AWARD NUMBER: W81XWH-13-1-0477

TITLE: MicroRNA in Prostate Cancer Racial Disparities and Aggressiveness

PRINCIPAL INVESTIGATOR: Cathryn Bock

CONTRACTING ORGANIZATION: Wayne State University
Detroit, MI 48202

REPORT DATE: October 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2017	Annual	30 Sept 2016-29 Sept 2017

4. TITLE AND SUBTITLE
MicroRNA in Prostate Cancer Racial Disparities and Aggressiveness

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-13-1-0477

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Cathryn Bock

E-Mail: bockc@med.wayne.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

WAYNE STATE UNIVERSITY (INC)
CAROLE BACH
5700 CASS AVE STE 4900
DETROIT MI 48202-3692

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

During this fourth year of the project, final DNA extraction (Task 1.b) was completed. Activities were focused on miRNA extraction (Task 1.c) and quantification (Task 4), which were completed at Exiqon. We evaluated associations between plasma levels of miRNAs and prostate cancer aggressiveness and submitted a manuscript describing the findings to the peer-reviewed journal (Task 5.b). Briefly, we demonstrate that a previously unreported circulating miRNA signature consisting of two distinct combinations of miRNAs (miR-17/miR-192) and (miR-146a/miR-20a/miR26b) is capable of segregating aggressive and non-aggressive prostate cancer in both AA and EA patients. Genotyping of DNA samples for Task 3 is complete, and analyses for Task 5.a assessing associations between SNPs and PCa aggressiveness are underway. Related to Task 2, we have actively followed all of the enrolled men in the cohort who did not have extensive disease at diagnosis for PSA outcomes. Mean follow-up time is currently 60 months. Follow-up of PSA test results through medical records and Caisis database were updated in August, 2017, and a linkage with Metropolitan Detroit SEER registry (MDCSS) will be repeated prior to final analyses.

15. SUBJECT TERMS
prostate cancer, microRNA, racial disparities, African American, genetic polymorphisms, biochemical recurrence, epidemiology

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE	UU	9	USAMRMC
					19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

1. Introduction

In the US, African American (AA) men are at 60% higher risk of developing prostate cancer (PCa) than European American (EA) men, and AA men are 2.4 times more likely to die from PCa than EA men.[1] The objective of this study is to identify novel genetic and epigenetic factors that might contribute significantly to racial/ethnic disparity in PCa risk and progression. We will examine the association of inherited polymorphisms in genes in the microRNA (miRNA) biogenesis pathway as well as the association of plasma miRNA levels with prostate cancer aggressiveness and biochemical recurrence (BCR) among 480 AA and 320 EA men with PCa from the Karmanos Cancer Institute (KCI) in Detroit, MI. Little is known about the role of microRNAs (miRNAs) and their biogenesis in prostate cancer (PCa), and less is understood about the possible race-specific role of miRNAs in PCa aggressiveness and outcomes. We hypothesize that polymorphisms in genes in the miRNA biogenesis pathway and plasma miRNA levels are potential prognostic indicators for PCa aggressiveness and/or outcome and that these associations may be linked to race. The specific aims of this project are to 1) determine the associations between polymorphisms in genes within the miRNA biogenesis pathway and (a) PCa aggressiveness and (b) biochemical recurrence in AA and EA men with PCa, 2) determine the associations between plasma levels of PCa-related miRNAs and PCa aggressiveness, and 3) determine the associations between genetic polymorphisms in miRNA biogenesis pathway genes and plasma levels of miRNAs known to regulate genes in prostate cancer pathways. To increase the potential for translating our results into disease management strategies, we will include miRNAs with cell-line evidence of transcriptional regulation by miRNA promoter methylation and evidence of gene-expression regulation within prostate carcinogenic pathways. This project is built on a previously funded study of metabolic syndrome, PCa aggressiveness, and outcomes (CDMRP award W81XWH-09-1-0203, PI: Isaac Powell, MD). We built on that study's infrastructure to enroll additional patients recently diagnosed PCa, ~60% of whom are AA and ~35% of whom have aggressive disease. During Years 1-3, 688 study subjects were enrolled, 668 men completed surveys (*Tasks 1.a and 1.d: completed*), 586 participants provided blood samples for DNA extraction (*Task 1.b*), and 116 men had a plasma sample collected prior to initiation of prostate cancer treatment (*Task 1.c*). Following national trends, the number of prostate cancer patients eligible for the study declined in the clinic over the course of the study. Our enrollment rate among eligible men was ~95%, with no differences by race. Because many miRNAs are regulated by promoter methylation, they are potential targets for treatment with demethylating agents to prevent or slow PCa carcinogenesis;[2, 3] target miRNAs may vary by race. Identifying risk profiles of men who may benefit from such treatment, based on race, inherited genotypes and/or plasma miRNA levels, will provide momentum for developing the field of personalized medicine.

2. Keywords

prostate cancer, microRNA, racial disparities, African American, genetic polymorphisms, biochemical recurrence, epidemiology

3. Overall Project Summary

Current Objectives

Since final recruitment and data collection activities were completed near the end of year 3, final DNA extractions from blood samples were obtained in year 4 (*Task 1.b: completed*). Further activities during the fourth year of the project were focused on determining circulating miRNA levels in pre-treatment blood, genotyping, analyzing data and preparing manuscripts for publication. We sent the 116 samples that were obtained prior to treatment initiation to Exiqon (with an approved material transfer agreement in place) where miRNA was extracted from plasma (*Task 1.c: completed*). miRNA was quantified by Exiqon using their cancer miRNA panel (*Task 4: completed*). We performed genotyping on DNA samples from 480 men (*Task 3: completed*). The originally proposed genotyping platform (Illumina custom SNP panel) was no longer available, so we genotyped a dense panel of genome-wide SNPs using the Illumina Mega panel. This panel has the advantage of good gene coverage of all of our genes of interest in the miRNA biogenesis pathway, and also includes ancestry informative markers. The cost, however, was substantially higher than for the custom panel, so we selected all EA participants, all high risk AA men, and a random sample of low risk AA men) for inclusion in the genotyping sample.

As stated in *Task 2* of our statement of work, we have been actively following all of the enrolled men in the cohort (from the previously funded study and from the current protocol) who did not have extensive disease at diagnosis for PSA outcomes. Mean follow-up time is currently 60 months (as of August, 2017). We have abstracted the most recent follow-up of PSA test results through medical records and Caisis database, and will perform a final linkage with Metropolitan Detroit SEER registry (MDCSS) for vital status in year 5.

Results, Progress and Accomplishments with Discussion.

We evaluated associations between plasma levels of miRNAs and prostate cancer aggressiveness and submitted a manuscript describing the findings to the peer-reviewed journal *Carcinogenesis*. (*Task 5.c).* We received our initial reviews from the journal, and are revising for resubmission in November, 2017. A brief summary of our findings follows.

We investigated the patterns of expression of 68 cancer-related plasma-derived microRNAs (miRNAs) in a cohort of African American (AA) and European American (EA) prostate cancer patients (n=114, Table 1). miRNA qPCR results were evaluated for association with aggressive disease using a novel extension of CART methods. Aggressive disease was defined as Gleason sum $\geq$ 4+3, and non-aggressive disease was defined as Gleason sum $\leq$ 3+4. Our data demonstrate that a previously unreported circulating miRNA signature consisting of two distinct combinations of interacting miRNAs (miR-17/miR-192, Table 2 and Figure 1) and (miR-146a/miR-20a/miR26b, Table 3 and Figure 2) is capable of segregating aggressive and non-aggressive 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. A manuscript describing the methods and results in greater detail is under review.

Table 1.

Variable	Level	Non-aggressive	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
	African American	68(0.84)	25(0.76)	
	European American	13(0.16)	8(0.24)	

Table 2.

Group	Non-aggressive	Aggressive	% Aggressive
C1	65	14	17.7
C2	13	7	35.0
C3	3	12	80.0

Figure 1.

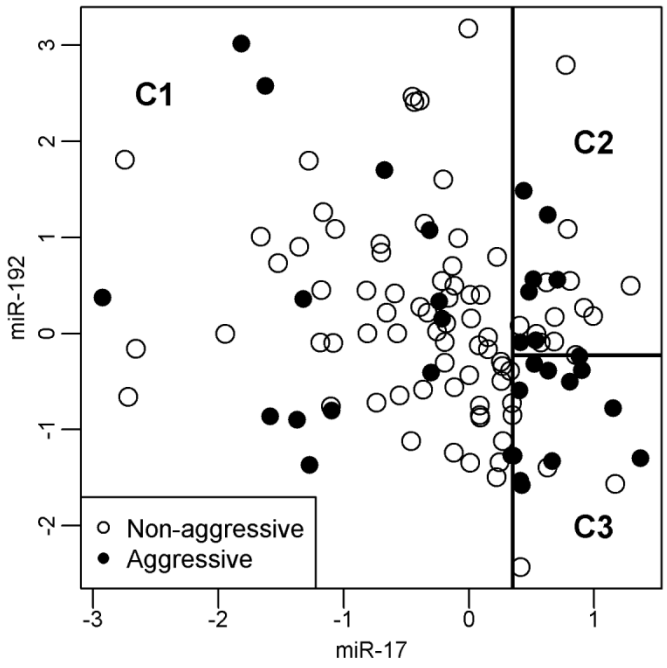
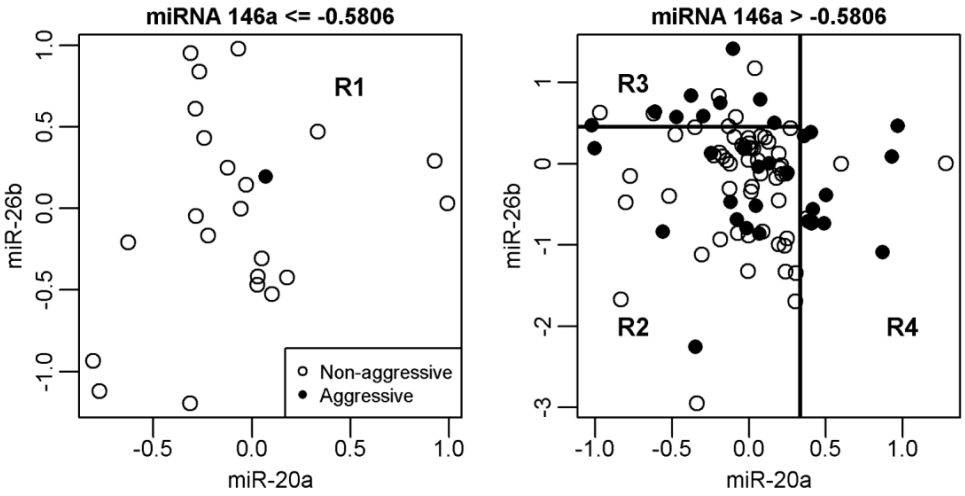


Table 3.

Group	Non-aggressive	Aggressive	% Aggressive
R1	22	1	4.3%
R2	50	13	20.6%
R3	5	9	64.3%
R4	4	10	71.4%

Figure 2.



We have begun our analyses of the SNP data in association with prostate cancer aggressiveness (*Task 5a*), and anticipate completing these and submitting a manuscript for publication soon. We will then evaluate the SNP data in association with prostate cancer recurrence (Task 5b) and examine associations between SNPs and miRNA levels (Task 5d) and prepare manuscripts describing the results.

4. Key research Accomplishments

Nothing to report.

5. Conclusion

Our results suggest that circulating miRNAs may constitute novel biomarkers of prostate cancer aggressiveness in both races. *A manuscript describing the methods and results in greater detail is under revision for resubmission to Carcinogenesis*.

We will analyze the data as outlined in *Task 5* of the statement of work for publication in the next year. Planned analyses are as follows:

a. Perform logistic regression analyses of associations between SNPs, haplotypes and PCa aggressiveness and prepare related manuscript (analyses underway).
b. Perform Cox proportional hazard analyses of associations between SNPs, haplotypes and PCa recurrence and prepare related manuscript
c. [Perform logistic regression analyses of associations between plasma levels of miRNAs and aggressiveness and prepare related manuscript (completed, manuscript in review process).]
d. Perform Analysis of Covariance to associate genetic polymorphism with plasma levels of miRNAs, and prepare related manuscript

6. Publications, Abstracts, and Presentations

Presentations:

(None new in Year 4)

1. MicroRNA and Prostate Cancer Disparities, Karmanos Cancer Institute Prostate Cancer Research Team seminar, April 22, 2016

2. Exploring Genes and MicroRNAs in Prostate Cancer Disparities, Karmanos Cancer Institute Population Studies and Disparities Research Program Seminar, March 4, 2016

3. MicroRNA in Prostate Cancer Racial Disparities and Aggressiveness, Karmanos Cancer Center Prostate Cancer Research Team SPORE planning retreat, June 25, 2013

7. Inventions, Patents and Licenses

Nothing to report.

8. Reportable Outcomes

Nothing to report.

9. Other Achievements

The miRNA expression data was used in a manuscript describing the novel extension of CART methods used in this study; this manuscript is under peer review.

A review paper describing miRNA as a theranostic in prostate cancer has been written, and we are hoping to get it published soon.

10. References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014;64: 9-29.

2. Kong D, Banerjee S, Ahmad A, et al. Epithelial to mesenchymal transition is mechanistically linked with stem cell signatures in prostate cancer cells. PLoS One. 2010;5: e12445.

3. Kong D, Heath E, Chen W, et al. Epigenetic silencing of miR-34a in human prostate cancer cells and tumor tissue specimens can be reversed by BR-DIM treatment. Am J Transl Res. 2012;4: 14-23.

11. Appendices

Nothing to report.