Urologic Oncology: Seminars and Original Investigations 37 (2019) 814.e1−814.e7

UROLOGIC
ONCOLOGY

Laboratory-Prostate cancer

Circulating microRNAs in plasma before and after radical prostatectomy

Alicia C. McDonald, Ph.D., M.P.H.[1],*, Jay D. Raman, M.D.[2], Jing Shen, Ph.D.[3], Jason Liao, Ph.D.[1], Bhavyata Pandya, M.Sc.[1], Manish A. Vira, M.D.[4]

[1] *Department of Public Health Sciences, Pennsylvania State University College of Medicine, Hershey, PA*
[2] *Division of Urology, Penn State Health Milton S. Hershey Medical Center, Hershey, PA*
[3] *Gertrude H. Sergievsky Center, Columbia University Medical Center, New York, NY*
[4] *Smith Institute for Urology, Zucker School of Medicine at Hofstra/Northwell, Northwell Cancer Institute, New Hyde Park, NY*

Received 15 April 2019; received in revised form 11 June 2019; accepted 1 July 2019

Abstract

Purpose: MicroRNAs (miRNAs/miRs) as circulating biomarkers for prostate cancer have yet to be determined. We examined whether circulating miRNAs in plasma could be employed as biomarkers of disease among men treated for prostate cancer by radical prostatectomy (RP).

Methods: The expression of 17 preselected circulating miRNAs associated with prostate cancer (miR-381, -34a, -365, -122, -375, -1255b, -34b, -450b-5p, -885-5p, -1260, -150, -378, -671-3p, -148a, and -224) or high-grade prostate cancer (miR-28 and -100) in plasma at prostate biopsy was examined in pre- and post-RP plasma of prostate cancer patients using real-time PCR and compared using Wilcoxon signed-ranked test. Wilcoxon rank sum test was used to compare the expression of miRNAs in pre-RP plasma between pathologic tumor stage (T2 vs. T3) and Gleason score (6−7 [3 + 4] vs. ≥ 7 [4 + 3]) groups. Partial correlation coefficient between the expression of miRNAs in pre-RP plasma and serum prostate-specific antigen (PSA) level at RP, adjusting for age, was calculated.

Results: Twenty-nine men, aged 43 to 77 years, were included. Median follow-up time after RP was 55 days. There was no significant change in the expression of miRNAs in plasma from before to after RP. However, higher expression of miR-34a, -378, and 450b-5p in pre-RP plasma was observed among T3 compared to T2 patients (P values = 0.01). Overall, there were no statistically significant relationships observed between the expression of these circulating miRNAs and Gleason score and serum PSA at RP.

Conclusions: There was no significant change in the expression of circulating miRNAs in plasma from before to approximately 2 months after RP. This finding may be due to the lack of immediate effect RP may have on the expression of circulating miRNAs. However, higher expression of miR-34a, -378, and -450b-5p in plasma was found among patients with more advanced disease at RP. A longer follow-up time after RP is warranted to investigate RP's possible influence on circulating miRNAs among men treated for prostate cancer and to evaluate miRNAs' diagnostic potential for prostate cancer.

Keywords: Prostate cancer; MicroRNAs; Biomarker; Radical prostatectomy

---

1. Introduction

Serum prostate-specific antigen (PSA) and digital rectal examination are diagnostic screening tests used for the early detection of prostate cancer; however, both are limited in their diagnostic ability due to poor specificity and sensitivity, respectively [1]. Small noncoding RNAs known as microRNAs (miRNAs/miRs) may be potential prostate cancer diagnostic biomarkers due to their involvement in cancer processes such as differentiation, proliferation, and apoptosis [1−3]. Studies have found miRNAs associated with prostate cancer in tissue, blood, and urine [4,5]. However, these findings have been inconsistent and not reproducible due to differences in study design methods and study populations [1,6]. As a diagnostic screening biomarker, circulating miRNAs in blood (i.e., serum and plasma) have been found to be stable [7,8]; they are less invasive to collect compared to tissue; and, they have been shown to be correlated with miRNAs identified in prostatic

---

Funding: This study was supported the National Institutes of Health [R21CA178864].

***Corresponding author.** Tel: 717-531-2973; fax: 717-531-0480.
E-mail addresses: amcdonald3@phs.psu.edu, acm42@psu.edu (A.C. McDonald).

https://doi.org/10.1016/j.urolonc.2019.07.001
1078-1439/

tumor tissue [1,6]. As a result, circulating miRNAs may be ideal biomarkers for prostate cancer screening.

Few studies have examined whether circulating miRNAs associated with prostate cancer change from before to after treatment [1,9−11]. In previous studies, we identified circulating miRNAs in plasma that were most associated with prostate cancer [12] and high-grade prostate cancer [13] at the time of prostate biopsy. To further evaluate whether these miRNAs have diagnostic potential for prostate cancer, we conducted a prospective study to investigate whether the expression of 17 circulating miRNAs most associated with prostate cancer or high-grade prostate cancer in plasma identified from our previous studies changes from before to after radical prostatectomy (RP) in a cohort of men treated for prostate cancer.

2. Materials and methods

2.1. Study population

Men with clinically localized prostate cancer, aged ≥40 years, were recruited from Northwell Health (New Hyde Park, NY) and Penn State Health Milton S. Hershey Medical Center (Hershey, PA) at the time of their preoperative RP (pre-RP) visit. Men were excluded if they had factors that may influence miRNA expression such as having a cancer diagnosis other than prostate cancer or nonmelanoma skin cancer. Men were also excluded if they did not have both pre- and postoperative RP (post-RP) blood collected at their visit. A baseline questionnaire that collected demographic information, medical history (i.e., comorbidities), family history of any cancer, and lifestyle behaviors (i.e., cigarette smoking) was administered at their pre-RP visit. Blood was collected at pre-RP visit (~21 ml) and post-RP visit (~16 ml) approximately 6 to 8 weeks but within 1 year after RP during their postoperative evaluation. Medical records were used to collect clinical information such as serum PSA results and pathologic information such as Gleason score and tumor stage.

To participate in the study, all study participants had to sign an informed consent form. This study was approved by the Institutional Review Boards of Northwell Health and The Pennsylvania State University College of Medicine.

2.2. Laboratory analysis

Blood (~16 ml) collected at pre- and post-RP visits was processed in plasma. Total serum PSA (3−5 ml) was determined using Elecsys Total PSA Immunoassay (Roche, Switzerland) at Northwell Health and 2-step Chemiluminescent Microparticle Immunoassay (Abbott Architect**,** Abbott Park, IL) at Penn State Health. Total RNA, including miRNAs, was isolated from 250 μl plasma using miRNeasy Mini Kit (Qiagen, Germantown, MD) according to the manufacturer's protocol. Each plasma sample spiked in 100 fmol synthesized cel-miR-39 (Invitrogen, Carlsbad, CA) to normalize the RNA extraction, RT and qRT-PCR processes. Based on our previous studies [12,13], the 17 circulating miRNAs in plasma most associated with prostate cancer with the lowest statistically significant P value $\leq$ 0.05 (miR- 381, -34a, -523, -365, -122, -375, -1255b, -34b, -450b-5p, -639, -885-5p, -1260, -150, -378, -671-3p, -148a, and -224) and the top 2 statistically significant miRNAs that had a higher expression (miR-708 and -1298) and a lower expression (miR-28 and -100) in high-grade compared to low-grade prostate cancer patients, after adjusting for age, were evaluated in the present study. These 21 miRNAs were identified by TaqMan Low Density Arrays (Applied Biosystem, Foster City, CA) and were quantified by TaqMan MicroRNA Assays (Thermo Fisher Scientific, Grand Island, NY) following manufacturer's protocols. In brief, 3 μl extracted RNA as input was reverse transcribed for each candidate miRNA by using TaqMan miR Reverse Transcription Kit (Thermo Fisher Scientific, Grand Island, NY). The miRNA specific stem-loop RT primer for each candidate miRNA was used in the RT reaction. Then, 2 times diluted RT product was mixed with TaqMan miRNA assay and 2× Universal PCR Master Mix (No AmpErase UNGa). Finally, the real-time PCR was carried out on 7900 HT Fast Real-Time PCR system (Applied Biosystems, Grand Island, NY) for the quantification. U6 snRNA was used as an endogenous control to normalize the relative expression level of each candidate miRNA. The laboratory technician was blinded to sample status (pre- and post-RP). All data were output with SDS Relative Quantification Software version 2.2.2 (Applied BioSystems, Foster City, CA) to determine the cycle threshold (Ct). Samples were run in triplicate to calculate the mean Ct value.

2.3. Data analysis

The summary statistics of patient characteristics (i.e., demographics, self-reported medical history, and clinical and pathologic information) were calculated. Ct values of the expression of miRNAs were log-based 2 transformed before analysis. Of the 21 miRNAs that were evaluated, the frequency of a detectable miRNA expression was calculated; and, miRNAs that were detected in <50% of the patients' plasma samples were excluded (miR-523, -708, -1298, and -639), leaving 17 miRNAs that were included in the final analysis. For the remaining 17 miRNAs, patients that had undetectable levels were assigned the lowest value for that particular miRNA. Wilcoxon signed ranked test was used to compare the expression of each miRNA between pre-RP and post-RP plasma samples. Wilcoxon rank sum test was used to compare the expression of each miRNA in pre-RP plasma samples between pathologic tumor stage groups (T2 vs. T3) and between Gleason score groups (6−7 [3 + 4] vs. ≥ 7 [4 + 3]). Partial correlation coefficient was calculated individually between the expression of miRNAs in pre-RP plasma samples and serum PSA level at RP, adjusting for age.

Data analysis was conducted in R software version 3.4.3 (R Statistical Foundation) [14] and SAS version 9.4 (Cary, NC). Statistical significance was at alpha level 0.05, 2-sided.

3. Results

Of the 39 men recruited, 29 men, aged 43 to 77 years, were included in the final analysis: 12 from Northwell Health and 17 from Penn State Health. There was no difference in patient characteristics listed in Table 1 between men from Northwell Health and Penn State Health (Data not shown). As listed in Table 1, the majority of the study population were white men (79.3%), had ≥ 12 years of education (93.1%), were cigarette smokers of greater than six months (62.1%), and had a family history of any cancer (62.1%). The median pre-RP serum PSA was 6.25 ng/mL. The median follow-up time after RP was 55 days, ranging from 41 to 237 days.

Table 1
Patient characteristics of prostate cancer patients at radical prostatectomy.

	Patients ($N = 29$)
Median age (range)	64.0 y (43.0−77.0 y)
Median BMI[a] (25−75 percentile)	26.7 kg/m^2 (23.6−31.6 kg/m^2)
Race %	
White	79.3%
Black	3.5%
Asian	10.3%
Other	6.9%
Ethnicity%	
Not Hispanic	93.1%
Hispanic	6.9%
Education%	
≤11 y	6.9%
≥12 y	93.1%
Ever smoked cigarettes%	
Yes	62.1%
No	37.9%
Family history of any cancer%	
Yes	62.1%
No	37.9%
Ever prostatitis diagnosis%	
Yes	17.2%
No	82.8%
Ever BPH diagnosis%	
Yes	17.2%
No	82.8%
Median presurgery PSA (25−75 percentile)	6.25 ng/ml (4.88−10.61 ng/ml)
Pathologic Gleason score[b]%	
6	3.4%
7 (3 + 4)	51.7%
7 (4 + 3)	31.0%
8−9	10.3%
Missing	3.4%
Pathologic tumor stage%	
T2	41.4%
T3	58.6%

BMI = body mass index; BPH = benign prostatic hyperplasia; PSA = prostate-specific antigen.

[a] Sample size is 28 prostate cancer patients.

[b] Rounding error to 100%.

There was no statistically significant difference between the expression of the 17 circulating miRNAs in pre- and post-RP plasma samples among the prostate cancer patients as shown in Fig. 1. There were statistically significant differences in the expression of circulating miR-34a, -378, and -450b-5p in plasma between men with a pathologic tumor stage of T2 and T3 at RP (*P* values = 0.01) (Fig. 2). There was no statistically significant difference in circulating miRNA expression in plasma between men with Gleason score of ≥ 7 [4 + 3] compared to 6−7 [3 + 4] at RP (Data not shown). In addition, there was no statistically significant correlation between the expression of circulating miRNAs in pre-RP plasma and serum PSA at RP, after adjusting for age, among all prostate cancer patients (Data not shown).

4. Discussion

In the present study, we found no significant change in the circulating levels in plasma of 17 preselected miRNAs most associated with prostate cancer or high-grade prostate cancer from before to approximately 2 months after RP. There were circulating miR-34a, -378, and -450b-5p in plasma that differ by pathologic tumor stage in which higher expressions were observed among T3 compared to T2 patients at RP. Because of the relationship observed between the expression of miRNAs and higher pathologic tumor stage, our data suggest that circulating miRNAs may have the potential to serve as diagnostic biomarkers of aggressive disease status in patients with prostate cancer.

Few studies have examined changes in the expression of miRNAs prior to and following RP among men treated for prostate cancer [1,9−11]. The present study's finding of no statistically significant change in the expression of miRNAs from before to 2 months after RP is consistent with a previous study that showed no difference in the expression of preselected miRNAs (miR-21 and mir-141) between pre- and post-30-day RP serum samples of prostate cancer patients, even though an initial change in expression was observed 5 days after RP [9]. However, in this previous study, it is not clear how these miRNAs were selected. An initial change in the expression of miRNAs was also observed for preselected miRNAs that were shown to be up-regulated in prostate cancer (miR-16, miR-26a, and mir-195) in serum sample of prostate cancer patients 7 days after RP [1]. Studies have suggested increased expression of miRNAs immediately after RP may be due to a postinflammatory response to the surgery [1,10]. In the present study, the expression of miRNAs was examined approximately 1.8 months after RP, the typical time for postoperative evaluation of RP. This timing may be too short to examine the true effects RP may have on the expression of circulating miRNAs. As a result, a longer follow-up time after RP to evaluate the expression of circulating miRNAs

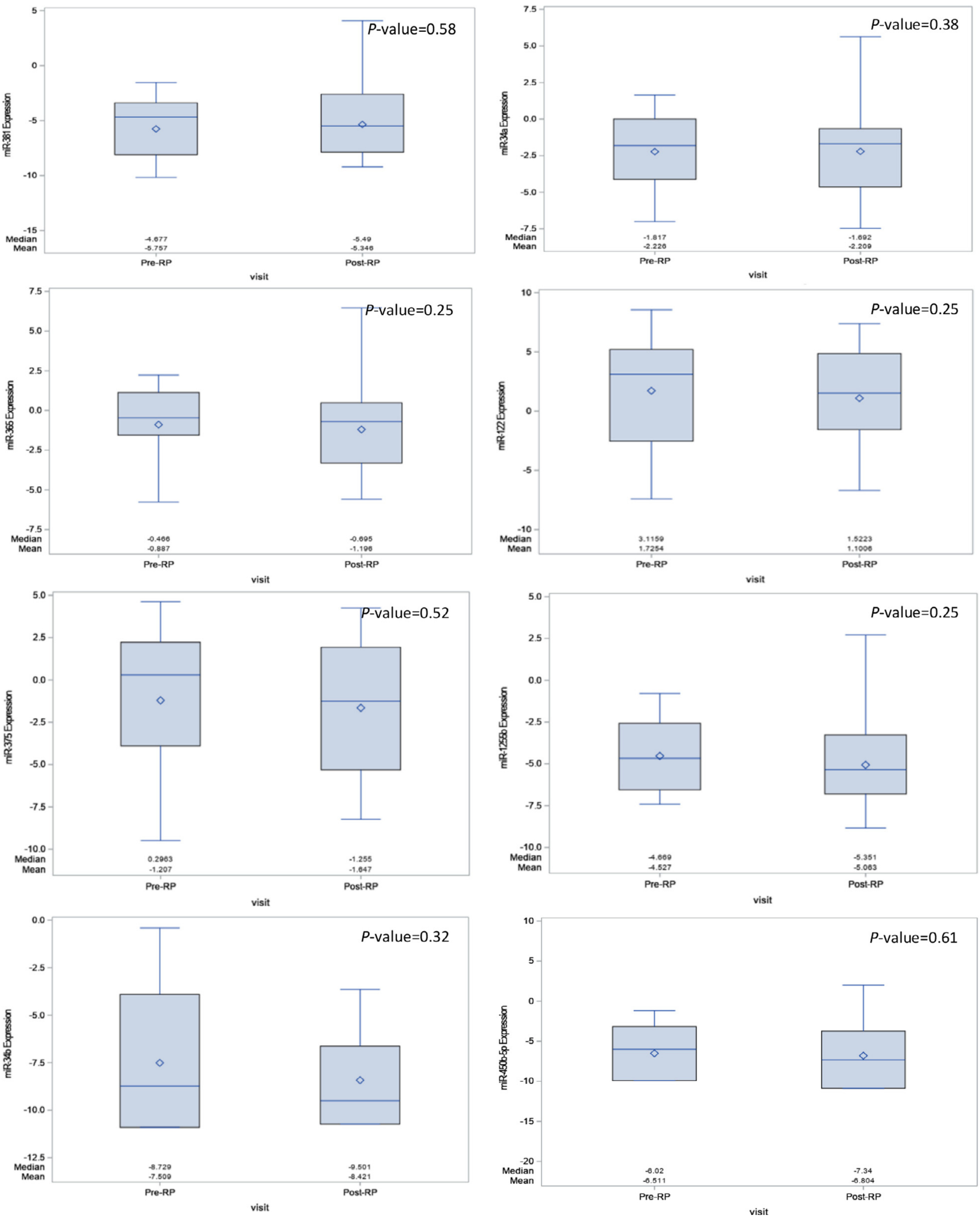
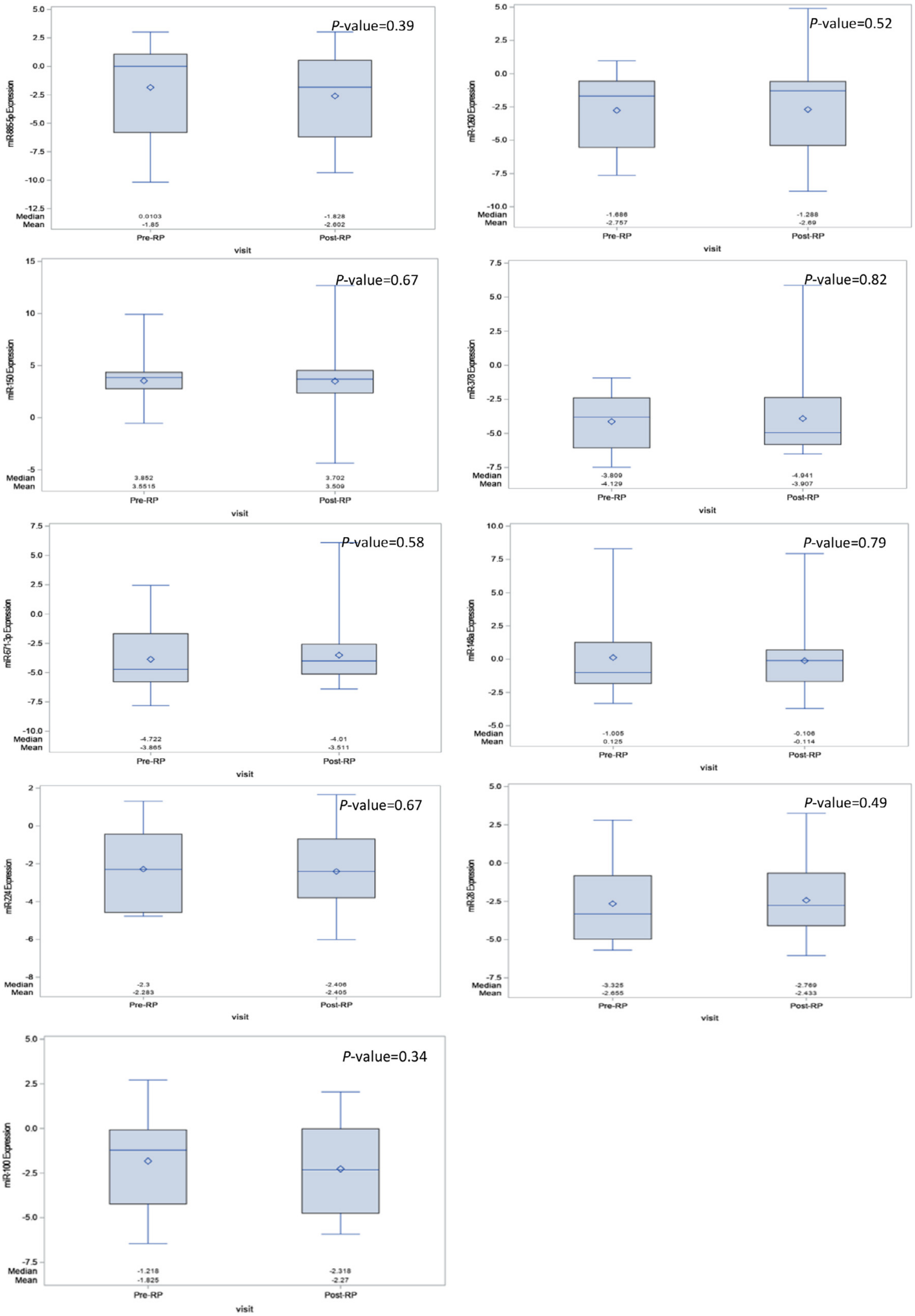
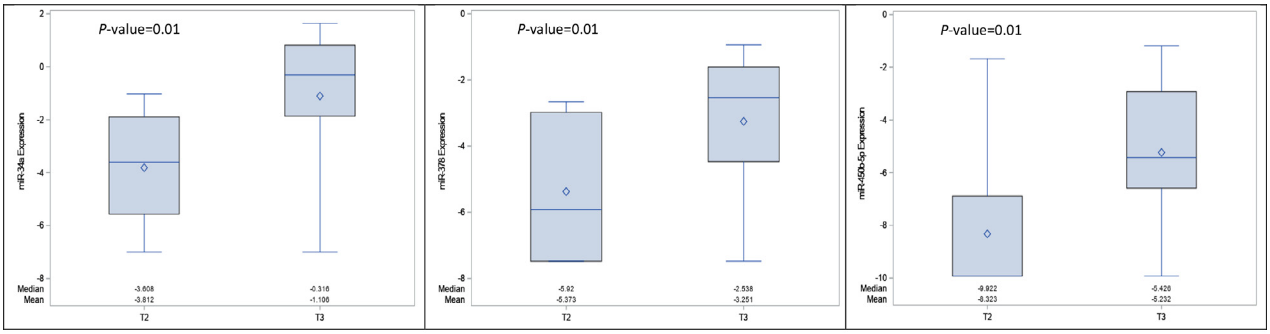


Fig. 1. Expression of circulating microRNAs in plasma before and after radical prostatectomy among prostate cancer patients. miR = microRNA; RP = radical prostatectomy;◊ = mean.



Note: miR=microRNA; RP=radical prostatectomy;◊=mean

Fig. 1 Continued.



Note: miR=microRNA; ◊=mean

Fig. 2. Differentially expressed circulating microRNAs in preradical prostatectomy plasma samples between prostate cancer patients with pathologic tumor stages T2 and T3. miR = microRNA; ◊ = mean.

may provide useful information on RP's possible impact on miRNAs. Another possible reason for no difference in the expression of circulating miRNAs between pre- and post-RP plasma in the present study could be that the circulating miRNAs that were evaluated were not tumor specific in that the miRNAs were not correlated with the levels found in the tissue. However, prior studies have shown miRNAs identified in pre-RP serum correlated with miRNAs identified prostatic tumor tissue [1]; and, miRNAs identified in serum correlated with miRNAs identified in plasma [7]. Finally, removal of the prostate by RP may have no effect on the expression of circulating miRNAs. Even though there was no statistically significant change in the expression of circulating miRNAs in plasma from before to 2 months after RP in the present study, several circulating miRNAs have been found to be associated with prostate cancer [4,5], suggesting that they could serve as potential biomarkers for prostate cancer. Further validation of their diagnostic potential for prostate cancer is warranted.

In the present study, higher expression of circulating miR-34a, -450b-5p, and -378 in pre-RP plasma were associated with more advanced disease of T3 compared to T2 at RP, an indicator of aggressive disease. Of these miRNAs, miR-34a and -378 have been linked to prostate cancer in previous studies [15−18]. For example, lower expression of miR-34a in prostate tumors has been linked to poor prostate cancer outcomes such as biochemical recurrence and metastatic prostate cancer [15,16], which is inconsistent with the present study's finding of higher expression among prostate cancer patients with more advanced disease. In addition, miR-34a has been shown to be down-regulated in primary tumors of prostate cancer cases with higher compared to lower Gleason scores [15]. In the present study, there was no statistically significant relationship found between Gleason score and the expression of circulating miRNAs in plasma, possibly due to a small sample size in each Gleason score group. As for miR-378, conflicting results in previous studies have been reported on its relationship with indicators of aggressive prostate cancer [17,18]. For example, 1 study reported lower expression of miR-378 in prostate tumor tissues correlated with higher Gleason scores, elevated serum PSA, and larger tumors as well as a shorter time to prostate cancer recurrence among cases identified at high-risk for recurrence [17], therefore suggesting lower expression of miR-378 is associated with prostate cancer progression. In the present study, there was no statistically significant correlation found between serum PSA and the expression of circulating miR-378 as well as for miR-34a and -450b-5p in pre-RP plasma samples, after adjusting age, among all prostate cancer patients and prostate cancer patients stratified by tumor stage. Another study found castrate resistant prostate cancer cases had a higher expression of circulating miR-378 in serum samples compared to localized, low-risk prostate cancer cases [18], therefore suggesting higher expression of miR-378 is associated with more advanced disease which supports the present study's finding of higher expression among men with advanced disease. Further evaluation of these miRNAs over time, in particular, along with prostate cancer progression may provide better information on their relationship with aggressive disease.

Even though the present study was unique in examining the expression of circulating miRNAs in plasma before and after RP, there were limitations. Our cohort of men came from 2 different institutions; however, there was no difference in patient characteristics between these 2 groups. The method of serum PSA testing differed between the 2 cohorts. There were 4 patients who were lost to follow-up; therefore, we did not collect post-RP blood which reduced our sample size. In addition, our sample size was small in which the study had <80% statistical power. Nevertheless, the present study was an exploratory study to examine whether the expression of miRNAs in plasma changed from before to after RP (the removal of the prostate tumor) among these prostate cancer patients in order to investigate their diagnostic potential. Another limitation was that the timing of pre-RP blood collection was not consistent among the men; however, the majority of the men's post-RP plasma (69%) was collected within 2 months after RP for their postoperative visit. In addition, there was no significant change in the expression of circulating miRNAs in plasma of men who had their blood collected from before to 2, 6, or 8 months after RP. Because the present study's follow-up time was short (less than 1 year), we did not collect prostate cancer outcome information after RP such as biochemical recurrence or metastasis which require a longer follow-up time. Finally, we did not examine miRNAs in tumor tissue to confirm their tumor specificity. In the present study, we were interested in determining which circulating miRNA, a less invasive way of profiling miRNAs, could be used as diagnostic biomarkers for prostate cancer.

5. Conclusions

In summary, there was no significant change in the circulating levels of the 17 preselected miRNAs in plasma from before to approximately 2 months after RP among men treated for prostate cancer. This no change in the expression of circulating miRNAs in plasma from before to after treatment may suggest that RP may not immediately influence their expression. Nevertheless, there were circulating miRNAs identified in the pre-RP plasma samples that were associated with a higher pathologic stage at RP, a more advanced stage of disease. Future prospective studies that examine the expression of circulating miRNAs over a longer period of time after treatment may provide a better insight on the impact treatment may have on circulating miRNAs as well as on miRNAs' diagnostic potential for prostate cancer.

Acknowledgments

Special thanks to research assistants Alexia Watson from Northwell Health and Kathleen Lehman from The Pennsylvania State University College of Medicine for data collection and administrative support for this study.

References

[1] Mahn R, Heukamp LC, Rogenhofer S, von Ruecker A, Muller SC, Ellinger J. Circulating microRNAs (miRNA) in serum of patients with prostate cancer. Urology 2011;77(5):1265.e9-16.

[2] Zhong X, Coukos G, Zhang L. miRNAs in human cancer. Methods in Mol Biol 2012;822:295–306.

[3] Leite KRM, Sousa-Canavez JM, Reis ST, Tomiyama AH, Camara-Lopes LH, Sanudo A, et al. Change in expression of miR-let7c, miR-100, and miR-218 from high grade localized prostate cancer to metastasis. Urol Oncol 2011;29(3):265–9.

[4] Luu HN, Lin HY, Sorensen KD, Ogunwobi OO, Kumar N, Chornokur G, et al. miRNAs associated with prostate cancer risk and progression. BMC Urol 2017;17(1):18.

[5] Cannistraci A, Di Pace AL, De Maria R, Bonci D. MicroRNA as new tools for prostate cancer risk assessment and therapeutic intervention: results from clinical data set and patients' samples. Biomed Res Int 2014;146170(10):16.

[6] He Y, Lin J, Kong D, Huang M, Xu C, Kim TK, et al. Current state of circulating microRNAs as cancer biomarkers. Clin Chem 2015;61(9):1138–55.

[7] Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S Am 2008;105(30):10513–8.

[8] Cortez MA, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA. MicroRNAs in body fluids−the mix of hormones and biomarkers. Nat Rev Clin Oncol 2011;8(8):467–77.

[9] Egidi MG, Cochetti G, Serva MR, Guelfi G, Zampini D, Mechelli L, et al. Circulating microRNAs and kallikreins before and after radical prostatectomy: are they really prostate cancer markers? Biomed Res Int 2013;2013:241780.

[10] Al-Qatati A, Akrong C, Stevic I, Pantel K, Awe J, Saranchuk J, et al. Plasma microRNA signature is associated with risk stratification in prostate cancer patients. Int J Cancer 2017;141(6):1231–9.

[11] Kelly BD, Miller N, Sweeney KJ, Durkan GC, Rogers E, Walsh K, et al. A circulating MicroRNA signature as a biomarker for prostate cancer in a high risk group. J Clin Med 2015;4(7):1369–79.

[12] McDonald AC, Vira M, Shen J, Sanda M, Raman JD, Liao J, et al. Circulating microRNAs in plasma as potential biomarkers for the early detection of prostate cancer. Prostate 2018;78(6):411–8.

[13] McDonald AC, Vira M, Walter V, Shen J, Raman JD, Sanda MG, et al. Circulating microRNAs in plasma among men with low-grade and high-grade prostate cancer at prostate biopsy. Prostate 2019;79(9):961–8.

[14] Team RC. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2017.

[15] Lichner Z, Ding Q, Samaan S, Saleh C, Nasser A, Al-Haddad S, et al. miRNAs dysregulated in association with Gleason grade regulate extracellular matrix, cytoskeleton and androgen receptor pathways. J Pathol 2015;237(2):226–37.

[16] Watahiki A, Wang Y, Morris J, Dennis K, O'Dwyer HM, Gleave M, et al. MicroRNAs associated with metastatic prostate cancer. PLoS One 2011;6(9):30.

[17] Avgeris M, Stravodimos K, Scorilas A. Loss of miR-378 in prostate cancer, a common regulator of KLK2 and KLK4, correlates with aggressive disease phenotype and predicts the short-term relapse of the patients. Biol Chem 2014;395(9):1095–2104.

[18] Nguyen HC, Xie W, Yang M, Hsieh CL, Drouin S, Lee GS, et al. Expression differences of circulating microRNAs in metastatic castration resistant prostate cancer and low-risk, localized prostate cancer. Prostate 2013;73(4):346–54.