Carcinogenesis, 2018, Vol. 39, No. 4, 556–561

doi:10.1093/carcin/bgy025
Advance Access publication February 17, 2018
Original Article

ORIGINAL ARTICLE

A study of circulating microRNAs identifies a new potential biomarker panel to distinguish aggressive prostate cancer

Batoul Farran[1], Gregory Dyson[1], Douglas Craig[1], Alan Dombkowski[2], Jennifer L.Beebe-Dimmer[1], Isaac J.Powell[3], Izabela Podgorski[4], Lance Heilbrun[1], Susan Bolton[1] and Cathryn H.Bock[1,*]

[1]Department of Oncology, [2]Department of Pediatrics, [3]Department of Urology and [4]Department of Pharmacology, Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, USA

*To whom correspondence should be addressed. Tel: +1 313 578 4203; Fax +1 313 578 4306; Email: bockc@wayne.edu

Abstract

Prostate cancer is one of the most common cancers in men worldwide. Currently available diagnostic and prognostic tools for this disease, such as prostate specific antigen, suffer from lack of specificity and sensitivity, resulting in over- and misdiagnosis. Hence, there is an urgent need for clinically relevant biomarkers capable of distinguishing between aggressive and nonaggressive forms of prostate cancer to aid in stratification, management and therapeutic decisions. To address this unmet need, we investigated the patterns of expression of a panel of 68 plasma-derived microRNAs (miRNAs) in a cohort of African American (AA) and European American (EA) prostate cancer patients (n = 114). miRNA qPCR results were analyzed using in-depth statistical methods, and a bioinformatics analysis was conducted to identify potential targets of the differentially expressed miRNAs. Our data demonstrate that a new previously unreported circulating miRNA signature consisting of a combination of interacting miRNAs (miR-17/miR-192) and an independent miRNA (miR-181a) are capable of segregating aggressive and nonaggressive prostate cancer in both AA and EA patients. The interacting miRNAs outperformed independent miRNAs in identifying aggressiveness. Our results suggest that these circulating miRNAs may constitute novel biomarkers of prostate cancer aggressiveness in both races and warrant further investigation.

Introduction

Prostate cancer is the second most frequently diagnosed cancer and the fifth leading cause of mortality among men worldwide (1). In 2016, an estimated 180 890 new prostate cancer cases were diagnosed in the USA alone, representing up to 10.7% of reported cancer cases, with over 3 million men living with the disease in the USA (2). The highest rates of prostate cancer incidence across five continents occur consistently among African-American (AA) populations (2). Incidence rates are also relatively high in black populations in Africa and the Caribbean, despite the lack of prostate specific antigen (PSA) screening, indicating that genetic predisposition could influence the risk of developing this disease (1,3). While the exact causes of prostate cancer remain largely unknown, established risk factors encompass advancing age, black race, family history of the disease, excess body weight and economic development (1).

MicroRNAs (miRNAs) are small noncoding RNA molecules (~22 nucleotides) that repress protein expression by cleaving mRNA or inhibiting its translation, thus modulating the expression of key oncogenes and tumor suppressor genes implicated in cell-cycle progression, cell growth and apoptosis. Recent studies have revealed the critical impact of alterations in the expression of cancer-related miRNAs on tumor progression. Pioneering research suggests that miRNA expression might be modified during the initial stages of carcinogenesis (4), rendering them early drivers of genomic instability and highlighting

Received: June 22, 2017; **Revised:** January 16, 2018; **Accepted:** February 12, 2018

Abbreviations

AA	African American
CART	classification and regression trees
EA	European American
miRNAs	microRNAs.

their potential as promising biomarkers for several solid cancers, including prostate cancer (5). Building on these findings, Calin and Croce (6) suggested that variations in the expression of miRNAs could be inherited in the germline, thus contributing to cancer predisposition by deregulating the intricate balance between tumor suppressor genes and oncogenes. Applying these ground-breaking concepts to the study of prostate cancer disparities between AA and European-American (EA) males, Calin and Croce (6) were the first to identify a set of miRNAs that are differentially expressed in AA versus EA patients (miR-301, miR-219, miR-26a, miR-1b-1 and miR-30c-1).

In 2008, Mitchell *et al.* published an innovative study demonstrating that miRNAs originating in human prostate cancer xenografts are secreted into the circulation and can be detected in patient plasma (7,8), thus providing the original proof of the presence of miRNA in the plasma of prostate cancer patients. This ground-breaking discovery has ushered in a new age of research aimed at uncovering sensitive noninvasive biomarkers of diagnosis and prognosis that could lessen the shortcomings of PSA-based screening and allow accurate prediction of disease aggressiveness, recurrence and chemoresistance. Improved diagnosis could reduce the mortality of prostate cancer by informing effective targeted therapy choices, but also curtail over-diagnosis and over-treatment, which pose other health threats. Circulating miRNAs constitute attractive diagnosis markers for various reasons. First, miRNAs appear to be frequently deregulated in cancer, where they display unique expression patterns in comparison with healthy controls and contribute to aberrant cell differentiation, proliferation and progression (7,8). They are stable, possess greater longevity than mRNA, are impermeable to degradation by nucleases due to their short sequence length and packaging in sheltering lipid microvesicles and are amenable to reliable extraction and measurement (5). Furthermore, circulating miRNAs overcome the limitations of clonal heterogeneity that reduce the diagnostic precision of tissue markers. They reflect the entire tumor mosaic by encapsulating the sum of heterotypic cellular and clonal interactions with the tumor microenvironment (9). Finally, miRNAs can be utilized as a low-cost noninvasive detection procedure and quantified by a variety of standard techniques, such as qRT-PCR, microarray or small RNA sequencing (5,7).

Recent profiling studies have endeavored to find a discriminatory circulating miRNA signature that can identify patients at risk of developing aggressive prostate disease that requires effective treatment, castration-resistant or chemotherapy refractory disease or simply to guide therapeutic strategies. These efforts have led to the detection of a few recurrent miRNA alterations that associate with prostatic carcinogenesis. In fact, three diagnostic and prognostic biomarkers emerge repeatedly in at least 17 studies (miR-141, miR-375 and miR-21), where one or more miRNAs were found to be concomitantly upregulated in the serum, plasma or urine of prostate cancer patients (7,8). Although discordant results have been published for a number of miRNAs (8), the quest for clinically relevant markers has not subsided as the need for novel diagnostic tools is still unmet. The goal of this study was to examine the association between circulating miRNA levels and prostate cancer aggressiveness to identify a panel of miRNAs that better distinguishes aggressive from nonaggressive disease. The cohort included both AA and EA prostate cancer patients to evaluate potential differences by race in miRNA associations with prostate cancer aggressiveness.

Materials and methods

Sample collection

The current study population was part of a larger prostate cancer cohort study. Cases diagnosed with prostate cancer between January 2004 and December 2016 were recruited through Karmanos Cancer Institute (KCI) clinics in metropolitan Detroit, MI. All participants self-identified as AA or non-Hispanic white were aged 75 years or younger at date of diagnosis and did not have a history of any invasive cancer prior to prostate cancer diagnosis. KCI cases were identified primarily through the hospital's Caisis database as eligible and approached for enrollment into the study at the time of their clinic appointment with consent of their treating physician. All participants provided written informed consent, and the study protocol was approved by the Wayne State University Institutional Review Board.

Participants completed a written, self-administered questionnaire at the time of their consent to participate in the study. This captured demographics, family history of prostate cancer, medical history and prostate cancer screening behaviors. At the time of study entry, each patient had their height, weight, waist and hip circumference measured according to a standardized protocol by trained study personnel. Blood was collected in CPT tubes for plasma isolation. Samples were stored at –80°C.

All medical records related to each patient's prostate cancer diagnosis, treatment and follow-up were reviewed. Age at diagnosis, prediagnostic PSA, clinical stage, biopsy Gleason grade, primary treatment, tumor pathologic stage, margin status and pathologic Gleason grade (for radical prostatectomy patients), nodal status and evidence for metastases were obtained from medical records. Aggressive disease was defined by Gleason score (4 + 3 and higher) from radical prostatectomy (if performed), otherwise from diagnostic biopsy.

RNA sample preparation

Plasma samples were thawed on ice and centrifuged at 3000*g* for 5 min in a 4°C microcentrifuge. An aliquot of 200 µl per sample was transferred to a FluidX tube. Sixty microliters of lysis solution BF, containing 1 µg carrier-RNA per 60 µl lysis solution BF, and RNA spike-in template mixture was added to the sample, mixed for 1 min and incubated for 7 min at room temperature, followed by the addition of 20 µl Protein Precipitation Solution BF. Total RNA was extracted from the samples using miRCURY RNA isolation Kit–Biofluids (Exiqon, Vedbaek, Denmark) in an automated 96-well format. The purified total RNA was eluted in a final volume of 50 µl.

miRNA real-time qPCR

RNA, 4 µl, was reverse transcribed in 20 µl reactions using the miRCURY LNA™ Universal RT miRNA PCR, Polyadenylation and cDNA synthesis kit (Exiqon). cDNA was diluted 50× and assayed in 10 µl PCR reactions according to the protocol for miRCURY LNA™ Universal RT miRNA PCR; each miRNA was assayed once by qPCR on the miRNA Ready-to-Use PCR, CANCer Focus panel using EXILENT SYBR® Green Master mix. Including 5 RNA spike-ins controls and 2 small noncoding RNAs (endogenous controls), 92 miRNAs were measured on the miRNA panel. Negative controls excluding templates from the reverse transcription reaction were performed and profiled like the samples. The amplification was performed in a LightCycler® 480 Real-Time PCR System (Roche) in 384-well plates. The amplification curves were analyzed using the Roche LC software, both for determination of Cq (by the second derivative method) and for melting curve analysis. Internal RNA isolation and cDNA synthesis controls indicated that the extraction efficiency was similar for all samples and that none harbored inhibitors. miR-103, miR-23a and miR-30c, which are known to be expressed at a steady level in many sample types, were employed as controls to assess the miRNA content of the analyzed samples. To verify that the miRNAs detected in our samples did not originate from blood contamination through hemolysis but were shed by the prostatic tumor burden itself, thus accurately mirroring neoplasm stage, two miRNAs were used: miR-451, which is highly expressed in red blood cells, and miR-23-a, which is relatively stable in serum and plasma and not

affected by hemolysis. The dCp value was calculated as the ratio of miR-451 relative miR-23a for all plasma samples.

Statistical methods

Aggressive and nonaggressive cases were evaluated for differences in age, BMI, PSA at diagnosis and race using the chi-square test. Any characteristics that were statistically significant at 0.05 level were included in further models.

The median polish method (10) was used to estimate miRNA and person-specific effects that will allow for normalization of the miRNA array data. These quantities, along with the overall median C_q value across all samples, were subtracted from the observed C_t values, resulting in a score describing the deviation of the observed C_t from expectation. To ease the interpretation (so that higher score values indicate higher miRNA expression), the scores were multiplied by −1. As a result of the median polish, the miRNA values are centered within each miRNA.

We used classification and regression trees (11) (CART) to identify miRNAs along with patient characteristics, which were associated with high or low prostate cancer aggressiveness. CART was implemented using the rpart function in the rpart package (12) in the R programming environment (13). We used the default parameters except that we forced the minimum size of a terminal node to consist of at least 10 observations. Categorized age, race and BMI were included as potential splitting factors in the CART analyses. Standard logistic regression was also used to associate each individual miRNA with the aggressiveness phenotype. Multiple approaches were used to allow for the possibility that heterogeneous mechanisms may lead to aggressive prostate cancer.

In the absence of publically available circulating miRNA datasets on prostate cancer patients, bootstrapping was used to estimate prediction accuracy. Briefly, for each bootstrap sample, a model was developed using the same variables and input parameters as when constructing the model for the original database. Predictions using the model built from the bootstrap sample were made to the observations not included in the bootstrap sample to compute model accuracy. The prediction accuracy was defined as the median accuracy across all bootstrap samples. Given the relatively small sample size, we anticipated that both approaches would have a tendency to overfit the data. We utilized bioinformatic tools (described in the next section) to corroborate any miRNA relationship that we observe.

Targets and pathway analysis

Distinct sets of miRNAs identified as being associated with disease aggressiveness were used to identify associated gene targets in the KEGG (14) prostate cancer pathway (hsa05215) using the DIANA-miRPath v3.0 (15) KEGG reverse search feature. For each set composed of an individual miRNA, a list of gene targets for that miRNA was compiled. For the two signature sets containing multiple miRNAs, we compiled a list of gene targets common to all miRNAs in the set.

Results

Participant characteristics

Plasma samples collected prior to prostate cancer treatment initiation were available for 116 cohort members. One sample was excluded due to suggested hemolysis (dCp > 8) and one sample was excluded because >25% of miRNAs were not detected. After excluding 17 miRNAs that were missing for at least 25% of the samples, 68 plasma-derived miRNAs in 114 patients, n = 93 AA and n = 21 EA, were eligible for inclusion in our analyses. On average, 66 miRNAs were detected per sample, and 44 miRNAs were detected in all 114 samples. Table 1 describes age, BMI, PSA and race characteristics in our sample. Only PSA at the time of diagnosis was significantly associated with aggressiveness.

Table 1. Summary of the patient cohort under investigation in this study

Variable	Level	Nonaggressive	Aggressive	χ^2 P-value
Age				0.685
	≤60	37(0.46)	13(0.39)	
	>60	44(0.54)	20(0.61)	
BMI				0.760
	≤25	17(0.21)	8(0.24)	
	(25, 30)	33(0.41)	11(0.33)	
	>30	31(0.38)	14(0.42)	
Race				0.449
	AA	68(0.84)	25(0.76)	
	EA	13(0.16)	8(0.24)	
PSA				<0.001
	<10	75 (0.93)	14 (0.42)	
	>10	6 (0.07)	19 (0.58)	

A circulating miR-17a/miR-192 signature is associated with disease aggressiveness

CART analysis segregated the prostate cancer cases into three distinct groups based on the combined effects of two miRNAs: miR-17 and miR-192. Age, BMI, PSA and race were not utilized by CART to discriminate aggressive and nonaggressive prostate cancer. We found that the upregulation of miR-192 exerted a moderating effect on prostate cancer aggressiveness for patients with upregulated miR-17. As shown in Figure 1 and Table 2, patients with reduced miR-17 levels (group C1) were classified as having low likelihood of being aggressive, regardless of miR-192 expression level (17.7% aggressive). Patients with upregulated miR-17 and miR-192 levels, shown in group C2, were more likely to have aggressive disease (35% aggressive). Patients who had high levels of miR-17 but reduced levels of miR-192, shown in group C3, were most likely to have aggressive disease (80% aggressive).

Individual miRNAs with marginal association with disease aggressiveness

Standard logistic regression was subsequently used to associate each miRNA with the aggressiveness phenotype in the prostate cancer samples while adjusting for race. As shown in Figure 2, we observed an association between the aberrant expression of three circulating miRNAs (miR-150a, miR-181a and miR-22) and prostate cancer aggressiveness. Plasma miR-181a level [odds ratio = 0.28 (relative to a 1-unit increase in the miR), P = 0.0004] and plasma miR-150a (odds ratio = 0.67, P = 0.032) level were negatively associated with disease aggressiveness. Plasma miR-22 (odds ratio = 1.94, P = 0.017) level was positively associated with disease aggressiveness. When adjusting for age, BMI, PSA at diagnosis and race, we observed only two significant miRNA (miR-181a & miR-93). Plasma miR-181a level (odds ratio = 0.24, P = 0.001) remained negatively associated with disease aggressiveness, while plasma miR-93 (odds ratio = 3.76, P = 0.042) level was positively associated with disease aggressiveness.

The dysregulated miRNAs affect key signaling pathways in prostate cancer development

To identify potential target genes and/or pathways of our miRNAs, we established their putative biological targets using the DianaTools-mirPath v.3 KEGG Reverse Search platform. Focusing on the prostate cancer pathway, we observed that the miR-17/miR-192 and miR-146a/miR-20a/miR-26b signatures affected

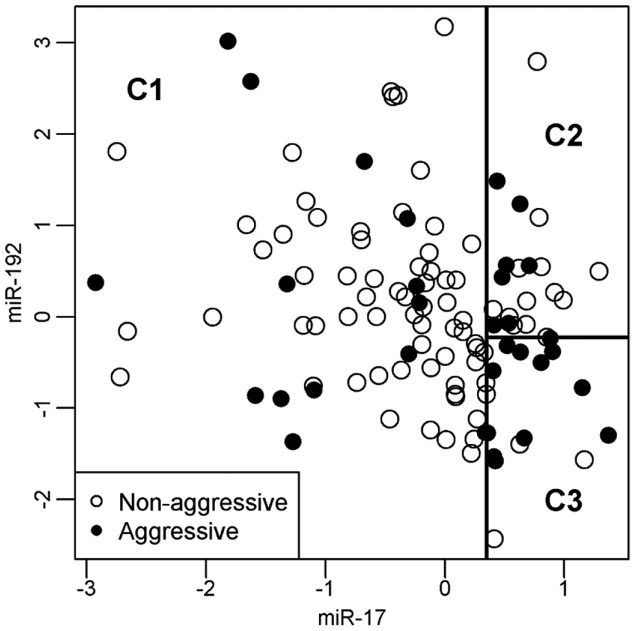

Figure 1. A miR-17/miR-192 signature is associated with prostate cancer aggressiveness. Scatter plot illustrating the graphical relationship between levels of expression of circulating miR-17 and miR-192 and prostate cancer aggressiveness in three patient subgroups constructed by the CART analysis. The error rate from bootstrapping was 36% for this model. Higher values on the x- and y-axis scores indicate higher miRNA expression.

Table 2. Percent of aggressive versus nonaggressive patients in CART subgroups

Group	Nonaggressive	Aggressive	% Aggressive
C1	65	14	17.7
C2	13	7	35.0
C3	3	12	80.0

different tumorigenesis pathways. miR-17 had 31 target genes, encompassing most of the genes in the prostate cancer pathway, while miR-192 possessed 9 targets. These two miRNAs shared three common gene targets, EP300, IGF1R and MDM2. On the other hand, miR-146a possessed 4 potential gene targets, while miR-20a and miR-26b had 23 and 21 targets, respectively. The three miRNAs shared two common gene targets, CDKN1A and MDM2, while miR-146a and miR-26b shared another target, NFKB1. We also analyzed the putative gene targets of our three independent markers and identified three interesting targets of miR-150, CDKN1B, EP300 and FGFR1. miR-181a had 25 gene targets, including the oncogenes NRAS, KRAS, HRAS, HSP90, PI3K3R and BCL2. Finally, miR-22 was predicted to interact with 18 gene targets, including EP300, CDKN1A, PTEN and TP53. The complete lists of target genes identified for each miRNA are shown in Supplementary Material, available at *Carcinogenesis* Online.

Discussion

In this study, we have shown that a previously unreported combination of interacting miRNAs (miR-17/miR-192) and three independent miRNA markers can distinguish between patients with aggressive versus nonaggressive prostate cancer in a cohort of AA and EA men. Bioinformatics analysis unmasked key target genes and cancer pathways potentially impacted by these differentially altered miRNAs. The novelty of our approach lies in the analytical and statistical tools we employed to assess the association between groups of miRNAs and disease aggressiveness, as opposed to identifying individual miRNA targets.

Based on CART analysis, the miR-17/miR-192 pair identified disease aggressiveness according to three distinct expression cut points (downregulated/upregulated; upregulated/upregulated; upregulated/downregulated), indicating that the progressive induction of miR-17a and loss of miR-192a translated into increased incidence of aggressive cancer. Although we established a diagnostic connection between the impaired expression of specific interacting miRNA signatures and prostate cancer stage in both races, we did not identify discrepancies in miRNA expression in AA versus EA men, as suggested by previous studies (9). This limitation indicates that the relatively smaller size of our cohort could have diluted the influence of miRNA variances on racial disparities in prostate cancer incidence, signifying that a larger population might be required to better assess these differences. Furthermore, our miRNA panel only measured a fraction of the total known miRNAs, implying that we might have missed an important miRNA interaction. The lack of circulating miRNA measurements in publically available prostate cancer datasets constituted another limitation. We employed bioinformatics tools to confirm the mechanisms and suggest potential targets. However, we will need to corroborate our findings in another dataset of prostate cancer. Despite these limitations, our findings indicate that the miR-17/miR-192 could provide novel clinically relevant information that might improve the diagnosis and management of prostate cancer in both AA and EA patients. These findings warrant validation in a larger cohort to ascertain their clinical utility.

The upregulation of miR-17 has been consistently reported in various hematopoietic and solid malignancies including breast, lung, pancreas, prostate, stomach cancers and B-cell lymphomas (16). This miRNA belongs to a cluster of six oncogenic miRNAs, miR-17–92 (17), known as oncomir-1 or first oncomir (18). miR-17 has been repeatedly found to be enriched in prostate cancer cells, exosomes (19,20), tissue (21) and the serum of high-risk patients (22,23), indicating that our results agree with previous findings (24). miR-17 exerts its oncogenic effect by inhibiting an intricate network of tumor suppressors, antiproliferative, antiapoptotic and cell cycle genes, including PTEN (25), the loss of which constitutes one of the hallmarks of prostate cancer (26), HIF1A (27) and CDKN1A (28).

miR-192 downregulation has been documented in several cancer types, including multiple myeloma, colorectal, ovarian and renal cell carcinomas (29) but has only been observed in prostate cancer cell lines (30). Our study is the first to report reduced miR-192 levels in the plasma of aggressive prostate cancer patients. miR-192 is directly induced by p53 (31) and functions as a tumor suppressor implicated in the regulation of the angiogenic switch (29) and the cell cycle (32). Its targets comprise genes overexpressed in aggressive prostate cancer, including the oncogene BCL2 (32) and MYLK, described as one of the most informative cancers for prostate tumorigenesis (33).

Our bioinformatics analysis unraveled two common putative target pathways of miR-17 and miR-192, comprising genes such as EP300, IGF1R and MDM2. This suggests that the dysregulation of this miRNA pair could contribute to aberrant G_1 and G_2 arrest, reduced apoptosis and genomic instability through impaired p53 signaling, as well as increased PI3K-AKT signaling. Previous work has shown that the acetyltransferase EP300 is a major promoter of prostate cancer development. It is thought to exercise its oncogenic activity by promoting epithelial–mesenchymal transition (34) and has been linked to worse disease

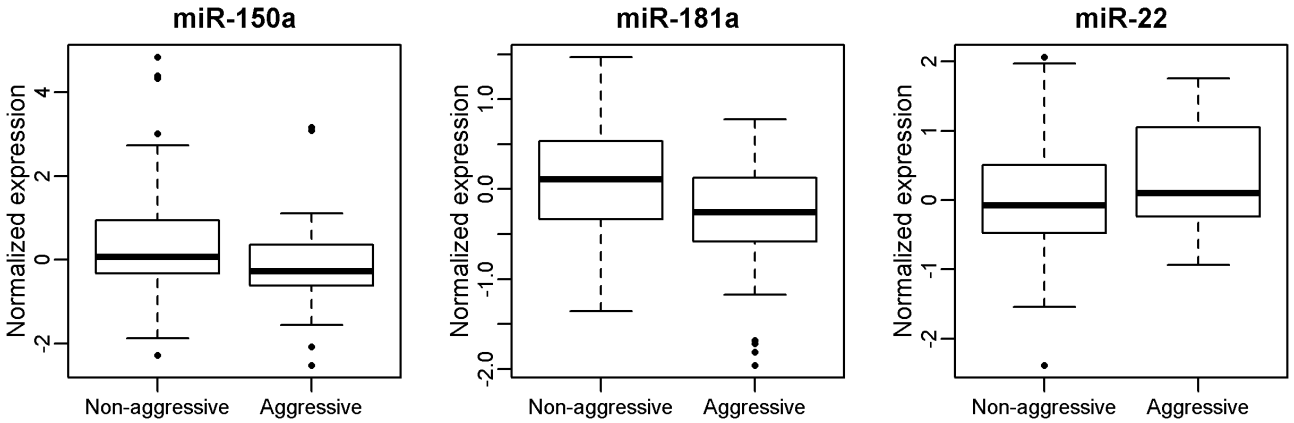

Figure 2. Circulating levels of miR-150a, miR-181a and miR22 are independently associated with prostate cancer aggressiveness by logistic regression. Here, we present normalized expression levels of these three miRNAs by prostate cancer aggressiveness.

prognosis (35). Although the miR-17–92 cluster has been shown to regulate EP300 expression in the myocardium (36), the interaction between miR-192 and EP300 has not been investigated. We hypothesize that miR-17 and miR-192 might be exerting opposing effects by inhibiting key tumor suppressors (miR-17) and upregulating oncogenes (miR-192) in this pathway, resulting in a synergistic effect that promotes tumorigenesis. The paradoxical relation between altered miR-17/miR-192 and EP300 in prostate cancer thus warrants further examination.

To complement our diagnostic model, we identified three independent markers of disease aggressiveness, miR-150a, miR-181a and miR-22. Plasma miR-150 levels were decreased in aggressive prostate cancer cases, in agreement with tissue (37) and urine-based studies (8). Pathway analysis identified three important targets of miR-150, including EP300, FGFR1 and the tumor suppressor CDKN1B (p27), found to be significantly upregulated in clinical samples of prostate cancer (38). Furthermore, reduced levels of circulating miR-181a were associated with disease aggressiveness in our cohort, in agreement with previous findings (39). Putative targets of miR-181a included NRAS, KRAS, HRAS, HSP90, PI3K3R and BCL2, highlighting its involvement in a wide web of signaling pathways, including the PI3K-AKT and MAPK cascades. The final marker, miR-22, has been described as a proto-oncogene in prostate cancer cells (40). However, our study is the first to report its upregulation in the plasma of prostate cancer patients. After adjusting for age, BMI, PSA at diagnosis and race, the statistically significant negative association between miR-181a and aggressiveness was not appreciably changed; however, miR-150a and miR-22 were no longer associated with aggressiveness, and miR-93 was marginally associated with disease aggressiveness.

In this pilot study, we have identified a novel panel of circulating miRNAs, consisting of a combination of interacting miRNAs and three independent markers. Our study has several limitations. First, our cohort of 116 patients is relatively small, which diluted discrepancies in miRNA expression in AA versus EA men. Second, our miRNA panel only measured a fraction of the total known miRNAs. Third, the miRNAs detected in this study were not validated in independent samples. Despite these limitations, our panel was able to successfully distinguish between aggressive versus nonaggressive prostate cancer. Bioinformatics analysis revealed that the dysregulated miRNAs regulate key cellular functions and could potentially drive an aggressive neoplastic phenotype. Hence, this novel miRNA signature warrants further investigation in a larger cohort to assess its potential as a diagnostic tool for prostate cancer.

Supplementary material

Supplementary data can be found at *Carcinogenesis* online.

Funding

Congressionally Directed Medical Research Programs (W81XWH-10-2-0068 to I.P. and W81XWH-13-1-0477 to C.B.); the Michigan Fund for Cancer Research (to C.B).

Acknowledgments

The authors thank Karen Slaven and Nicole Kubinec for their tireless subject recruiting efforts.
Conflict of Interest Statement: None declared.

References

1. Zhou, C.K. *et al.* (2016) Prostate cancer incidence in 43 populations worldwide: an analysis of time trends overall and by age group. Int. J. Cancer, 138, 1388–1400.
2. Howlader, N. *et al.* (1975–2014) SEER Cancer Statistics Review. National Cancer Institute, Bethesda, MD.
3. Parkin, D.M. *et al.* (2014) Cancer in Africa 2012. Cancer Epidemiol. Biomarkers Prev., 23, 953–966.
4. Calin, G.A. *et al.* (2004) Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc. Natl. Acad. Sci. USA, 101, 2999–3004.
5. Fabris, L. *et al.* (2016) The potential of microRNAs as prostate cancer biomarkers. Eur. Urol., 70, 312–322.
6. Calin, G.A. *et al.* (2006) MicroRNA signatures in human cancers. Nat. Rev. Cancer, 6, 857–866.
7. Mitchell, P.S. *et al.* (2008) Circulating microRNAs as stable blood-based markers for cancer detection. Proc. Natl. Acad. Sci. USA., 105, 10513–10518.
8. Filella, X. *et al.* (2016) miRNAs as novel biomarkers in the management of prostate cancer. Clin. Chem. Lab Med. 55, 715–736.
9. Yates, C. *et al.* (2017) miRNAs as drivers of TMPRSS2-ERG negative prostate tumors in African American men. Front. Biosci. (Landmark Ed.), 22, 212–229.
10. Tukey, J.W. (1977) Exploratory Data Analysis. Addison-Wesley, Reading, MA.
11. Breiman, L.F.J. *et al.* (1984) Classification and Regression Trees. Wadsworth International Group, Belmont, CA.
12. Therneau, T.M. *et al.* (2014) rpart: Recursive Partitioning and Regression Trees. R package. https://CRAN.R-project.org/package=rpart
13. R Development Core Team (2011) R: A Language and Environment for Statistical Computing. The R Foundation for Statistical Computing, Vienna, Austria

14. Kanehisa, M. *et al.* (2017) KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res., 45, D353–D361.
15. Vlachos, I.S. *et al.* (2015) DIANA-miRPath v3.0: deciphering microRNA function with experimental support. Nucleic Acids Res., 43, W460–W466.
16. van Haaften, G. *et al.* (2010) Tumorigenicity of the miR-17-92 cluster distilled. Genes Dev., 24, 1–4.
17. Ota, A. *et al.* (2004) Identification and characterization of a novel gene, C13orf25, as a target for 13q31-q32 amplification in malignant lymphoma. Cancer Res., 64, 3087–3095.
18. Mogilyansky, E. *et al.* (2013) The miR-17/92 cluster: a comprehensive update on its genomics, genetics, functions and increasingly important and numerous roles in health and disease. Cell Death Differ., 20, 1603–1614.
19. Dhar, S. *et al.* (2015) Resveratrol and pterostilbene epigenetically restore PTEN expression by targeting oncomiRs of the miR-17 family in prostate cancer. Oncotarget, 6, 27214–27226.
20. Ahadi, A. *et al.* (2016) Long non-coding RNAs harboring miRNA seed regions are enriched in prostate cancer exosomes. Sci. Rep., 6, 24922.
21. Volinia, S. *et al.* (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. Proc. Natl. Acad. Sci. USA, 103, 2257–2261.
22. Sapre, N. *et al.* (2014) Curated microRNAs in urine and blood fail to validate as predictive biomarkers for high-risk prostate cancer. PLoS One, 9, e91729.
23. Feng, S. *et al.* (2017) Combinations of elevated tissue miRNA-17-92 cluster expression and serum prostate-specific antigen as potential diagnostic biomarkers for prostate cancer. Oncol. Lett., 14, 6943–6949.
24. Shukla, K.K. *et al.* (2017) Recent scenario of microRNA as diagnostic and prognostic biomarkers of prostate cancer. Urol. Oncol., 35, 92–101.
25. Luan, Y. *et al.* (2017) MiR-17 targets PTEN and facilitates glial scar formation after spinal cord injuries via the PI3K/Akt/mTOR pathway. Brain Res. Bull., 128, 68–75.
26. Wise, H.M. *et al.* (2017) Prostate cancer, PI3K, PTEN and prognosis. Clin. Sci. (Lond.), 131, 197–210.
27. Taguchi, A. *et al.* (2008) Identification of hypoxia-inducible factor-1 alpha as a novel target for miR-17-92 microRNA cluster. Cancer Res., 68, 5540–5545.
28. Li, S. *et al.* (2017) miR-3619-5p inhibits prostate cancer cell growth by activating CDKN1A expression. Oncol. Rep., 37, 241–248.
29. Wu, S.Y. *et al.* (2016) A miR-192-EGR1-HOXB9 regulatory network controls the angiogenic switch in cancer. Nat. Commun., 7, 11169.
30. Sun, J. *et al.* (2016) MiR-192 suppresses the tumorigenicity of prostate cancer cells by targeting and inhibiting nin one binding protein. Int. J. Mol. Med., 37, 485–492.
31. Hünten, S. *et al.* (2015) p53-regulated networks of protein, mRNA, miRNA, and lncRNA expression revealed by integrated pulsed stable isotope labeling with amino acids in cell culture (pSILAC) and next generation sequencing (NGS) analyses. Mol. Cell. Proteomics, 14, 2609–2629.
32. Georges, S.A. *et al.* (2008) Coordinated regulation of cell cycle transcripts by p53-Inducible microRNAs, miR-192 and miR-215. Cancer Res., 68, 10105–10112.
33. Fujita, A. *et al.* (2008) Multivariate gene expression analysis reveals functional connectivity changes between normal/tumoral prostates. BMC Syst. Biol., 2, 106.
34. Isharwal, S. *et al.* (2008) p300 (histone acetyltransferase) biomarker predicts prostate cancer biochemical recurrence and correlates with changes in epithelia nuclear size and shape. Prostate, 68, 1097–1104.
35. Ghosh, A.K. *et al.* (2007) The transcriptional coactivator and acetyltransferase p300 in fibroblast biology and fibrosis. J. Cell. Physiol., 213, 663–671.
36. Shehadeh, L.A. *et al.* (2013) MicroRNA-20a constrains p300-driven myocardial angiogenic transcription by direct targeting of p300. PLoS One, 8, e79133.
37. Kumar, B. *et al.* (2016) MicroRNA expression and function in prostate cancer: a review of current knowledge and opportunities for discovery. Asian J. Androl., 18, 559–567.
38. Lynch, S.M. *et al.* (2016) miR-24 regulates CDKN1B/p27 expression in prostate cancer. Prostate, 76, 637–648.
39. Bryant, R.J. *et al.* (2012) Changes in circulating microRNA levels associated with prostate cancer. Br. J. Cancer, 106, 768–774.
40. Budd, W.T. *et al.* (2015) Dual action of miR-125b as a tumor suppressor and oncomiR-22 promotes prostate cancer tumorigenesis. PLoS One, 10, e0142373.