AD_____

Award Number: W81XWH-14-1-0011

TITLE:
Identification of Prostate Cancer-Specific microDNAs

PRINCIPAL INVESTIGATOR:
Yin-Yuan Mo

CONTRACTING ORGANIZATION:
University of Mississippi Medical Center
Jackson, MS 39216

REPORT DATE:
February 2016

TYPE OF REPORT:
Final

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
February 2016	Final	25 Nov 2013 - 24 Nov 2015

4. TITLE AND SUBTITLE
Identification of prostate cancer-specific microDNAs

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0011

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Yin-Yuan Mo

E-Mail: ymo@umc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

University of Mississippi Medical Center
2500 State Street, G652
Jackson, MS 39216

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

Emerging evidence has suggested that eukaryotic cells can express a special group of extrachromosomal circular DNAs (eccDNAs), called microDNAs. Unlike previously reported eccDNAs, microDNAs are relatively small in length, map to unique DNA sequence, and arise from genes, mostly likely resulting from microdeletions. Since they are usually in a circular form, they are more resistant to exonuclease than linear DNAs and can be stably present in the cells or even possibly in the circulating system. Therefore, overall goal of this application is to determine whether prostate cancer cells express such microDNAs which can be used to serve as valuable biomarkers for prostate cancer diagnosis or prognosis.

15. SUBJECT TERMS
microDNA, biomarker

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

Table of Contents

Introduction

MicroDNAs are a special group of extrachromosomal circular DNAs (eccDNAs) derived from chromosomal repetitive sequences, intermediates of mobile elements or viral genomes. They are generally small, can be mapped to unique DNA sequences, and arise from various genes through deletions and circulations. Since they are circular, they are resistant to exonuclease and are more stable present in the cells or even possibly in the circulating system. Based on these findings, we hypothesize that prostate cancer may exploit this mechanism for its own advantage and thus may express a very different microDNA pattern from normal prostate tissue. This different pattern can be detected by currently advanced technology such as deep sequencing. Therefore, overall goal of this application was to determine whether prostate cancer cells express specific microDNAs which may contribute to prostate cancer pathogenesis and thus they may serve potential biomarkers for prostate cancer diagnosis or prognosis.

Body

Task 1. Determine whether prostate cancer cells display different patterns of microDNAs from those of normal tissue or indolent diseases

Results

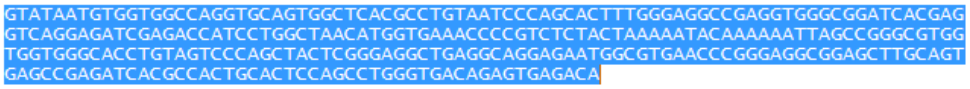
Little is known about microDNAs, and it is not clear whether prostate cancer cells carry potential microDNAs. Thus our goal was to demonstrate the existence of microDNAs in prostate cancer. We adopted multiple displacement amplification (MDA) with random

primers for enriched circular DNA by rolling circle amplification (RCA) and then amplified DNA fragments were subject to deep sequencing.

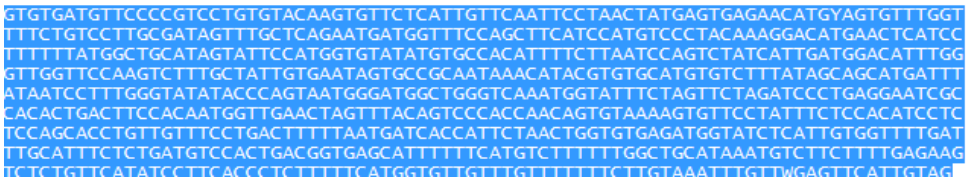
Deep sequencing of the amplified DNA fragments identified several potential microDNA sequences. The detailed sequences of 4 microDNAs were shown in Fig. 1. All 4 microDNAs were within 1kb in length. In particular, PCA-microDNA 7 carries AA, AT or TT dinucleotides, a feature of microDNAs; in addition, its GC content is relatively high (58.4%), another feature of microDNAs.

PCA-microDNA-1; 971bp

PCA-microDNA-7; 286 bp



PCA-microDNA-9; 710 bp



PCA-microDNA-11; 450 bp

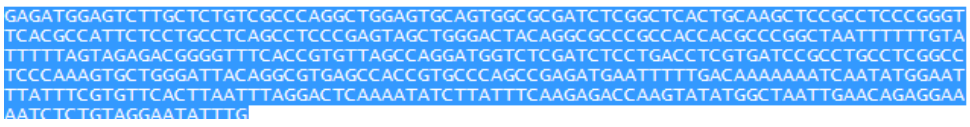


Fig. 1 Nucleotide sequences of 4 potential microDNAs based on deep sequencing data.

Task 2. Determine whether prostate cancer cells display different patterns of microDNAs and their role in tumor cell growth in cell culture models

Next, we detected their expression in normal prostate cell line RWPE-1 and prostate cancer cell line LNCaP and found that expression of PCA-microDNA 7 is higher in LNCaP cells than in RWPE-1 cells (Fig. 2), whereas there is no difference for the other three microDNAs between these cell lines, suggesting that PCA-microDNA-7 may play an oncogenic role. To test this hypothesis, we cloned PCA-microDNA-7 in pCDH expression vector. MTT assays support the oncogenic role of microDNA-7 in tumor cell growth (Fig. 3).

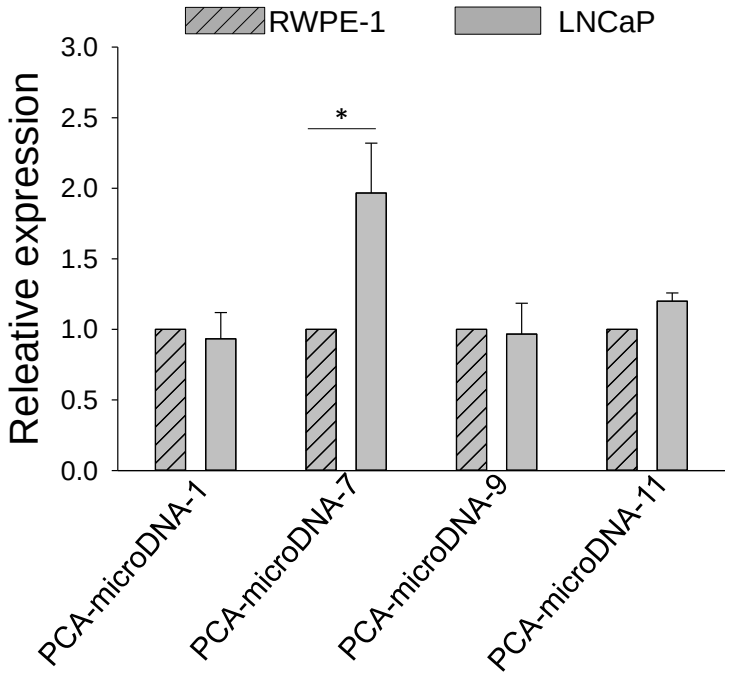


Fig. 2 Expression of microDNAs in RWPE-1 and LNCaP cells.

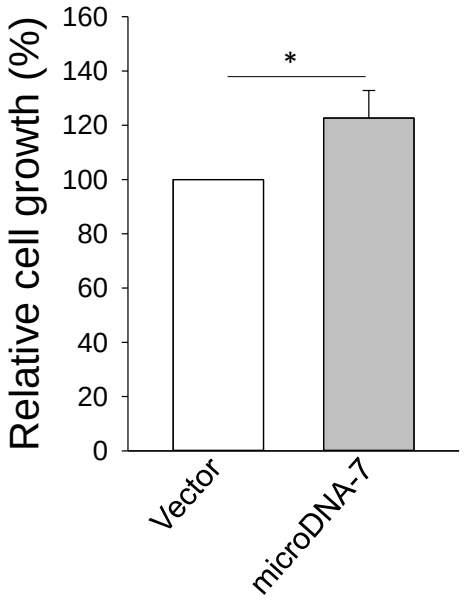


Fig. 3 PCA-microDNA-7 promotes tumor cell growth in LNCaP cells. The cell growth was measured by MTT assays.

Task 3. Detect microDNAs in blood/serum samples from healthy and prostate cancer patients

Therefore, we focused on PCA-microDNA-7 from clinical specimens. We used 5 normal (healthy donor) and 5 prostate cancer patient serum samples from a commercial source. qPCR analysis suggested that the level of PCA-microDNA-7 is higher in patients than in normal healthy donors (Fig. 4). Thus, it would be interesting to determine whether PCA-microDNA-7 can serve as a novel biomarker for prostate cancer.

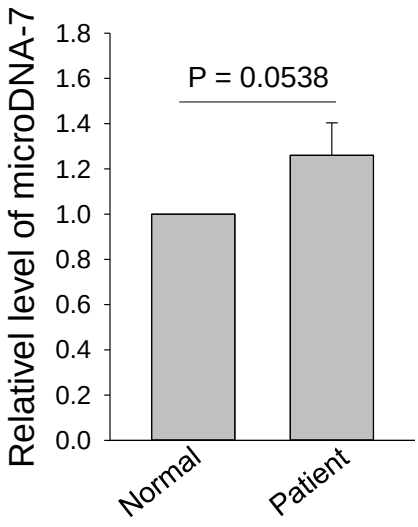


Fig. 4 Detection of PCA-microDNA-7 in serum samples of prostate cancer patients (n = 5) as compared to healthy donors (n = 5)

Key Research Accomplishments

- We identified several potential microDNAs from prostate cancer cells through multiple displacement amplification and next generation sequencing.

- PCA-microDNA-7 is the top candidate which is highly expressed in prostate cancer LNCaP cells as compared to normal prostate cell line RWPE-1 cells.
- Overexpression of PCA-microDNA-7 in prostate cancer cells promotes tumor cell growth, suggesting an oncogenic role
- The level of PCA-microDNA-7 is higher in serum samples from prostate cancer patients than from healthy donors, suggesting PCA-microDNA-7 as a potential biomarker for prostate cancer.

Reportable Outcomes

A manuscript on PCA-microDNA-7 is in preparation.

Conclusions

Deep sequencing of MDA samples from prostate cancer cells has identified 31 potential microDNA candidates. Clone #7 is a top candidate based on size, dinucleotide repeats and high GC content. Furthermore, we overexpressed PCA-microDNA-7 in prostate cancer cells and MTT assays suggest that PCA-microDNA-7 plays an oncogenic role. Finally, we detect a high level of PCA-microDNA-7 in serum samples of prostate cancer patients as compared to healthy donors. Together, these results suggest that microDNAs may serve as novel biomarkers for prostate cancer. Therefore, further investigation of these microDNAs in large samples is warranted.