of cancer, or a subsequent diagnosis of metastatic prostate cancer after prostate biopsy[55]. Patients were included if they had a positive screening test (abnormal DRE, high age-specific serum PSA rate, or serum PSA rate of >0.35 ng/mL per year). A questionnaire ascertaining demography information, medical history (including comorbidities), family history of cancer, prostate cancer screening, urology, and lifestyle (e.g., smoking, and physical activity) was administered. Ultrasound biopsy was performed to determine the following disease groups: (1) men with benign prostatic hyperplasia (BPH); (2) men without a prostate cancer diagnosis but with high-grade prostatic intraepithelial neoplasia (PIN) or prostatic atypical small acinar proliferation (ASAP)/atypia (PL); (3) men with a prostate cancer diagnosis (PC). The studies were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each subject before the study.

2.2. RNA Extraction

Blood samples were collected during the prostate biopsy, and plasma immediately separated. All plasma samples were stored at -80 °C from the collection until measurement. The total RNA from 250 µL of plasma was extracted with Mirvana MIRNA ISO Kit 10-40ISO (Life Technologies, Carlsbad, California, USA) according to the manufacturer's protocol. The final elution volume was 15 µL with an RNA yield of 5.2-8.0 µg misused by NanoDrop™ One/OneC Microvolume UV-Vis (Thermo Fisher Scientific Inc.). Three individual miRNAs, miR-145, miR-148, and miR-185 were selected based on previous studies [46 48 51 56]

2.3. Quantitative-PCR Analysis

The concentration level of mature miRNAs was assessed by quantitative real-time PCR (qPCR) using TaqMan® MicroRNA Reverse Transcription Kit, (Life Technologies), for the reverse transcription. TaqMan® Universal Master Mix II, Life Technologies, was used for PCR according to the manufacturer's instructions; 45 amplification cycles were performed. miRNA concentration levels were quantified using the IQ5, BIORAD, instrument, (Milan Italy). U6snRNA was used for data normalization [57 58 59]. Real-time PCR was performed in duplicate. Sequences and identification symbols were retrieved from miRbase and are reported in Table 1.

2.4. Real-Time PCR Data Analysis

The raw Ct values for each miRNA and U6snRNA were checked for normal distribution. The Kruskal-Wallis test was applied to compare the groups in each target. All the analyses and graphics were obtained through the SPSS software version 17.0. Reverse transcription followed by polymerase chain reaction (RT-PCR) is the most suitable method for the detection and quantification of miRNA. It provides high sensitivity, good reproducibility, and a wide range quantification. Several mathematical algorithms have been developed to calculate a ratio of expression based on real-time PCR efficiency and the crossing point deviation of an unknown sample against a control.

2.5. Statistical analysis.

Baseline hematological parameters (i.e., blood cell counts), anthropometric measures (BMI), as well as clinical parameters evaluated for prostate cancer screening (i.e., PSA, SIRI, AISI, IPSS, IIEF, TRUS, Charlson score), were tested for significant differences among study groups (i.e., BPH, PL and PC) using the non-parametric Wilcoxon's signed-rank test. Categorical variables of interest (namely, smoking, alcohol consumption and G6PDH deficiency) were compared between groups using Pearson's $\chi 2$ test. Differences in miRNAs expression levels across study groups were assessed by means of the Wilcoxon's test.P-values <0.05 were considered as significant. SIMCA-P software version 13.0, (Umetrics AB, Umea°, Sweden) was used for PCA and OPLS-DA. Outliers are excluded from Dmodx (Distance to model is an estimate of how far from the model plane) and T2 range (a large T2-range value for a given observation, i.e., a value far above the critical limits, indicates that the observation is far from the others in the selected range of components in the score-space). Supervised analysis was carried out by applying the orthogonal partial least square discriminant analysis (OPLS-DA), which implies a rotation of the corresponding PLS-DA models and simplifies them concentrating the information in one predictive component, meanwhile maintaining the same predictive ability. To define outliers, we used a plot between the distance from the model (DmodX) and T2 range Hotelling (T2range) considering as outliers the samples with DmodX > 1.4 and T2Range > 95% simultaneously (serious outliers). The number of orthogonal components in the models was determined by the ''autofit'' routine of SIMCA-P. To avoid model overfitting, the OPLS-DA model was validated by 300 times permutation tests. The prediction strength of the model was evaluated by "Leave out" analysis. Variable Importance Parameter (VIP) values were used to assess the overall contribution of each X variable to the model, summed over all components and weighted according to the Y variation accounted for by each component. The number of terms in the sum depends on the number of OPLS-DA components found to be significant in distinguishing the classes. The Y axis indicates the VIP scores corresponding to each variable on the X axis. Bars indicate the factors with the highest VIP scores and thus are the most contributory variables in class discrimination in the OPLS-DA model.

Receiver operating characteristics (ROC) curves were generated to compare sensitivity, specificity, and the area under the curve (AUC). Logistic regression was used to calculate the sensitivity and specificity of individual and combinations of all three miRNAs in discriminating between PC from both BPH and PL patients, and PL from BPH patients. Calculations were performed using the R package pROC version 1.17.0.1. Harrel's bootstrap method was used to adjust for optimism/overfitting of the ROC curve using 200

bootstraps[60].

Results

2.1 Clinical Data

We analysed clinical data collected and gleaned from each patient, from the anamnesis, through biochemical measurements and physical examination, which are routinely generated to diagnose prostate cancer, and investigate any potential concurrent symptoms (Table 2). Diagnosis is primarily based on prostate-specific antigen (PSA) testing, and transrectal ultrasound-guided (TRUS) prostate tissue biopsies, although PSA testing for screening remains controversial [61 62]. The detection of PCa is traditionally based on digital rectal examination (DRE), serum prostate-specific antigen (PSA) level, and transrectal ultrasound (TRUS)–guided biopsies [63]. TRUS-guided biopsy result is the diagnostic standard for PCa, but in this way 30% of cancers are missed. Patients with a suspected false-negative result on biopsy are a diagnostic challenge, because there is a progressively lower diagnostic yield from subsequent prostate biopsies. Other clinical parameters are the Charlson Comorbidity Index, predicting the ten-year mortality for a patient who may have a range of comorbid conditions; and INDEX, a ratio between free PSA and total PSA: when this ratio is between 0 and 10percent, the risk of having cancer may be as high as 56 percent.

PC samples showed significant differences with PL for PSA ($p = 1.53x10^{-3}$), INDEX ($p = 8.72x10^{-3}$), WBC ($p = 7.18x10^{-3}$), neutrophils ($p = 1.99x10^{-2}$), Charlson index ($p = 8.09x10^{-8}$) IIEF ($p = 1.37x10^{-2}$).

In the BPH group, PSA levels were significantly lower than in the PC group ($p = 1.67x10^{-4}$) but did not differ between PL and BPH groups.

On the other hand, patients with BPH showed statistically significant differences from PC patients for INDEX ($p = 1.40x10^{-3}$), age (p = 4.15x10-3), Charlson index ($p = 5.36x10^{-11}$).

2.2 Differential miRNA expression

We observed some statistically significant differences in miRNA expression among the three patients' subgroups (Figure 1). Particularly, we observed a significant trend of increasing expression of mir-145 from PC to BPH and PL patients (Figure 1A). Significant upregulation of miR-148 was also observed in both PL andBPH, compared to PC patients (Figure 1B). Finally, a significant trend of decreasing expression of mir-185 levels was observed among the PC, PL, and BPH groups (Figure 1C).

2.3 Diagnostic value of miRNA in prostate cancer

To investigate the ability of these miRNA measured in plasma to discriminate among the three groups of patients, we analyzed miRNAs' expression and clinical data through partial orthogonal analysis of minimum square discrimination (OPLS-DA). PCA was initially applied to the complete data set to identify potential outliers, using the above criterion 30 samples were identified as outliers and removed from the analysis. OPLS-DA is an approach of discriminating analysis used to optimize the separation among different groups. The

OPLS-DA score plot (Figure 2A) showed a clear separation among the three groups of patients. It's used a Hotelling T2 test and the distance to the model in the X-data (DModX) plot to identify possible outliers. Outliers lie within the 95% confidence limit for T2, and with a DMoX<2. The Variable Importance in Projection (VIP) coefficients (Figure 2B) summarize the overall contribution of each X-variable to the model, summed over all components and weighted according to the Y variation accounted for by each component. The Y axis in 2B indicates the VIP scores corresponding to each variable on the X axis, where, as a general rule, we considered important contributors to the model those variables showing a VIP >1. To test the validity of the model we perform misclassication table, figure 3A that shows proportion of correct classification observation of 98.58%; figure 3B shows results from permutation test on the OPLS-DA). Results showed that the model was statistically valid, with a Q2 intercept value of -0.624 and an R2 of 0.726.

The AUC ROC curve for each individual miRNA and for the combined miRNAs was unsurprisingly close to 1 for PC versus BPH+PL patients, given the highly significant differences in miRNA level between these two groups, thus indicating significant ability to distinguish prostate cancer patient samples from benign prostatic hyperplasia and precancerous lesions patients (Supplementary Figure 1). Similarly, miR-145 and miR-185 were able to discriminate PL from BPH patients, with AUC>0.9 for each of the two miRNAs and their combination, while the AUC for miR-148 was about 0.5, as this miRNA is unable to discriminate between the two classes (Supplementary Figure 2).

3. Discussion

Prostate cancer is one of the deadliest cancers in the world [64]. Early detection has reduced mortality in the recent decades [65]. Non-invasive biomarkers with high sensitivity and specificity are urgently needed for clinical management of the patients. Owing to inherent limitations of the prostate-specific antigen (PSA) biomarker, a large number of BPH and PL patients with PSA levels in the "grey area" (4–10 ng/mL) currentlysuffer from unnecessary biopsy [66]. DRE fails to detect a substantial proportion of cancers and identifies predominantly large tumours [67], and, while TRUS-guided biopsy is the diagnostic standard [68], some cancers are missed when performing biopsies or interpreted as high-risk cases [69]. Patients with a suspected false- negative result on biopsy have a progressively lower diagnostic yield from subsequent prostate biopsies [70]. Therefore, PSA levels, DRE, and TRUS-guided biopsy are not always able to distinguish between PL and BPHpatients [71]. MicroRNAs (miRNAs) are regulators of gene expression and play a key role in the pathophysiology of various disease processes [72 73], particularly in tumours [74 75 76 77 78]. Several studies have suggested that microRNAscould be used as diagnostic biomarkers in different types of cancer [79 80 81]. Particularly, altered expression of some microRNAs seems to be indicative of cancer progression [82 83 84 85]. The aim of this study was to find promisingbiomarkers that can meet the unmet clinical needs in prostate cancer patient management. Such biomarkers would provide actionable guidance for patient's risk assessment and are expected to contribute to the development of personalized medicine. Thus, the initial assumption we made was that miRNAs in the circulation system might function as feasible biomarkers for PCa. Mitchell et al. [86], demonstrated the utility ofcirculating miRNAs as biomarkers using plasma and serum samples from PCa patients. These promising assumptions have led to the

design of the present project for miRNAs potential use as minimally invasive biomarkers, which would be a considerable improvement in disease monitoring [84]. Clinical data shown in Table 2 indicate that age markedly influences the progression of pathology, in accordance with observationalstudies [87]. Increased PSA and INDEX herald clinical progression and have been an accepted indicator of worsening disease [88]. Systemic inflammation and immune responses were reported to be associated with progressive prostate cancer. Accordingly, we also observed increased serum WBC count in patients with prostate cancer [89]. The reason why neutrophils have higher counts in PCs than in PLs may be related to the fact that they respond to chemotactic substances released by tumour cells [90]. Moreover, we observed higher Charlson index in PC subjects, as is often observed in affected patients [91]. Finally, International Index of Erectile Function (IIEF) is correlated a tool to study the erectile dysfunction, and scores were correlated in patients with prostate cancer [92].

Previous studies have shown up-regulated miR-145 levels between patients with PC and patients with BPH [47, 93], or between patients with PC and healthy controls [94]. Here, we show that miR-145 levels may be helpful to distinguish between PC, PL, and BPH patients.

The high levels observe in PC patients may indicate secretion of miRNA from cancer cells as elimination route of tumour- or metastasis-suppressor miRNAs [95]. Alternatively, it has been speculated that the high expression in plasma may reflect changes occurring in circulation [96]. Patients with hight plasma levels of miR-145 have been shown to have significantly shorter median overall survival rate [47].

MiR-148 is down-regulated in PC subjects compared to PL and BPH patients. There is no difference in expression between PL and BPH patients, so miR-148 is not useful in differentiating these two groups. Increased levels of miR-148 have been previously observed in in prostate cancer [97], with studies suggesting poor prognosis for PC patients with low miR-148 levels [98]. miR-148 has been shown to positively regulate cell apoptosis and inhibit tumor growth [48 49 98 99]

Low miR-185 levels have been previously observed in prostate cancer tissue and cells [100 101]. Here, we show a significant decreasing trend between BPH, PL, and PC patients, and may be thus helpful to distinguish between these three groups of patients. Studies indicate miR-185 targets the two anti-apoptotic *BCL2* and *BCL2L1* genes [51 76]. Some authors hypothesized that miR-185 over-expression may be able to induce apoptosisin PCa cell lines by sub-regulating *BCL2* and *BCL2L1* anti-apoptotic genes [51].

We combined both non-invasive clinical data and invasive tests with levels from the three miRNAs to assess their ability in distinguish among the three groups of patients. We used OPLS-DA, a relatively simple statistical technique, which is robust to multicollinearity that may be present in these data. Individuals from the three patient classes were clearly separated into three distinct clusters by the OPLS-DA analysis (Figure 2A), indicating an R2 of 0.726) and a Q2 of 0.624. An R2 value >0.5 is a usually considered as a condition for a good classification model, while a Q2 value >0.5 indicates good predictability. The contribution score graph showing the relative importance of each variable in differentiating the three classes of patients (Figure 2B) indicated miR-145, miR-185 miR-148 as the main contributor to the model. Therefore, the levels of these three miRNAs are good biomarkers to distinguishing between the three classes of patients, with a potential higher predictive power compared to clinical biomarkers usually measured in clinical practice. Finally, the validity of the OPLS-

DA model was verified through permutations.

This work paves the way for the search for miRNAs that can be used as diagnostics and for personalized medicine. MiRNAs may help patient stratification into risk classes, which from a clinical point of view would avoid unnecessary diagnostic tests in patients at low risk for PCa, and particularly repeated biopsies in patients at high risk for this tumour, such as those belonging to the PL group. Use of suitable biomarkers would not only rationalize available resources but have also the potential to help early identification of prostate cancer cases or ideally of precancerous lesions.

Since the discovery of circulating miRNAs in 2008, they have been advocated as a potential new generation of biomarkers for the diagnosis and evaluation of several diseases. Prostate cancer is one of the deadliest malignant tumors, the first among males. The search for new and more effective biomarkers constitutes the challenge and the most important need in the clinical management of these patients. Intensive efforts are currently directed towards the search for prostate cancer biomarkers that can predict disease progression to drive clinical decisions. In this work, we have shown the ability of miR-145 and miR-148 and miR-185 in distinguishing between BPH, PL, and PC patients. miRNAs could be a useful help to overcome inherent limitations of the prostate-specific antigen (PSA) biomarker in clinical practice, and help developing a clinical prediction model. Further studies are needed to explore the utility of this class of biomarkers in the prediction of (1) disease risk, (2) developmental trajectory, and (3) response to treatment and risk for severe adverse reactions to therapy.

References

1. Behm-Ansmant I, Rehwinkel J, Izaurralde E: MicroRNAs silence gene expression by repressing protein expression and/or by promoting mRNA decay. Cold Spring Harbor symposia on quantitative biology: Cold Spring Harbor Laboratory Press, 2006. pp. 523-530.
2. Martinez NJ, Walhout AJ: The interplay between transcription factors and microRNAs in genome-scale regulatory networks. Bioessays 2009, 31:435-445.
3. Makeyev EV, Maniatis T: Multilevel regulation of gene expression by microRNAs. Science 2008, 319:1789-1790.
4. Pedroza-Torres A, Romero-Córdoba SL, Justo-Garrido M, Salido-Guadarrama I, Rodríguez-Bautista R, Montaño S, Muñiz-Mendoza R, Arriaga-Canon C, Fragoso-Ontiveros V, Álvarez-Gómez RM: MicroRNAs in tumor cell metabolism: roles and therapeutic opportunities. Frontiers in oncology 2019:1404.
5. Nakamura K, Sawada K, Yoshimura A, Kinose Y, Nakatsuka E, Kimura T: Clinical relevance of circulating cell-free microRNAs in ovarian cancer. Molecular cancer 2016, 15:1-10.
6. Schwarzenbach H, Nishida N, Calin GA, Pantel K: Clinical relevance of circulating cell-free microRNAs in cancer. Nature reviews Clinical oncology 2014, 11:145-156.
7. Javidi MA, Ahmadi AH, Bakhshinejad B, Nouraee N, Babashah S, Sadeghizadeh M: Cell-free microRNAs as cancer biomarkers: the odyssey of miRNAs through body fluids. Medical oncology 2014, 31:1-11.
8. Armand-Labit V, Pradines A: Circulating cell-free microRNAs as clinical cancer biomarkers. Biomolecular concepts 2017, 8:61-81.
9. Hooten NN, Fitzpatrick M, Wood 3rd WH, De S, Ejiogu N, Zhang Y, Mattison JA, Becker KG, Zonderman AB, Evans MK: Age-related changes in microRNA levels in serum. Aging (Albany NY) 2013, 5:725.
10. Olivieri F, Rippo MR, Procopio AD, Fazioli F: Circulating inflamma-miRs in aging and age-related diseases. Frontiers in genetics 2013, 4:121.

11. Ozen M, Creighton C, Ozdemir M, Ittmann M: Widespread deregulation of microRNA expression in human prostate cancer. Oncogene 2008, 27:1788-1793.
12. Medina-Villaamil V, Martínez-Breijo S, Portela-Pereira P, Quindós-Varela M, Santamarina-Cainzos I, Antón-Aparicio L, Gómez-Veiga F: Circulating MicroRNAs in blood of patients with prostate cancer. Actas Urológicas Españolas (English Edition) 2014, 38:633-639.
13. Porkka KP, Pfeiffer MJ, Waltering KK, Vessella RL, Tammela TL, Visakorpi T: MicroRNA expression profiling in prostate cancer. Cancer research 2007, 67:6130-6135.
14. Zhang W, Zang J, Jing X, Sun Z, Yan W, Yang D, Guo F, Shen B: Identification of candidate miRNA biomarkers from miRNA regulatory network with application to prostate cancer. Journal of translational medicine 2014, 12:1-12.
15. Ambs S, Prueitt RL, Yi M, Hudson RS, Howe TM, Petrocca F, Wallace TA, Liu C-G, Volinia S, Calin GA: Genomic profiling of microRNA and messenger RNA reveals deregulated microRNA expression in prostate cancer. Cancer research 2008, 68:6162-6170.
16. Singh PK, Campbell MJ: The interactions of microRNA and epigenetic modifications in prostate cancer. Cancers 2013, 5:998-1019.
17. Saini S: PSA and beyond: alternative prostate cancer biomarkers. Cellular Oncology 2016, 39:97-106.
18. Selth L, Townley S, Bert A, Stricker P, Sutherland P, Horvath L, Goodall G, Butler L, Tilley W: Circulating microRNAs predict biochemical recurrence in prostate cancer patients. British journal of cancer 2013, 109:641-650.
19. Casanova-Salas I, Rubio-Briones J, Fernández-Serra A, López-Guerrero JA: miRNAs as biomarkers in prostate cancer. Clinical and Translational Oncology 2012, 14:803-811.
20. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians 2018, 68:394-424.
21. Rawla P: Epidemiology of prostate cancer. World journal of oncology 2019, 10:63.
22. Rawla P, Sunkara T, Gaduputi V: Epidemiology of pancreatic cancer: global trends, etiology and risk factors. World journal of oncology 2019, 10:10.
23. Bostwick DG, Burke HB, Djakiew D, Euling S, Ho Sm, Landolph J, Morrison H, Sonawane B, Shifflett T, Waters DJ: Human prostate cancer risk factors. Cancer: Interdisciplinary International Journal of the American Cancer Society 2004, 101:2371-2490.
24. Kash DP, Lal M, Hashmi AH, Mubarak M: Utility of digital rectal examination, serum prostate specific antigen, and transrectal ultrasound in the detection of prostate cancer: a developing country perspective. Asian Pacific Journal of Cancer Prevention 2014, 15:3087-3091.
25. Mistry K, Cable G: Meta-analysis of prostate-specific antigen and digital rectal examination as screening tests for prostate carcinoma. The Journal of the American Board of Family Practice 2003, 16:95-101.
26. Woo S, Suh CH, Eastham JA, Zelefsky MJ, Morris MJ, Abida W, Scher HI, Sidlow R, Becker AS, Wibmer AG: Comparison of magnetic resonance imaging-stratified clinical pathways and systematic transrectal ultrasound-guided biopsy pathway for the detection of clinically significant prostate cancer: a systematic review and meta-analysis of randomized controlled trials. European urology oncology 2019, 2:605-616.
27. Smith RA, Andrews KS, Brooks D, Fedewa SA, Manassaram-Baptiste D, Saslow D, Wender RC: Cancer screening in the United States, 2019: A review of current American Cancer Society guidelines and current issues in cancer screening. CA: a cancer journal for clinicians 2019, 69:184-210.
28. Krishnamurthy N, Spencer E, Torkamani A, Nicholson L: Liquid biopsies for cancer: coming to a patient near you. Journal of clinical medicine 2017, 6:3.
29. Brönimann S, Pradere B, Karakiewicz P, Abufaraj M, Briganti A, Shariat SF: An overview of current and emerging diagnostic, staging and prognostic markers for prostate cancer. Expert Review of Molecular Diagnostics 2020, 20:841-850.
30. Carlsson SV, Roobol MJ: Improving the evaluation and diagnosis of clinically significant prostate cancer in 2017. Current opinion in urology 2017, 27:198.
31. Loeb S: Biomarkers for Prostate Biopsy and Risk Stratification of Patients with Newly Diagnosed Prostate Cancer. Urology practice 2017, 4:315-321.
32. Kiebish MA, Cullen J, Mishra P, Ali A, Milliman E, Rodrigues LO, Chen EY, Tolstikov V, Zhang L, Panagopoulos K: Multi-omic serum biomarkers for prognosis of disease progression in prostate cancer. Journal of translational medicine 2020, 18:1-10.
33. Loeb S, Dani H: Whom to biopsy: prediagnostic risk stratification with biomarkers, nomograms, and

risk calculators. Urologic Clinics 2017, 44:517-524.
34. Mao Z, Ji A, Yang K, He W, Hu Y, Zhang Q, Zhang D, Xie L: Diagnostic performance of PCA3 and hK2 in combination with serum PSA for prostate cancer. Medicine 2018, 97.
35. Darson MF, Pacelli A, Roche P, Rittenhouse HG, Wolfert RL, Young CY, Klee GG, Tindall DJ, Bostwick DG: Human glandular kallikrein 2 (hK2) expression in prostatic intraepithelial neoplasia and adenocarcinoma: a novel prostate cancer marker. Urology 1997, 49:857-862.
36. Bosland M: Dissociation between androgen responsiveness for malignant growth vs. expression of prostate specific differentiation markers PSA, hK2, and PSMA in human prostate cancer models. Denmeade SR, Sokoll LJ, Dalrymple S, Rosen DM, Gady AM, Bruzek D, Ricklis RM, Isaacs JT, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD 21231: Prostate 2003; 54 (4): 249–257. Urologic Oncology: Seminars and Original Investigations: Elsevier, 2003. pp. 414-415.
37. Denmeade SR, Sokoll LJ, Dalrymple S, Rosen DM, Gady AM, Bruzek D, Ricklis RM, Isaacs JT: Dissociation between androgen responsiveness for malignant growth vs. expression of prostate specific differentiation markers PSA, hK2, and PSMA in human prostate cancer models. The Prostate 2003, 54:249-257.
38. McGarty TP: Prostate Cancer Prognostic Tests: Pre and Post Diagnosis. Hal, 2015.
39. Borrebaeck CA: Precision diagnostics: moving towards protein biomarker signatures of clinical utility in cancer. Nature Reviews Cancer 2017, 17:199-204.
40. Helo P, Cronin AM, Danila DC, Wenske S, Gonzalez-Espinoza R, Anand A, Koscuiszka M, Väänänen R-M, Pettersson K, Chun FK: Circulating prostate tumor cells detected by reverse transcription-PCR in men with localized or castration-refractory prostate cancer: concordance with CellSearch assay and association with bone metastases and with survival. Clinical chemistry 2009, 55:765-773.
41. Yu R, d'Aquin M, Gasevic D, Kimmerle J, Herder E, Ewerth R: Lile2019: 8th international workshop on learning and education with web data. Companion Publication of the 10th ACM Conference on Web Science, 2019. pp. 15-16.
42. Lu H, Ouyang W, Huang C: Inflammation, a key event in cancer development. Molecular cancer research 2006, 4:221-233.
43. Erre GL, Buscetta G, Mangoni AA, Castagna F, Paliogiannis P, Oggiano M, Carru C, Passiu G, Zinellu A: Diagnostic accuracy of different blood cells-derived indexes in rheumatoid arthritis: A cross-sectional study. Medicine 2020, 99.
44. McNally CJ, Ruddock MW, Moore T, McKenna DJ: Biomarkers that differentiate benign prostatic hyperplasia from prostate cancer: A literature review. Cancer Management and Research 2020, 12:5225.
45. Coradduzza D, Cruciani S, Arru C, Garroni G, Pashchenko A, Jedea M, Zappavigna S, Caraglia M, Amler E, Carru C: Role of miRNA-145, 148, and 185 and Stem Cells in Prostate Cancer. International Journal of Molecular Sciences 2022, 23:1626.
46. Larne O, Hagman Z, Lilja H, Bjartell A, Edsjö A, Ceder Y: miR-145 suppress the androgen receptor in prostate cancer cells and correlates to prostate cancer prognosis. Carcinogenesis 2015, 36:858-866.
47. Avgeris M, Stravodimos K, Fragoulis E, Scorilas A: The loss of the tumour-suppressor miR-145 results in the shorter disease-free survival of prostate cancer patients. British journal of cancer 2013, 108:2573-2581.
48. Feng F, Liu H, Chen A, Xia Q, Zhao Y, Jin X, Huang J: miR-148-3p and miR-152-3p synergistically regulate prostate cancer progression via repressing KLF4. Journal of cellular biochemistry 2019, 120:17228-17239.
49. Walter BA, Valera VA, Pinto PA, Merino MJ: Comprehensive microRNA profiling of prostate cancer. Journal of Cancer 2013, 4:350.
50. Tian C, Deng Y, Jin Y, Shi S, Bi H: Long non-coding RNA RNCR3 promotes prostate cancer progression through targeting miR-185-5p. American journal of translational research 2018, 10:1562.
51. Ostadrahimi S, Valugerdi MA, Hassan M, Haddad G, Fayaz S, Parvizhamidi M, Mahdian R, Esfahani PF: miR-1266-5p and miR-185-5p promote cell apoptosis in human prostate cancer cell lines. Asian Pacific journal of cancer prevention: APJCP 2018, 19:2305.
52. Tewari AK, Whelan P, Graham JD: Prostate cancer: diagnosis and clinical management: John Wiley & Sons, 2013.
53. Leone A, Gershman B, Rotker K, Butler C, Fantasia J, Miller A, Afiadata A, Amin A, Zhou A, Jiang Z: Atypical small acinar proliferation (ASAP): is a repeat biopsy necessary ASAP? A multi-institutional review. Prostate Cancer and Prostatic Diseases 2016, 19:68-71.
54. Zhou M: High-grade prostatic intraepithelial neoplasia, PIN-like carcinoma, ductal carcinoma, and

intraductal carcinoma of the prostate. Modern Pathology 2018, 31:71-79.
55. McDonald AC, Vira M, Shen J, Sanda M, Raman JD, Liao J, Patil D, Taioli E: Circulating microRNAs in plasma as potential biomarkers for the early detection of prostate cancer. The Prostate 2018, 78:411-418.
56. Ozen M, Karatas OF, Gulluoglu S, Bayrak OF, Sevli S, Guzel E, Ekici ID, Caskurlu T, Solak M, Creighton CJ: Overexpression of miR-145–5p inhibits proliferation of prostate cancer cells and reduces SOX2 expression. Cancer investigation 2015, 33:251-258.
57. Peltier HJ, Latham GJ: Normalization of microRNA expression levels in quantitative RT-PCR assays: identification of suitable reference RNA targets in normal and cancerous human solid tissues. Rna 2008, 14:844-852.
58. Shen J, Wang Q, Gurvich I, Remotti H, Santella RM: Evaluating normalization approaches for the better identification of aberrant microRNAs associated with hepatocellular carcinoma. Hepatoma research 2016, 2:305.
59. Maioli M, Rinaldi S, Pigliaru G, Santaniello S, Basoli V, Castagna A, Fontani V, Ventura C: REAC technology and hyaluron synthase 2, an interesting network to slow down stem cell senescence. Scientific reports 2016, 6:1-8.
60. Harrell Jr FE, Lee KL, Mark DB: Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Statistics in medicine 1996, 15:361-387.
61. Harvey C, Pilcher J, Richenberg J, Patel U, Frauscher F: Applications of transrectal ultrasound in prostate cancer. The British journal of radiology 2012, 85:S3-S17.
62. Sadeghi-Nejad H, Simmons M, Dakwar G, Dogra V: Controversies in transrectal ultrasonography and prostate biopsy. Ultrasound quarterly 2006, 22:169-175.
63. Chun FK-H, Steuber T, Erbersdobler A, Currlin E, Walz J, Schlomm T, Haese A, Heinzer H, McCormack M, Huland H: Development and internal validation of a nomogram predicting the probability of prostate cancer Gleason sum upgrading between biopsy and radical prostatectomy pathology. European urology 2006, 49:820-826.
64. Pernar CH, Ebot EM, Wilson KM, Mucci LA: The epidemiology of prostate cancer. Cold Spring Harbor perspectives in medicine 2018, 8:a030361.
65. Merriel SW, Funston G, Hamilton W: Prostate cancer in primary care. Advances in therapy 2018, 35:1285-1294.
66. Salido-Guadarrama AI, Morales-Montor JG, Rangel-Escareño C, Langley E, Peralta-Zaragoza O, Cruz Colin JL, Rodriguez-Dorantes M: Urinary microRNA-based signature improves accuracy of detection of clinically relevant prostate cancer within the prostate-specific antigen grey zone. Molecular medicine reports 2016, 13:4549-4560.
67. Naji L, Randhawa H, Sohani Z, Dennis B, Lautenbach D, Kavanagh O, Bawor M, Banfield L, Profetto J: Digital rectal examination for prostate cancer screening in primary care: a systematic review and meta-analysis. The Annals of Family Medicine 2018, 16:149-154.
68. Smeenge M, Barentsz J, Cosgrove D, De La Rosette J, De Reijke T, Eggener S, Frauscher F, Kovacs G, Matin SF, Mischi M: Role of transrectal ultrasonography (TRUS) in focal therapy of prostate cancer: report from a Consensus Panel. BJU international 2012, 110:942-948.
69. Scattoni V, Raber M, Abdollah F, Roscigno M, Dehò F, Angiolilli D, Maccagnano C, Gallina A, Capitanio U, Freschi M: Biopsy schemes with the fewest cores for detecting 95% of the prostate cancers detected by a 24-core biopsy. European urology 2010, 57:1-8.
70. Wolters T, van der Kwast TH, Vissers CJ, Bangma CH, Roobol M, Schröder FH, van Leenders GJ: False-negative prostate needle biopsies: frequency, histopathologic features, and follow-up. The American journal of surgical pathology 2010, 34:35-43.
71. Obralic N, Kulovac B: High grade intraepithelial neoplasia of prostate is associated with values of prostate specific antigen related parameters intermediate between prostate cancer and normal levels. Bosnian Journal of Basic Medical Sciences 2011, 11:223.
72. Balzano F, Campesi I, Cruciani S, Garroni G, Bellu E, Dei Giudici S, Angius A, Oggiano A, Rallo V, Capobianco G: Epigenetics, stem cells, and autophagy: exploring a path involving miRNA. International Journal of Molecular Sciences 2019, 20:5091.
73. Balzano F, Garroni G, Cruciani S, Bellu E, Dei Giudici S, Oggiano A, Capobianco G, Dessole S, Ventura C, Maioli M: Behavioral changes in stem-cell potency by HepG2-exhausted medium. Cells 2020, 9:1890.
74. Zhou X, Zhao F, Wang Z-N, Song Y-X, Chang H, Chiang Y, Xu H-M: Altered expression of miR-152 and miR-148a in ovarian cancer is related to cell proliferation. Oncology reports 2012, 27:447-454.

75. Zhang J-g, Shi Y, Hong D-f, Song M, Huang D, Wang C-y, Zhao G: MiR-148b suppresses cell proliferation and invasion in hepatocellular carcinoma by targeting WNT1/β-catenin pathway. Scientific reports 2015, 5:1-9.
76. Ostadrahimi S, Fayaz S, Parvizhamidi M, Abedi-Valugerdi M, Hassan M, Kadivar M, Teimoori-Toolabi L, Asgari M, Shahrokh H, Abolhasani M: Downregulation of miR-1266-5P, miR-185-5P and miR-30c-2 in prostatic cancer tissue and cell lines. Oncology letters 2018, 15:8157-8164.
77. Qu F, Cui X, Hong Y, Wang J, Li Y, Chen L, Liu Y, Gao Y, Xu D, Wang Q: MicroRNA-185 suppresses proliferation, invasion, migration, and tumorigenicity of human prostate cancer cells through targeting androgen receptor. Molecular and cellular biochemistry 2013, 377:121-130.
78. Yao Q, Chen Y, Zhou X: The roles of microRNAs in epigenetic regulation. Current opinion in chemical biology 2019, 51:11-17.
79. Kelly BD, Miller N, Sweeney KJ, Durkan GC, Rogers E, Walsh K, Kerin MJ: A circulating microRNA signature as a biomarker for prostate cancer in a high risk group. Journal of clinical medicine 2015, 4:1369-1379.
80. Kosaka N, Iguchi H, Ochiya T: Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer science 2010, 101:2087-2092.
81. Zen K, Zhang CY: Circulating microRNAs: a novel class of biomarkers to diagnose and monitor human cancers. Medicinal research reviews 2012, 32:326-348.
82. Wang H, Peng R, Wang J, Qin Z, Xue L: Circulating microRNAs as potential cancer biomarkers: the advantage and disadvantage. Clinical epigenetics 2018, 10:1-10.
83. Farazi TA, Hoell JI, Morozov P, Tuschl T: MicroRNAs in human cancer. MicroRNA cancer regulation 2013:1-20.
84. Condrat CE, Thompson DC, Barbu MG, Bugnar OL, Boboc A, Cretoiu D, Suciu N, Cretoiu SM, Voinea SC: miRNAs as biomarkers in disease: latest findings regarding their role in diagnosis and prognosis. Cells 2020, 9:276.
85. Leichter AL, Sullivan MJ, Eccles MR, Chatterjee A: MicroRNA expression patterns and signalling pathways in the development and progression of childhood solid tumours. Molecular cancer 2017, 16:1-17.
86. Tiberio P, Callari M, Angeloni V, Daidone MG, Appierto V: Challenges in using circulating miRNAs as cancer biomarkers. BioMed research international 2015, 2015.
87. Attard G, Parker C, Eeles R: Schroff der F, Tomlins SA, Tannock I, Drake CG, de Bono JS. 2016. Prostate cancer The Lancet, 387:70-82.
88. Hussain M, Goldman B, Tangen C, Higano CS, Petrylak DP, Wilding G, Akdas AM, Small EJ, Donnelly BJ, Sundram SK: Prostate-specific antigen progression predicts overall survival in patients with metastatic prostate cancer: data from Southwest Oncology Group Trials 9346 (Intergroup Study 0162) and 9916. Journal of clinical oncology 2009, 27:2450.
89. Hayashi T, Fujita K, Tanigawa G, Kawashima A, Nagahara A, Ujike T, Uemura M, Takao T, Yamaguchi S, Nonomura N: Serum monocyte fraction of white blood cells is increased in patients with high Gleason score prostate cancer. Oncotarget 2017, 8:35255.
90. Bahig H, Taussky D, Delouya G, Nadiri A, Gagnon-Jacques A, Bodson-Clermont P, Soulieres D: Neutrophil count is associated with survival in localized prostate cancer. BMC cancer 2015, 15:1-8.
91. Lin Y-T, Lee MT-S, Huang Y-C, Liu C-K, Li Y-T, Chen M: Prediction of recurrence-associated death from localized prostate cancer with a charlson comorbidity index–reinforced machine learning model. Open Medicine 2019, 14:593-606.
92. Rhoden E, Telöken C, Sogari P, Vargas Souto C: The use of the simplified International Index of Erectile Function (IIEF-5) as a diagnostic tool to study the prevalence of erectile dysfunction. International journal of impotence research 2002, 14:245-250.
93. Song CJ, Chen H, Chen LZ, Ru GM, Guo JJ, Ding QN: The potential of microRNAs as human prostate cancer biomarkers: a meta-analysis of related studies. Journal of cellular biochemistry 2018, 119:2763-2786.
94. Watahiki A, Wang Y, Morris J, Dennis K, O'Dwyer HM, Gleave M, Gout PW, Wang Y: MicroRNAs associated with metastatic prostate cancer. PloS one 2011, 6:e24950.
95. Cui SY, Wang R, Chen LB: Micro RNA-145: a potent tumour suppressor that regulates multiple cellular pathways. Journal of cellular and molecular medicine 2014, 18:1913-1926.
96. Jackson BL, Grabowska A, Ratan HL: MicroRNA in prostate cancer: functional importance and potential as circulating biomarkers. BMC cancer 2014, 14:1-10.

97. Andl T, Ganapathy K, Bossan A, Chakrabarti R: MicroRNAs as Guardians of the Prostate: Those Who Stand before Cancer. What Do We Really Know about the Role of microRNAs in Prostate Biology? International Journal of Molecular Sciences 2020, 21:4796.
98. Li Y, Deng X, Zeng X, Peng X: The role of Mir-148a in cancer. Journal of cancer 2016, 7:1233.
99. Coppola V, De Maria R, Bonci D: MicroRNAs and prostate cancer. Endocrine-related cancer 2010, 17:F1.
100. Kristensen H, Thomsen AR, Haldrup C, Dyrskjøt L, Høyer S, Borre M, Mouritzen P, Ørntoft TF, Sørensen KD: Novel diagnostic and prognostic classifiers for prostate cancer identified by genome-wide microRNA profiling. Oncotarget 2016, 7:30760.
101. Luu HN, Lin H-Y, Sørensen KD, Ogunwobi OO, Kumar N, Chornokur G, Phelan C, Jones D, Kidd L, Batra J: miRNAs associated with prostate cancer risk and progression. BMC urology 2017, 17:1-18.

Abbreviations

PCa	Prostate cancer
DRE	Digital rectal examination
PSA	Prostate-specific antigen
TRUS	Transrectal ultrasound
NLR	Neutrophil/lymphocyte ratio
NLR	Derivative [dNLR = neutrophils/(white blood cells - neutrophils)]
PLR	Platelet/lymphocyte ratio
MLR	Monocyte/lymphocyte ratio
SIRI	(neutrophil × monocyte)/lymphocyte ratio
AISI	(neutrophil × monocyte × platelet)/lymphocyte ratio
MiRNA	MicroRNAs
MiR	MicroRNAs
PC	Patients with prostate cancer
BPH	Patients with benign prostatic hyperplasia
PL	Patients with precancerous lesions
ASAP	Atypical small acinar proliferation
PIN	Prostatic Intraepithelial Neoplasia
IIEF	Erectile function
WBC	White blood count
RBC	Red blood cel
HGB	Hemoglobin
RDW	Red cell distribuition
HDW	Hemoglobin distribution width
PLT	Platelet count blood test
NEUT	Neutrophils
LYMPH	Lymphocyte
MONO	Monocytes
EOS	Eosinophil
BASO	Basophil
LUC	Large Unstained Cells

LMR	Blood lymphocyte-to-monocyte ratio
G6PDH	Glucose-6-phosphate dehydrogenase
BMI	Body mass index
IPSS	International Prostate Symptom Score

Author Contributions: Conceptualization, D.C., C.C., M.F., and M.M.; methodology, D.C., F.B.; validation, D.C., F.B. and N.C.; formal analysis D.C., N.C.; investigation, D.C.; resources, M.M.; data curation, D.C., M.F., N.C, N.R.; writing—original draft preparation, D.C. M.F., N.C.; writing—review and editing, D.C., S.M., M.F.; supervision, M.F., M.R.D.M., C.C.; project administration, C.C.; funding acquisition, C.C., M.M. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Funding: FAR UNISS 2020, Grant Recipient Full Professor Ciriaco Carru, Fondazione di Sardegna protocollo pratica U660.2022/AI645BE pratica 20220788.

Figure legends:

Figure 1. Jitter boxplots showing the distribution of miRNA145 (A), miRNA148 (B), and miRNA185 (C) among study subjects presenting with prostate cancer (PC, N=92), precancerous lesions (PL, N=26), or benign prostatic hyperplasia (BPH, N=49). The centre line of the boxplots indicates the median, limits of the box indicate the 25th and 75th percentile. The whiskers represent either 1.5 times the interquartile range (IQR) or the maximum/minimum data point if they are within 1.5 times the IQR. Wilcoxon's test was used to compare mean miRNA levels between study groups, and p-values are shown.

Figure 2. Combined miRNA and clinical-records data set. A) OPLS-DA (orthogonal partial least squares discriminant analysis) score scatter plot discriminating three classes of patients: Green for BPH patients, Blue for PL, and Red for PC patients. B) VIP (variable importance in the projection). The Y axis indicates the VIP scores corresponding to each variable on the X- axis.

Figure 3. Statistics of overall performance of the model: A) Misclassication table shows the proportion of correct classification observation, B) Permutation plots for the OPLS-DA model to validate the occurrence or absence of over-fitting.

Supplementary Figure 1. PC versus PL+BPH: the corresponding receiver operating characteristics curves and the value of the area under the curve (AUC) are shown for each individual miRNA, and for the three miRNAs combined.

Supplementary Figure 2. PL versus BPH: the corresponding receiver operating characteristics curves and the value of the area under the curve (AUC) are shown for each individual miRNA, and for the three miRNAs combined.

Tables:

Accession ID number	Symbol	Sequence
MIMAT0000243	hsa-miR-148a-3p	UCAGUGCACUACAGAACUUUGU
MIMAT0004611	hsa-miR-185-3p	AGGGGCUGGCUUUCCUCUGGUC
MIMAT0000437	hsa-miR-145-5p	GUCCAGUUUUCCCAGGAAUCCCU

Table 1. miRNA collection: accession identification number, symbol, and sequence used in this study for each analyzed miRNA.

	PC	**PL**	**BPH**	**P**
N	92	26	49	*n.s.*
AGE	68.95±10.68	67.65±7.87	65.39±8.17	**4.15x10-3
PSA	21.28±45.09	6.38±4.57	6.87±6.8	*1.53x10-3 **1.67x10-4
INDEX	12.73±5.59	20±11.11	19.48±10.18	*8.72x10-3 **1.40x10-3
WBC	7.73±2.14	6.46±1.45	7.33±2.26	*7.18x10-3
RBC	5.07±0.59	5.18±0.93	5.25±0.51	*n.s.*
HGB	14.23±1.66	14.67±2.16	14.75±1.26	*n.s.*
RDW	13.99±1.51	13.51±0.95	13.6±0.99	*n.s.*
HDW	2.64±0.41	2.55±0.35	2.52±0.3	*n.s.*
PLT	235.5±66.15	217.35±45.01	235.6±55.8	*n.s.*
NEUT	4.88±1.89	3.96±1.33	4.45±1.91	*1.99x10-2
LYMPH	1.97±0.79	1.77±0.5	2.04±0.79	*n.s.*
MONO	0.5±0.17	0.43±0.13	0.47±0.15	*n.s.*
EOS	0.2±0.14	0.17±0.1	0.23±0.15	*n.s.*
BASO	0.04±0.05	0.02±0.04	0.04±0.05	*n.s.*
LUC [#]	0.14±0.07	0.12±0.04	0.14±0.06	*n.s.*
LUC [%]	1.96±0.73	2.03±0.57	2.13±0.74	*n.s.*
LMR	4.16±1.5	4.49±1.8	4.47±1.4	*n.s.*
NLR	2.92±1.85	2.51±1.67	2.47±1.24	*n.s.*
PLR	137.69±63.58	131.05±42.02	132.45±58.93	*n.s.*
SIRI	1.5±1.28	1.15±0.99	1.19±0.94	*n.s.*

PSA/AISI [%]	0.1±0.26	0.04±0.03	0.04±0.04	**1.41x10-2
INDEX/SIRI	11.75±11.98	24.1±29.58	12.49±15.34	*n.s.*
INDEX/AISI [%]	0.06±0.07	0.12±0.16	0.06±0.07	***4.57x10-2
FAMILIARITY	7/92(7.61%)	6/26 (23.08%)	3/49 (6.12%)	*n.s.*
CHARLSON	5.22±1.62	2.74±1.19	2.75±1.37	*8.09x10-8 **5.36x10-11
G6PDH DEFICENCY	7/92 (7.61%)	3/26 (11.54%)	5/49 (10.2%)	*n.s.*
BMI	27.4±3.67	26.87±2.82	26.8±4.01	n.s.
IPSS	12.05±7.44	11.64±8.97	9.52±7.53	n.s.
IIEF	13.06±7.4	17.29±6.17	15.73±8.43	*1.37x10^{-2}
TRUSS	51.26±24.98	60.31±33.05	65.45±35.46	n.s.
SMOKING	32/92 (34.78%)	4/26 (15.38%)	13/49 (26.53%)	n.s.
ALCOHOL	1/92 (1.09%)	0/26 (0%)	2/49 (4.08%)	n.s.

Table 2. For each study group (PC: prostate cancer, PL: precancerous lesions, BPH: benign prostatic hyperplasia) sample characteristics are shown. Categorical values are reported as number and percentage, and compared using Pearson's χ2 test among study groups, while continuous values are reported as mean ± standard deviation and compared using Wilcoxon's. P-values >0.05 were considered as non-significant (n.s.). * PL vs PC, ** PC vs BPH, *** PL vs BPH