American Journal of Men's Health
September-October 1–10

DOI: 10.1177/15579883221120989
journals.sagepub.com/home/jmh
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Expression Analysis of miRNAs and Their Potential Role as Biomarkers for Prostate Cancer Detection

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Abstract

Prostate cancer (PCa) is the second most frequent cancer diagnosed in men worldwide. The detection methods for PCa are either unreliable, like prostate-specific antigen (PSA), or extremely invasive, such as in the case of biopsies. Therefore, there is an urgent necessity for reliable and less invasive detection procedures that can differentiate between patients with benign diseases and those with cancer. In this matter, microRNAs (miRNAs) are suggested as potential biomarkers for cancer. MiRNAs have been found to be dysregulated in several different cancers, and these genetic alterations may present specific signatures for a given malignancy. Here, we examined the expression of miR141-3p, miR145-5p, miR146a-5p, and miR148b-3p in human tissue samples of PCa ($n = 41$) and benign prostatic diseases (BPD) ($n = 30$) using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). We combined the expression results with patient clinicopathological characteristics in logistic regression models to create accurate PCa predictive models. A model including information of miR148b-3p and patient age showed relevant prediction results (area under the curve [AUC] = 0.818, precision = 0.763, specificity = 0.762, and accuracy = 0.762). A model including all four miRNAs and patient age presented outstanding prediction results (AUC = 0.918, precision = 0.861, specificity = 0.861, and accuracy = 0.857). Our results represent a potential novel procedure based on logistic regression models that utilize miRNA expressions and patient age to assist with PCa diagnosis.

Keywords

expression, miRNAs, biomarkers, predicted model, prostate cancer

Received May 12, 2022; revised August 1, 2022; accepted August 4, 2022

Introduction

Prostate cancer (PCa) is the second most diagnosed cancer in men, only behind lung cancer (Cancer Today, n.d.). Therefore, early detection of PCa is one of the main challenges for this pathology. Prostate-specific antigen (PSA) is a well-accepted biomarker for PCa follow-up, as its expression is highly sensitive to clinical conditions. PSA expression is modified in the presence of several prostate diseases and is not distinguishable between patients with and without a malignant disease, which in turn leads to a high percentage of unnecessary biopsies. Subjecting patients to an invasive procedure may cause bleeding, infections, and, in some extreme case, even death and psychological manifestations such as anxiety and depression (Byun et al., 2022; Cary & Cooperberg, 2013; Hendriks et al., 2017; Minervini et al., 2014; Wu et al., 2021). A robust method for early diagnosis is urgently needed. In this regard, microRNA (miRNA) expression profiling is a promising tool for the detection of various types of cancer due to the ease and reliability of extraction from biological samples, their stability, and measurement accuracy by standard techniques, and this is especially true for PCa, where the necessity for minimally invasive, sensitive, and specific biomarkers is both

fundamental and pressing (Ambrozkiewicz et al., 2020; Chang et al., 2021; Ignatiadis et al., 2021; Moya et al., 2019; Suer et al., 2019). MiRNAs are small noncoding RNA molecules that regulate the expression of various genes post-transcriptionally by binding to the 3′-untranslated region (UTR) region of target messenger RNAs (mRNAs) (Bartel, 2018).

To identify miRNAs as potential biomarkers, first, it is necessary to identify their specific expression profile in the tissue of patients with and without PCa, thus attributing this deregulation to the carcinogenic events that occur in the prostate and evaluating whether these miRNAs conserved the differential expression in some body fluid and whether this is related to their expression in the specific tissue. In addition, numerous studies have reported the utility of miRNAs for disease detection and prognosis (Byun et al., 2022; Giglio et al., 2021; Kim et al., 2021; Lyu et al., 2019; Porzycki et al., 2018; Suer et al., 2019). miRNAs miR141-3p (Osipov et al., 2016; Xiao et al., 2012), miR145-5p (Wang et al., 2015; Xu et al., 2019), miR146a-5p (Fredsøe et al., 2020; Huang et al., 2017), and miR148b-3p (Feng et al., 2019; Tomeva et al., 2022; Walter et al., 2013) have been suggested as predictive molecules for cancer, and especially in PCa for their role in carcinogenesis, tumor progression, and metastasis. This study analyzes the relative expression of miR141-3p, miR145-5p, miR146a-5p, and miR148b-3p, in tissue samples from patients diagnosed with and without PCa, to assess their potential as biomarkers to distinguish between PCa and benign prostate diseases.

Method

Patient Samples

Prostatic tissue samples were collected from patients with a first presumptive diagnosis of PCa through ultrasound-guided transrectal biopsy and stored in Qiazol (Qiagen) at −20°C for later analysis. Our study consisted of 71 samples: 41 samples from patients with PCa diagnosis (PCa group) and 30 samples from patients diagnosed with benign prostatic disease (BPD group). The BPD group included patients with benign prostatic hyperplasia ($n = 7$), prostatitis ($n = 18$), both hyperplasia and prostatitis ($n = 4$), and intraepithelial neoplasia ($n = 1$). All patients met the inclusion criteria: Mexican, ≥18 years, histopathological diagnosis of PCa or BPD, have not received chemotherapy or radiotherapy, and do not present any other type of cancer. All patients were recruited from the Alvarez & Arrazola Radiologists Clinic in Sinaloa, Mexico, from August 2016 to December 2021. Clinicopathological characteristics such as age, weight, height, body mass index (BMI), PSA, and family history of cancer were collected through direct questionnaires and the clinical database. An expert pathologist provided the diagnosis and Gleason score. All patients were approved by signing an informed consent reviewed and approved by the Ethics and Research Committee of Alvarez & Arrazola Radiologists Clinic (study approval number P-3103).

RNA Extraction and Reverse Transcription (RT) PCR

Tissue samples were used for RNA extraction. Total RNA, including miRNAs, was isolated using the miRNEasy kit (Qiagen) according to the manufacturer's protocol. The isolated RNA concentration was measured with a GENESYS 10S UV-Vis Spectrophotometer (Thermo Scientific™). It also allows for evaluating the integrity of the RNA, obtaining information on contaminants, analysis of low or atypical concentrations, and warning of invalid results. The analysis of concentration and integrity was performed in triplicate. RT was performed from 10 ng of total RNA with the TaqMan Advanced miRNA cDNA Synthesis kit (Applied Biosystems) in a T100 Thermal Cycler (Bio-Rad).

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Relative miRNA Expression

The real-time polymerase chain reaction (PCR) technique was carried out using the StepOnePlus™ thermal cycler (Applied Biosystems). For each of the miRNAs, standard curves of five points of serial dilutions 1:10 were made to obtain the efficiencies from the slope. Quantitative estimations for miR141-3p, miR145-5p, miR146a-5p, and miR148b-3p were performed using TaqMan MicroRNA Assays (Applied Biosystems). To select the normalizing miRNA, we used the RefFinder tool, which integrates different algorithms used for the selection of normalizers, identifying miR191-5p as the most appropriate miRNA for our study (Andersen et al., 2004; Pfaffl et al., 2004; Silver et al., 2006; Vandesompele et al., 2002). To obtain relative miRNA expression, we used the method proposed by Taylor et al. (2019) based on the ΔΔCt method ($2^{-\Delta\Delta Ct}$ algorithm) (Livak & Schmittgen, 2001). Finally, quantitative analyses using relative expression were performed using log-transformed relative normalized expression.

Statistical Analysis

Student's *t* test, Mann–Whitney *U* test, analysis of variance (ANOVA), and Kruskal–Wallis test (when appropriate) were used to compare differences between continuous variables. The chi-square test allowed the identification of relationships between categorical variables. The Pearson and Spearman tests (when appropriate) gave us the correlation coefficient between the following variables: miRNA expression, serum PSA, age, weight, and BMI. All the variables were contrasted among each other to observe relationships. Each miRNA's potential to aid in distinguishing between PCa and BPD groups was investigated using a receiver operating characteristic (ROC) curve analysis and Fagan's nomogram. The Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL) version 20 software was used for all statistical calculations. Predictive models integrating different variables were carried out through logistic regressions using the orange program, version 3.27.1. Results with a $p < .05$ were considered statistically significant.

Results

Clinicopathological Characteristics

The average age of PCa patients was 66.55 ± 12.13 years and of the BPD group was 45 ± 6.94 years ($p = .008$). BMI evaluation yielded an average of 24.94 ± 3.01 kg/m^2 for PCa patients and an average of 26.56 ± 2.65 kg/m^2 for BPD patients ($p = .399$). Concerning the family history of PCa, we found that 14.63% ($n = 6$) of our PCa patients had at least one family member affected by this disease. Regarding the BPD group, 23.33% ($n = 7$) had relatives with PCa. However, the analysis showed no relation between PCa family history and the presence of PCa ($p = .349$). PSA analysis showed that the PCa group had an average of 102.92 ± 283.56 µg/µL and the BPD group an average of 41.28 ± 55.73 µg/µL ($p = .945$). Classifying PCa patients using the five Gleason score groups (International Society of Urological Pathology [ISUP] grade), we observed that 4.88% ($n = 2$) were classified as 1 (Gleason score ≤6), 31.71% ($n = 13$) as 2 (Gleason score = 3+4), 31.71% ($n = 13$) as 3 (Gleason score = 4 + 3), 9.76% ($n = 4$) as 4 (Gleason score = 8), and 21.95% ($n = 9$) as 5 (Gleason score >8).

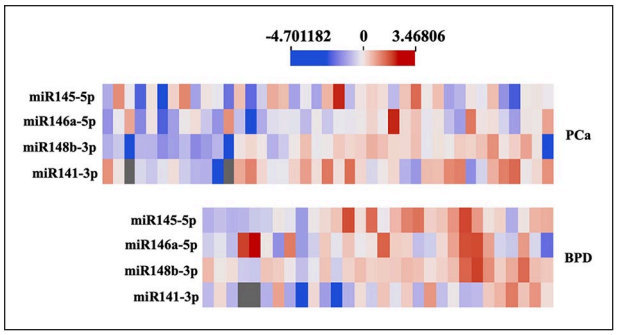

Figure 1. Heatmap Showing Differential miRNA Expression in PCa Samples Compared With BPD Samples.
Note. PCa = prostate cancer; BPD = benign prostatic diseases.

Relative miRNA Expression

The relative expression of miRNAs miR141-3p, miR145-5p, miR146a-5p, and miR 148b-3p was quantified in all samples, and the results are represented in Figure 1. MiR141-3p presented a 2.92 ± 0.48-fold expression in PCa patients compared with BPD patients; however, no statistical significance was observed ($p = .074$). When analyzing miR145-5p expression, an expression factor of 0.71 ± 0.15 was identified in PCa patients compared with BPD patients, showing a significant difference ($p = .033$). In the case of miR146-5p, the PCa patients had underexpression with an expression factor of 0.61 ± 0.19; however, no significant difference was identified between groups ($p = .051$). Finally, miR148b-3p was underexpressed in PCa patients with an expression factor of 0.44 ± 0.28 and a statistically significant difference between groups ($p = .001$) (Figure 2).

Correlation Analysis

Correlation analyses were performed to establish the relationship between relative miRNA expressions in PCa samples. The analyses did not show a relation between most relative miRNA expressions, as depicted in Figure 3.

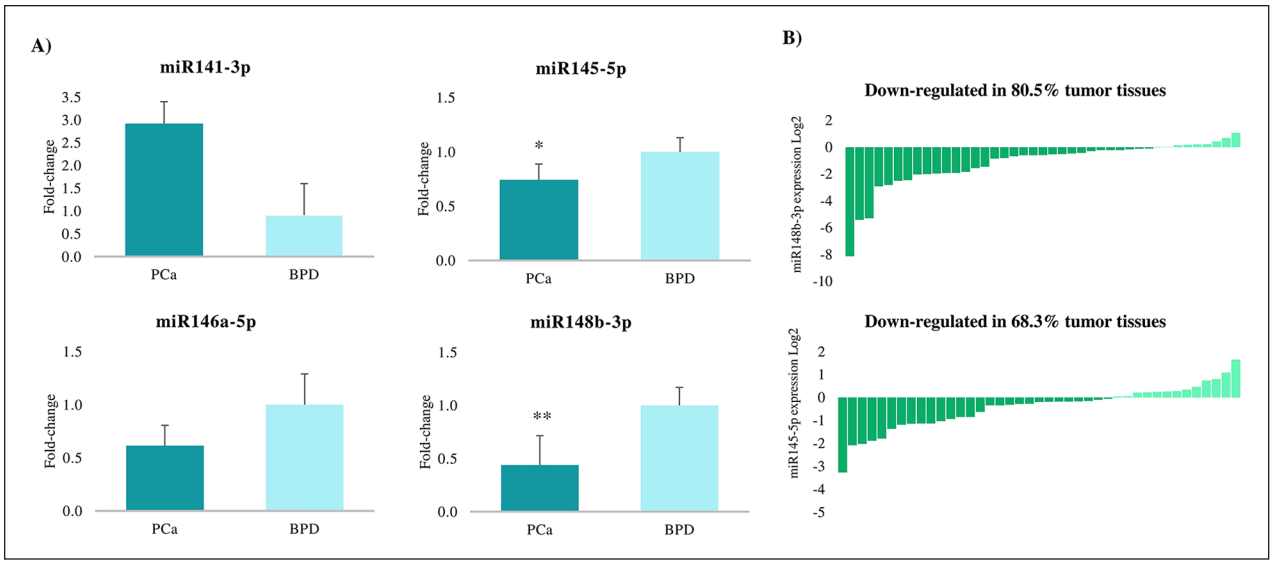

Figure 2. Relative miRNA Expression. (A) Comparison of Relative Expression Between PCa and BPD Groups. (B) Percentage of Samples With Underexpression in miR-145-5p and miR-148b-3p.
Note. PCa = prostate cancer; BPD = benign prostatic diseases; miRNA = microRNA.
*$p < .05$. **$p < .01$.

However, a correlation was found between miR145-5p and miR146a-5p, with a coefficient of 0.401 ($p = .009$). Correlations were also performed between relative miRNA expressions and different clinical characteristics of the groups (PSA, weight, age, and BMI); however, no statistical significance was observed (Table 1).

ROC Curves of Clinicopathological Characteristics

ROC curve analyses were performed to evaluate the capability of different clinicopathological characteristics to discriminate between PCa and BPD. In that regard, PSA had an area under the curve (AUC) of 0.507, which indicates a discrimination capacity of 50.7% ($p = .945$). Using the Youden index to identify the most appropriate cut-off point, we observed that values of 6.15 g/L have the best sensitivity and specificity (91.7% and 25%, respectively) (Figure 4A). Positive predictive value (PPV) and negative predictive value (NPV) were 62.52% and 68.83%, respectively. The BMI did not show statistical relevance to distinguish the PCa group, obtaining an AUC of 0.403 (Figure 4B).

Performing an ROC curve for age, an AUC of 0.691 was calculated. Results showed that age has a discrimination of 69.1%, which is statistically significant ($p = .008$) (Figure 4C). Using the Youden index to identify the best cut-off point, it was estimated that from 71 years onward, the sensitivity is 50% and the specificity is 93.1%. Performing the Fagan nomogram with this cut-off point, a positive likelihood ratio of 7.25 was calculated, going from a prior probability (odds) of 57% (1.3) to a posterior probability (odds) of 90% (9.5). Regarding the negative likelihood ratio, we obtained a value of 0.54, going from a prior probability (odds) of 57% (1.3) to a posterior probability (odds) of 41% (0.7) (Figure 4D). PPV and NPV were 90.81% and 57.72%, respectively.

ROC Curves of MiRNAs

ROC analyses were also performed for miR141-3p, miR145-5p, miR146a-5p, and miR148b-3p. Despite promising AUC results, no statistical significances were observed in the case of miR141-3p, miR145-5p, and miR146a-5p ($p = .086$, $p = .068$, and $p = .070$, respectively). However, for miR148b-3p, an AUC of .737 and a p value of .001 were calculated, establishing a discrimination capacity of 73.7% (Figure 5). The most appropriate cut-off point for miR148b-3p was obtained using the Youden index, set at −0.1958, with a sensitivity of 70.7% and a specificity of 70%. When performing the Fagan nomogram, a positive likelihood ratio of 2.36 was observed, going from a prior probability (odds) of 58% (1.4) to a posterior probability (odds) of 76% (3.2). As for the negative likelihood ratio, its value was 0.42, going from a prior probability (odds) of 58% (1.4) to a posterior probability (odds) of 36% (0.6). PPV and NPV were 76.27% and 63.89%, respectively.

Logistic Regression Analysis

Three logistic models were created using the best AUC results to assess the feasibility of distinguishing between

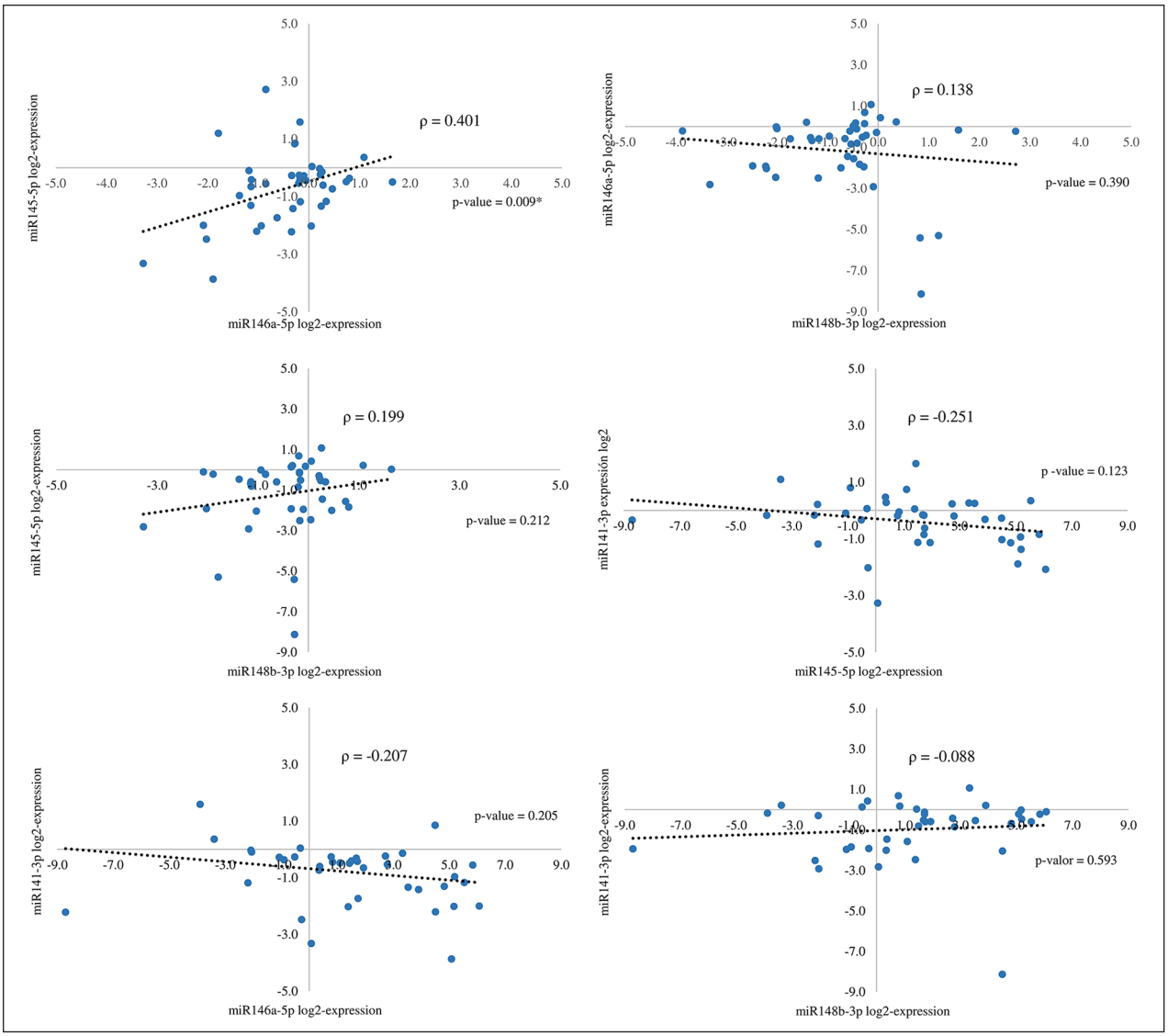

Figure 3. Correlation Analysis Between Different Relative miRNA Expressions in PCa Samples.

Table 1. Correlation Between Relative Expression of miRNAs and Clinicopathological Characteristics.

Variable	Statistics values	miR141-3p	miR145-5p	miR146a-5p	miR148b-3p
PSA	Correlation coefficient	.008	−.067	−.078	.353
	p-value	.970	.754	.718	.091
Weight	Correlation coefficient	−.131	−.069	.464	−.038
	p-value	.641	.800	.070	.889
Age	Correlation coefficient	−.069	−.120	.135	.094
	p-value	.690	.471	.419	.574
BMI	Correlation coefficient	.223	−.066	.488	−.006
	p-value	.443	.817	.065	.982

Note. PSA = prostate-specific antigen; BMI = body mass index.

PCa and BPD. Model 1 was composed of miR141-3p, miR145-5p, miR146a-5p, miR148b-3p, and age; model 2 was composed of miR141-3p, miR145-5p, miR146a-5p, miR148b-3p, age, and PSA; and model 3 consisted of

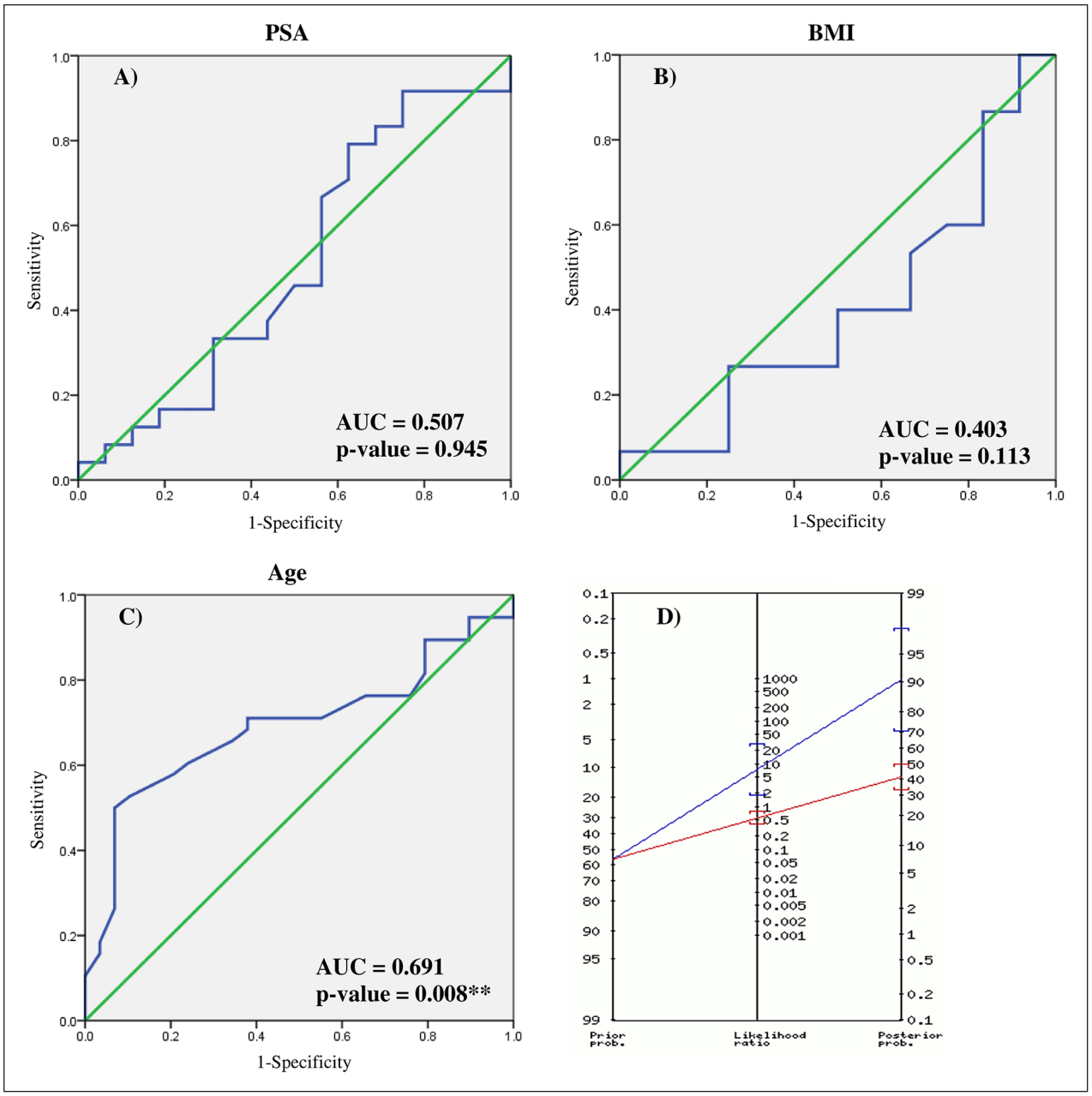

Figure 4. ROC Curve Analysis of Clinical Characteristics: (A) PSA ROC Curve, (B) BMI ROC Curve, (C) Age ROC Curve, and (D) Fagan's Nomogram of Age.
Note. ROC = receiver operating characteristic; PSA = prostate-specific antigen; BMI = body mass index.
**$p < .01$.

miR148b-3p and age. With the assistance of these three models of logistic regression discrimination, we observed that Model 1 had the best ability to distinguish between PCa and BPD, having the highest precision and specificity. In contrast, when incorporating PSA (Model 2), precision and specificity decreased. Importantly, Model 3, composed of only two variables, also yielded an acceptable discrimination capacity (Table 2).

Discussion

We identified that miRNAs miR145-5p and miR148b-3p were differentially expressed between patients with PCa and BPD, suggesting a potential capacity to discriminate between pathologies. When performing analyses to identify the predictive value of these two miRNAs, it was observed that miR148b-3p was the most relevant to distinguish between PCa and benign conditions. Several

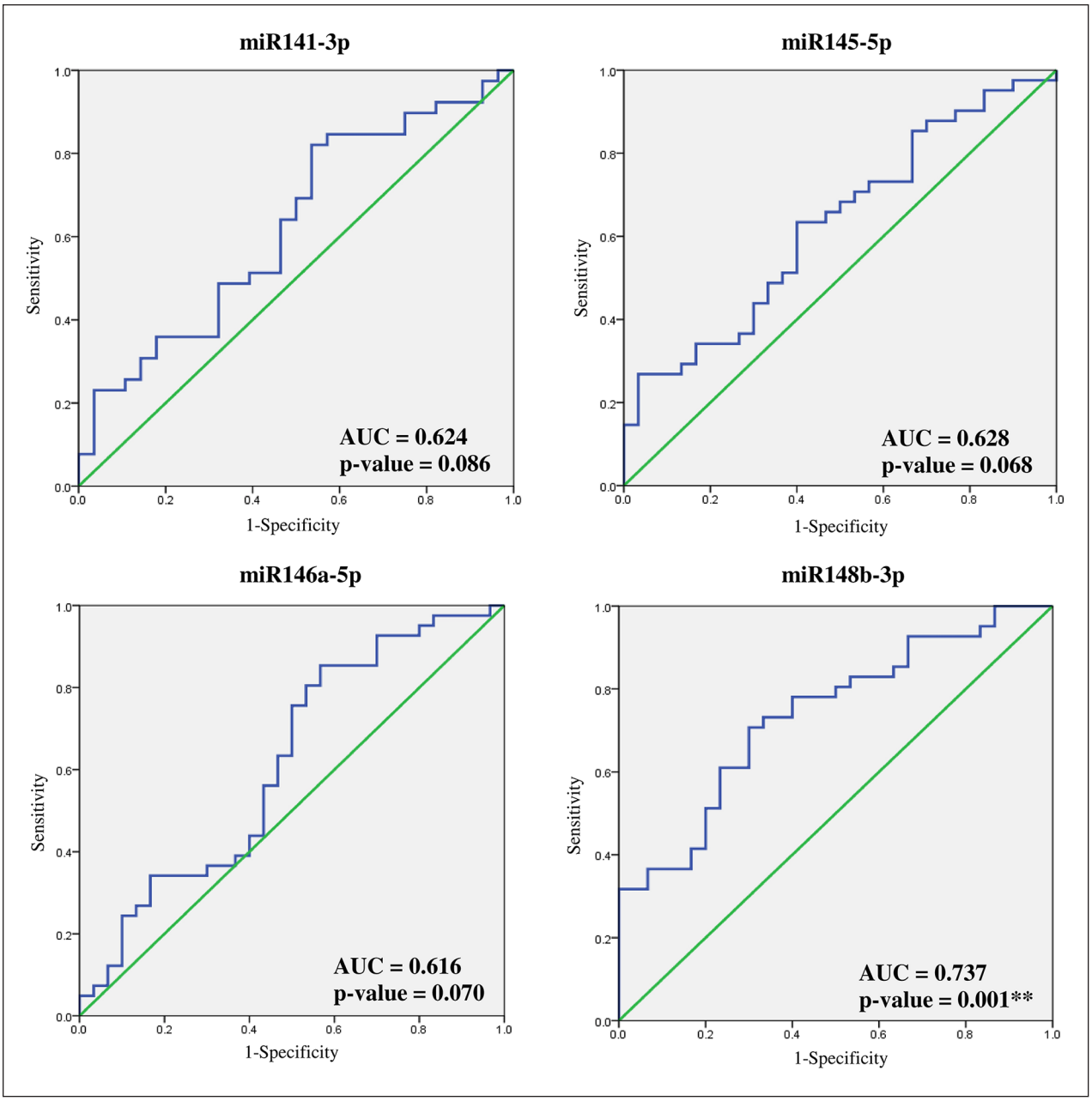

Figure 5. ROC Curve Analysis of miR141-3p, miR146a-5p and miR 148b-3p to Differentiate Between PCa and BPD.
Note. PCa = prostate cancer; BPD = benign prostatic diseases.
**$p < .01$.

Table 2. Logistic Regression Parameters of Three Models.

Model	AUC	Classification accuracy	Precision	Specificity	PPV (%)	NPV (%)
1	0.918	0.857	0.861	0.861	89.42	81.95
2	0.918	0.81	0.823	0.818	85.53	77.04
3	0.818	0.762	0.763	0.762	81.39	70.21

Note. AUC = area under the curve; PPV = positive predictive value; NPV = negative predictive value.

studies had focused on miR148b-3p expression in cancer; however, there are contrasting conclusions attributing it functions either as a tumor suppressor or as an oncogenic miRNA. Shan et al. (2021) observed that this miRNA was overexpressed in exosomes produced by cancer-associated fibroblasts. In lung cancer, miR148b-3p expression was significantly associated with tumor size and tumor grade; in addition, it was observed that patients with a high expression of miR148b-3p were in better health compared with those with a low expression. Thus, miR148b-3p was suggested as a possible prognosis biomarker (Huang et al., 2017). Notably, there are only a few studies involving miR148b-3p and PCa, all of which highlight this miRNA's potential as a biomarker. Walter et al. (2013) observed that miR148b-3p was significantly underexpressed in high-grade prostate tumors (Gleason scales of 4 + 4 and 4 + 5), suggesting its potential in prognosis for this pathology.

In the present study, we observed that miR148b-3p was significantly underexpressed in PCa patients when compared with BPD patients, which is consistent with previous studies (Feng et al., 2019; Tomeva et al., 2022; Walter et al., 2013). In the preceding works, we observed a correlation between miR148b-3p expression and genes PSA and PCA3, which are used as prognosis biomarkers for this disease (Arámbula-Meraz et al., 2020). However, no studies have analyzed its potential as a detection biomarker. Our results demonstrate that this miRNA has potential for PCa detection, yielding an AUC of 0.737 in the ROC curve, with a sensitivity of 70.7%, specificity of 70%, PPV of 76.27%, and NPV of 63.89%. MiR148b-3p is directly involved in mechanisms related to PCa development. Tomeva et al. (2022) observed that this miRNA was significantly underexpressed in tumors with mutations in the androgen receptor (AR). Moreover, Feng et al. (2019) observed that miR148b-3p was underexpressed in tumor tissue; in addition, they identified that it might bind to the 3′-UTR region of KLF4, a transcription factor that regulates gene expression. They also demonstrated that miR148b-3p inhibits the growth of PCa tumor cells in vivo. It is important to mention that in 2016, Siu et al. (2016) observed that KLF4 was overexpressed in PCa cell lines, directly promoting AR overexpression and exhibiting the close relationship between miR148b-3p deregulation and these fundamental mechanisms in the prostate. Although individually miRNAs miR141-5p, miR145-5p, and miR146a-5p were not statistically significant in the predictive analysis, they yielded better results than those obtained with PSA. This agreed with previous reports such as Giglio et al. (2021) and Yang et al. (2022), who obtained AUCs of 0.50 and 0.542 for PSA, respectively, manifesting the importance of exploring new and better biomarkers. By combining our most significant variables in a single predictive model and logistic regression, we obtained a higher AUC (0.918) with better precision and specificity; this model included all four studied miRNAs and patient age. When limited miRNA information is at hand, we proved that simpler predictive models might also be useful. In this matter, our Model 3, which only included miR148b-3p and age as variables, showed relevant specificity and accuracy values (with an AUC of 0.818). These are interesting results because obtaining such high AUC values is not common. Previous and valid works have reported their best miRNA's AUCs ranging from 0.620 to 0.720 for individual miRNAs and from 0.694 to 0.870 for models that combined multiple variables (Abramovic et al., 2021; Damodaran et al., 2021; Lyu et al., 2019; Suer et al., 2019). These results must be evaluated in other and larger populations to identify that the expression patterns are maintained. In addition, it is necessary to analyze these miRNAs in a body fluid to evaluate their potential to distinguish between benign disease and cancer.

Conclusion

Our results highlight the importance of analyzing miRNAs to distinguish between patients with and without PCa. Mainly, miRNA148 is a potential marker to identify patients with PCa. In addition, multiple markers that include the expression of miRNAs and clinical characteristics show high sensitivity and specificity to select patients with PCa.

Acknowledgments

This work was supported by "La Semilla de la Ciencia" from the Asociación de Distribuidores de Instrumentos para Uso Científico y Materiales para Laboratorio, DICLAB. We thank Yolanda Yhamylee Galvez Vazquez and Fernando Adrian Coronado Nieves for their help in collecting samples.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by Universidad Politécnica de Sinaloa and the Universidad Autónoma de Sinaloa.

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References

Abramovic, I., Vrhovec, B., Skara, L., Vrtaric, A., Nikolac Gabaj, N., Kulis, T., Stimac, G., Ljiljak, D., Ruzic, B.,

Kastelan, Z., Kruslin, B., Bulic-Jakus, F., Ulamec, M., Katusic-Bojanac, A., & Sincic, N. (2021). MiR-182-5p and miR-375-3p have higher performance than PSA in discriminating prostate cancer from benign prostate hyperplasia. *Cancers*, *13*(9), Article 2068. https://doi.org/10.3390/cancers13092068

Ambrozkiewicz, F., Karczmarski, J., Kulecka, M., Paziewska, A., Cybulska, M., Szymanski, M., Dobruch, J., Antoniewicz, A., Mikula, M., & Ostrowski, J. (2020). Challenges in cancer biomarker discovery exemplified by the identification of diagnostic MicroRNAs in prostate tissues. *BioMed Research International*, *2020*, Article e9086829. https://doi.org/10.1155/2020/9086829

Andersen, C. L., Jensen, J. L., & Ørntoft, T. F. (2004). Normalization of real-time quantitative reverse transcription-PCR data: A model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Research*, *64*(15), 5245–5250. https://doi.org/10.1158/0008-5472.CAN-04-0496

Arámbula-Meraz, E., Bergez-Hernández, F., Leal-León, E., Romo-Martínez, E., Picos-Cárdenas, V., Luque-Ortega, F., Romero-Quintana, J., Alvarez-Arrazola, M., & García-Magallanes, N. (2020). Expression of miR-148b-3p is correlated with overexpression of biomarkers in prostate cancer. *Genetics and Molecular Biology*, *43*(1), Article e20180330. https://doi.org/10.1590/1678-4685-GMB-2018-0330

Bartel, D. P. (2018). Metazoan microRNAs. *Cell*, *173*(1), 20–51. https://doi.org/10.1016/j.cell.2018.03.006

Byun, Y. J., Kang, H. W., Piao, X.-M., Zheng, C.-M., Moon, S.-K., Choi, Y. H., Kim, W. T., Lee, S.-C., Yun, S. J., & Kim, W.-J. (2022). Expression of hsv1-miR-H18 and hsv2-miR-H9 as a field defect marker for detecting prostate cancer. *Prostate International*, *10*(1), 1–6. https://doi.org/10.1016/j.prnil.2021.11.003

Cancer today. (n.d.). *larc.Fr*. Retrieved April 23, 2022, from https://gco.iarc.fr/today/home

Cary, K. C., & Cooperberg, M. R. (2013). Biomarkers in prostate cancer surveillance and screening: Past, present, and future. *Therapeutic Advances in Urology*, *5*(6), 318–329. https://doi.org/10.1177/1756287213495915

Chang, E. K., Gadzinski, A. J., & Nyame, Y. A. (2021). Blood and urine biomarkers in prostate cancer: Are we ready for reflex testing in men with an elevated prostate-specific antigen? *Asian Journal of Urology*, *8*(4), 343–353. https://doi.org/10.1016/j.ajur.2021.06.003

Damodaran, M., Dandapani, M. C., Raj, S. D., Sandhya, S., Venkat Ramanan, S., Ramachandran, I., & Venkatesan, V. (2021). Differentially expressed miR-20, miR-21, miR-100, miR-125a, and miR-146a as potential biomarkers for prostate cancer. *Molecular Biology Reports*, *48*(4), 3349–3356. https://doi.org/10.1007/s11033-021-06384-z

Feng, F., Liu, H., Chen, A., Xia, Q., Zhao, Y., Jin, X., & Huang, J. (2019). MiR-148-3p and miR-152-3p synergistically regulate prostate cancer progression via repressing KLF4. *Journal of Cellular Biochemistry*, *120*(10), 17228–17239. https://doi.org/10.1002/jcb.28984

Fredsøe, J., Rasmussen, A. K. I., Mouritzen, P., Bjerre, M. T., Østergren, P., Fode, M., Borre, M., & Sørensen, K. D. (2020). Profiling of circulating microRNAs in prostate cancer reveals diagnostic biomarker potential. *Diagnostics*, *10*(4), Article 188. https://doi.org/10.3390/diagnostics10040188

Giglio, S., De Nunzio, C., Cirombella, R., Stoppacciaro, A., Faruq, O., Volinia, S., Baldassarre, G., Tubaro, A., Ishii, H., Croce, C. M., & Vecchione, A. (2021). A preliminary study of micro-RNAs as minimally invasive biomarkers for the diagnosis of prostate cancer patients. *Journal of Experimental & Clinical Cancer Research*, *40*(1), Article 79. https://doi.org/10.1186/s13046-021-01875-0

Hendriks, R. J., van Oort, I. M., & Schalken, J. A. (2017). Blood-based and urinary prostate cancer biomarkers: A review and comparison of novel biomarkers for detection and treatment decisions. *Prostate Cancer and Prostatic Diseases*, *20*(1), 12–19. https://doi.org/10.1038/pcan.2016.59

Huang, Y., Tao, T., Liu, C., Guan, H., Zhang, G., Ling, Z., Zhang, L., Lu, K., Chen, S., Xu, B., & Chen, M. (2017). Upregulation of miR-146a by YY1 depletion correlates with delayed progression of prostate cancer. *International Journal of Oncology*, *50*(2), 421–431. https://doi.org/10.3892/ijo.2017.3840

Ignatiadis, M., Sledge, G. W., & Jeffrey, S. S. (2021). Liquid biopsy enters the clinic—Implementation issues and future challenges. *Nature Reviews Clinical Oncology*, *18*(5), 297–312. https://doi.org/10.1038/s41571-020-00457-x

Kim, J., Cho, S., Park, Y., Lee, J., & Park, J. (2021). Evaluation of micro-RNA in extracellular vesicles from the blood of patients with prostate cancer. *PLOS ONE*, *16*(12), Article e0262017. https://doi.org/10.1371/journal.pone.0262017

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Lyu, J., Zhao, L., Wang, F., Ji, J., Cao, Z., Xu, H., Shi, X., Zhu, Y., Zhang, C., Guo, F., Yang, B., & Sun, Y. (2019). Discovery and validation of serum MicroRNAs as early diagnostic biomarkers for prostate cancer in Chinese population. *BioMed Research International*, *2019*, Article 9306803. https://doi.org/10.1155/2019/9306803

Minervini, A., Vittori, G., Siena, G., & Carini, M. (2014). Morbidity and psychological impact of prostate biopsy: The future calls for a change. *Asian Journal of Andrology*, *16*(3), 415–417. https://doi.org/10.4103/1008-682X.126388

Moya, L., Meijer, J., Schubert, S., Matin, F., & Batra, J. (2019). Assessment of miR-98-5p, miR-152-3p, miR-326, and miR-4289 expression as biomarker for prostate cancer diagnosis. *International Journal of Molecular Sciences*, *20*(5), Article 1154. https://doi.org/10.3390/ijms20051154

Osipov, I. D., Zaporozhchenko, I. A., Bondar, A. A., Zaripov, M. M., Voytsitskiy, V. E., Vlassov, V. V., Laktionov, P. P., & Morozkin, E. S. (2016). Cell-Free miRNA-141 and miRNA-205 as prostate cancer biomarkers. In P. B. Gahan, M. Fleischhacker, & B. Schmidt (Eds.), *Circulating nucleic acids in serum and plasma—CNAPS IX* (pp. 9–12). Springer. https://doi.org/10.1007/978-3-319-42044-8_2

Pfaffl, M. W., Tichopad, A., Prgomet, C., & Neuvians, T. P. (2004). Determination of stable housekeeping genes, differentially regulated target genes, and sample integrity:

BestKeeper —Excel-based tool using pair-wise correlations. *Biotechnology Letters*, *26*(6), 509–515. https://doi.org/10.1023/b:bile.0000019559.84305.47

Porzycki, P., Ciszkowicz, E., Semik, M., & Tyrka, M. (2018). A combination of three miRNAs (miR-141, miR-21, and miR-375) is a potential diagnostic tool for prostate cancer recognition. *International Urology and Nephrology*, *50*(9), 1619–1626. https://doi.org/10.1007/s11255-018-1938-2

Shan, G., Zhou, X., Gu, J., Zhou, D., Cheng, W., Wu, H., Wang, Y., Tang, T., & Wang, X. (2021). Downregulated exosomal microRNA-148b-3p in cancer associated fibroblasts enhance chemosensitivity of bladder cancer cells by downregulating the Wnt/β-catenin pathway and upregulating PTEN. *Cellular Oncology (Dordrecht)*, *44*(1), 45–59. https://doi.org/10.1007/s13402-020-00500-0

Silver, N., Best, S., Jiang, J., & Thein, S. L. (2006). Selection of housekeeping genes for gene expression studies in human reticulocytes using real-time PCR. *BMC Molecular Biology*, *7*, Article 33. https://doi.org/10.1186/1471-2199-7-33

Siu, M.-K., Suau, F., Chen, W.-Y., Tsai, Y.-C., Tsai, H.-Y., Yeh, H.-L., & Liu, Y.-N. (2016). KLF4 functions as an activator of the androgen receptor through reciprocal feedback. *Oncogenesis*, *5*(12), Article e282. https://doi.org/10.1038/oncsis.2016.79

Suer, I., Guzel, E., Karatas, O. F., Creighton, C. J., Ittmann, M., & Ozen, M. (2019). MicroRNAs as prognostic markers in prostate cancer. *The Prostate*, *79*(3), 265–271. https://doi.org/10.1002/pros.23731

Taylor, S. C., Nadeau, K., Abbasi, M., Lachance, C., Nguyen, M., & Fenrich, J. (2019). The ultimate qPCR experiment: Producing publication quality, reproducible data the first time. *Trends in Biotechnology*, *37*(7), 761–774. https://doi.org/10.1016/j.tibtech.2018.12.002

Tomeva, E., Switzeny, O. J., Heitzinger, C., Hippe, B., & Haslberger, A. G. (2022). Comprehensive approach to distinguish patients with solid tumors from healthy controls by combining androgen receptor mutation p.H875Y with cell-free DNA methylation and circulating miRNAs. *Cancers*, *14*(2), Article 462. https://doi.org/10.3390/cancers14020462

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., & Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging multiple internal control genes. *Genome Biology*, *3*(7), Article RESEARCH0034. https://doi.org/10.1186/gb-2002-3-7-research0034

Walter, B. A., Valera, V. A., Pinto, P. A., & Merino, M. J. (2013). Comprehensive microRNA profiling of prostate cancer. *Journal of Cancer*, *4*(5), 350–357. https://doi.org/10.7150/jca.6394

Wang, C., Tao, W., Ni, S., Chen, Q., Zhao, Z., Ma, L., Fu, Y., & Jiao, Z. (2015). Tumor-suppressive microRNA-145 induces growth arrest by targeting SENP1 in human prostate cancer cells. *Cancer Science*, *106*(4), 375–382. https://doi.org/10.1111/cas.12626

Wu, Q., Li, F., Yin, X., Gao, J., & Zhang, X. (2021). Develop and validate a nomogram for predicting prostate cancer in patients with PSA ≤ 20ng/mL at initial biopsy. *Medicine*, *100*(50), Article e28196. https://doi.org/10.1097/MD.0000000000028196

Xiao, J., Gong, A.-Y., Eischeid, A. N., Chen, D., Deng, C., Young, C. Y. F., & Chen, X.-M. (2012). MiR-141 modulates androgen receptor transcriptional activity in human prostate cancer cells by targeting the small heterodimer partner protein. *The Prostate*, *72*(14), 1514–1522. https://doi.org/10.1002/pros.22501

Xu, W.-X., Liu, Z., Deng, F., Wang, D.-D., Li, X.-W., Tian, T., Zhang, J., & Tang, J.-H. (2019). MiR-145: A potential biomarker of cancer migration and invasion. *American Journal of Translational Research*, *11*(11), 6739–6753.

Yang, Z., Bai, H., Hu, L., Kong, D., Li, G., Zhao, C., Feng, L., Cheng, S., Shou, J., Zhang, W., & Zhang, K. (2022). Improving the diagnosis of prostate cancer by telomerase-positive circulating tumor cells: A prospective pilot study. *eClinicalMedicine*, *43*, Article 101161. https://doi.org/10.1016/j.eclinm.2021.101161