

Award Number: W81XWH-14-1-0384

**TITLE:**

Identification of prostate cancer-specific circular RNAs

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**REPORT DATE:** December 2016

**TYPE OF REPORT:** Final

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

**DISTRIBUTION STATEMENT:** Approved for Public Release;  
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<b>REPORT DOCUMENTATION PAGE</b>			<i>Form Approved</i> <i>OMB No. 0704-0188</i>		
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. <b>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</b>					
<b>1. REPORT DATE</b> December 2016		<b>2. REPORT TYPE</b> Final		<b>3. DATES COVERED</b> 09/15/2014 to 09/14/2016	
<b>4. TITLE AND SUBTITLE</b> Identification of prostate cancer-specific circular RNAs				<b>5a. CONTRACT NUMBER</b> W81XWH-14-1-0384	
				<b>5b. GRANT NUMBER</b> PC131272	
				<b>5c. PROGRAM ELEMENT NUMBER</b>	
<b>6. AUTHOR(S)</b> Yin-Yuan Mo  E-Mail: ymo@umc.edu				<b>5d. PROJECT NUMBER</b>	
				<b>5e. TASK NUMBER</b>	
				<b>5f. WORK UNIT NUMBER</b>	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  University of Mississippi Medical Center 2500 State Street, G652 Jackson, MS 39216				<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				<b>10. SPONSOR/MONITOR'S ACRONYM(S)</b>	
				<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>	
<b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited					
<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b> The major goal of this application is to determine whether newly identified circular RNAs can serve as novel biomarkers for prostate cancer diagnosis and prognosis. There are three specific aims. First, we will determine whether prostate cancer cells display different patterns of circular RNAs from those of normal tissue. Then, we will determine whether differential expression of circular RNAs can also be detected in cell culture models because we can easily manipulate the levels of circular RNAs in cell culture so that we are able to study their functions. Finally, we will determine whether circular RNAs are differentially expressed in blood/serum samples from healthy individuals and prostate cancer patients.					
<b>15. SUBJECT TERMS</b> microRNA, lncRNA, biomarker					
<b>16. SECURITY CLASSIFICATION OF:</b>			<b>17. LIMITATION OF ABSTRACT</b>	<b>18. NUMBER OF PAGES</b>	<b>19a. NAME OF RESPONSIBLE PERSON</b> USAMRMC
<b>a. REPORT</b> U	<b>b. ABSTRACT</b> U	<b>c. THIS PAGE</b> U			<b>19b. TELEPHONE NUMBER</b> (include area code)
			UU	15	

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## **Introduction**

Circular RNAs (circRNAs) are a new type of long non-coding RNAs (lncRNAs). Like classic lncRNAs, circRNAs do not code for protein. However, while classic lncRNAs are linear, circRNAs are circular often through back splicing. Moreover, they often have regulatory functions. For example, circRNAs can serve as endogenous microRNA sponges to neutralize the microRNA function. However, it is not clear whether prostate cancer can exploit this mechanism for its own advantage. We would like to determine whether circRNAs are aberrantly present in prostate cancer compared to normal tissue. Identification of such dysregulated circRNAs would lay a foundation for us to explore their role in prostate cancer and to identify novel prostate cancer biomarkers or therapeutic targets. We hypothesize that prostate cancer may exploit this mechanism for its own advantage and thus prostate cancer may display a very different circRNA pattern from normal prostate tissue. Therefore, the major goal of this application is to determine whether newly identified circular RNAs can serve as novel biomarkers for prostate cancer diagnosis and prognosis.

## **Body**

**CircRNAs are aberrantly expressed in prostate cancer.** As newly discovered molecules, circRNAs are poorly characterized. Little is known whether they are dysregulated in prostate cancer. Thus, our first step was to characterize these new molecules by profiling. Results indicate that a number of circRNAs are either upregulated (Table 1) or downregulated (Table 2) in tumor tissue as compared to normal tissue.

For example, 15 upregulated circRNAs have over 1.5-fold increase in tumor vs normal with p value <0.05. The expression level of hsa\_circRNA\_104595 was a 2.7-fold higher in tumor than in normal. To better illustrate how the circular form is formed, we provide the sequence for hsa\_circRNA\_002143, as shown in Fig. 1 as an example. The top part is the actual sequence and the bottom part is when a circle is formed. Two ends at the junction were highlighted by red and blue, respectively.

On the other hand, 18 circRNAs were at least 2-fold decrease in tumor vs normal. For example, hsa\_circRNA\_002143 was detected about a 3-fold downregulation in tumor as compared to normal (Table 2). We also provide schematic illustration of hsa\_circRNA\_002143, as shown in Fig. 2.

**CircRNAs are derived from various sources.** The origin of these circRNAs varies, ranging from intronic, intragenic to exonic. Intronic circRNAs originate from introns; intragenic circRNAs originate from the regions between two separate genes; and exonic circRNAs originate from exons. Furthermore, these exons can be for coding genes or non-coding genes.

**CircRNAs can potentially target microRNAs.** One of potential functions for circRNAs is the capability to serve as sponges to neutralize the endogenous microRNAs. In this regard, all of these circRNAs had the potential to target more than one microRNA. We listed one for each in Table 1 and Table 2. This suggests that aberrant expression of these circRNAs may affect the levels of these microRNAs, thus, contributing to prostate

tumorigenesis. For example, there are two binding sites for miR-412-3p and one binding site for miR-363-5p in hsa\_circRNA\_104595 (Fig. 3). On the other side, there are over 20 binding sites for miR-663a in hsa\_circRNA\_002143 probably because hsa\_circRNA\_002143 is much larger than hsa\_circRNA\_104595. We just listed five of them (Fig. 4). To determine whether overexpression of circRNAs can affect microRNA expression, we chose hsa\_circRNA\_002143. As shown in Fig. 5 A, miR-412-3p was downregulated in the cells with overexpression of hsa\_circRNA\_104595 as compared to vector control. For miR-363-5p, we only detected a slight downregulation in hsa\_circRNA\_104595 cells. Moreover, hsa\_circRNA\_104595 promoted cell growth (Fig. 5B), suggesting that it plays an oncogenic role.

**hsa\_circRNA\_104595 is upregulated in serum samples of prostate patients.** To

determine whether circRNAs are deregulated in serum samples, we chose both hsa\_circRNA\_104595 and hsa\_circRNA\_002143. We detected upregulation of hsa\_circRNA\_104595 in serum samples of breast patients (Fig. 6). In contrast, we were not able to deregulation of hsa\_circRNA\_002143

**Key Research Accomplishments**

- We identified 15 upregulated and 18 downregulated circRNAs from prostate cancer cells through profiling.
- All of these circRNAs carry microRNA binding sites, through which they may regulate the level of endogenous microRNAs.

- We found that hsa\_circRNA\_104595 can negatively regulate miR-412-3p and promote tumor cell growth.
- Finally, we showed that hsa\_circRNA\_104595 is upregulated in serum samples of breast cancer patients. Thus, hsa\_circRNA\_104595 may serve as a biomarker for prostate cancer.

### **Reportable Outcomes**

“Hsa\_circRNA\_104595 as a potential biomarker for prostate cancer” in preparation.

### **Conclusions**

Microarray profiling has identified 15 upregulated and 18 downregulated circRNAs from prostate cancer cells. Ectopic expression of hsa\_circRNA\_104595 downregulates expression of miR-412-3p and promotes tumor cell growth. Therefore, further characterization of circRNAs in prostate cancer will help identify novel circRNA-based biomarkers.

Table 1, Upregulation of circular RNAs in tumors

Name	Tumor/normal	P-value	circRNA_type	Potential miR binding
<a href="#">hsa_circRNA_104595</a>	2.717446	0.003168	exonic	<a href="#">hsa-miR-412-3p</a>
hsa_circRNA_100790	2.0369969	0.015794	exonic	<a href="#">hsa-miR-20b-3p</a>
hsa_circRNA_104927	1.9599525	0.044262	exonic	<a href="#">hsa-miR-500a-3p</a>
hsa_circRNA_102605	1.9344202	0.002482	exonic	<a href="#">hsa-miR-486-3p</a>
hsa_circRNA_000956	1.926276	0.022407	antisense	<a href="#">hsa-miR-765</a>
hsa_circRNA_000554	1.7791893	0.009459	intronic	<a href="#">hsa-miR-153-5p</a>
hsa_circRNA_100438	1.6898966	0.014578	exonic	<a href="#">hsa-miR-383-3p</a>
hsa_circRNA_101175	1.685527	0.033942	exonic	<a href="#">hsa-miR-374a-3p</a>
hsa_circRNA_103975	1.6771255	0.042994	exonic	<a href="#">hsa-miR-493-5p</a>
hsa_circRNA_103950	1.6427088	0.00164	exonic	<a href="#">hsa-miR-143-5p</a>
hsa_circRNA_102889	1.6353354	0.030357	exonic	<a href="#">hsa-miR-9-5p</a>
hsa_circRNA_102545	1.5942883	0.02303	exonic	<a href="#">hsa-miR-573</a>
hsa_circRNA_103417	1.55695	0.011534	exonic	<a href="#">hsa-miR-597-3p</a>
hsa_circRNA_100213	1.5444917	0.041263	exonic	<a href="#">hsa-miR-345-5p</a>
hsa_circRNA_105037	1.5315459	0.000508	exonic	<a href="#">hsa-miR-197-3p</a>



## Table 2, Downregulation of circular RNAs in tumors

Name	Tumor/normal	P-value	circRNA_type	Potential miR binding
<a href="#">hsa_circRNA_002143</a>	0.373893479	0.002055	intragenic	<a href="#">hsa-miR-663a</a>
hsa_circRNA_100477	0.373893479	0.004311	exonic	<a href="#">hsa-miR-134-5p</a>
hsa_circRNA_101164	0.373893479	0.003234	exonic	<a href="#">hsa-miR-103a-2-5p</a>
hsa_circRNA_101615	0.373893479	0.004198	exonic	<a href="#">hsa-miR-197-3p</a>
hsa_circRNA_000911	0.446551827	0.01212	intronic	<a href="#">hsa-miR-449c-3p</a>
hsa_circRNA_104084	0.446551827	0.012523	exonic	<a href="#">hsa-miR-506-3p</a>
hsa_circRNA_000780	0.461434466	0.018459	intronic	<a href="#">hsa-miR-651-3p</a>
hsa_circRNA_102701	0.461434466	0.015917	exonic	<a href="#">hsa-miR-369-3p</a>
hsa_circRNA_104121	0.461434466	0.019856	exonic	<a href="#">hsa-miR-203a-3p</a>
hsa_circRNA_104930	0.461434466	0.019407	exonic	<a href="#">hsa-miR-762</a>
hsa_circRNA_104204	0.478932053	0.03059	exonic	<a href="#">hsa-miR-619-5p</a>
hsa_circRNA_101213	0.485389422	0.033127	exonic	<a href="#">hsa-miR-431-3p</a>
hsa_circRNA_104666	0.486375944	0.034278	exonic	<a href="#">hsa-miR-1468-5p</a>
hsa_circRNA_100750	0.496412771	0.037023	exonic	<a href="#">hsa-miR-1301-3p</a>
hsa_circRNA_000881	0.499530875	0.044016	intronic	<a href="#">hsa-miR-557</a>
hsa_circRNA_102445	0.499530875	0.042097	exonic	<a href="#">hsa-miR-644a</a>
hsa_circRNA_103134	0.499530875	0.044068	exonic	<a href="#">hsa-miR-644a</a>
hsa_circRNA_101336	0.501698841	0.04572	exonic	<a href="#">hsa-miR-320b</a>

CCCAGCCCTGGGGAGC CCCTGTGGAAGTGGAGTCCTTCCCTGGTCCACCCCGGTGACCTGCT  
GCAGCTTCGCTGTCGGCTGCGGGACGATGTGCAGAGCATCAACTGGCTGCGGGACGGGGTG  
CAGCTGGCGGAAAGCAACCGCACCCGCATCACAGGGGAGGAGGTGGAGGTGCAGGACTCCG  
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CTACTTCTCCGTCAATGTTTCAG

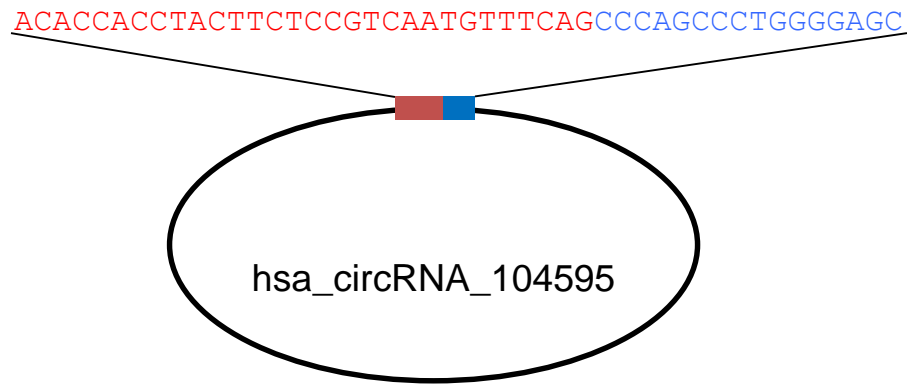
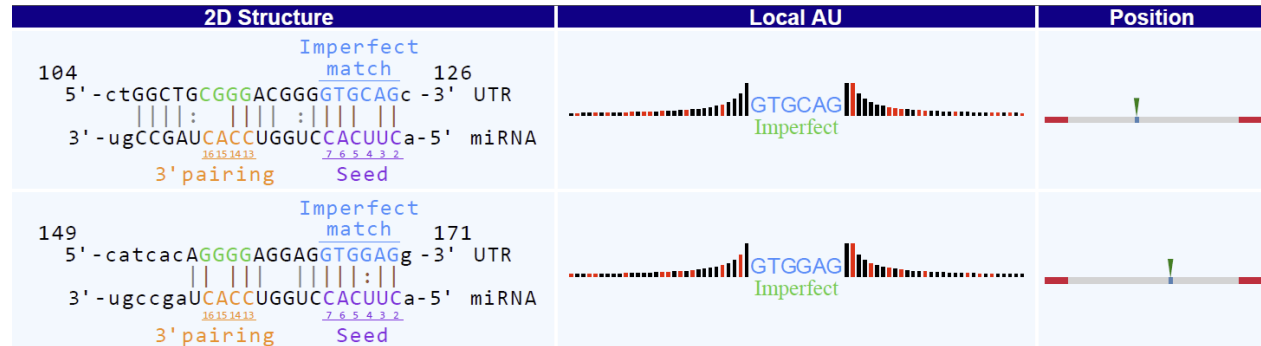


Fig. 1 DNA sequence of hsa\_circRNA\_104595. The junction of two ends is highlighted by red and blue, respectively.



# MicroRNA binding sites in hsa\_circRNA\_104595

## hsa-miR-412-3p



## hsa-miR-363-5p

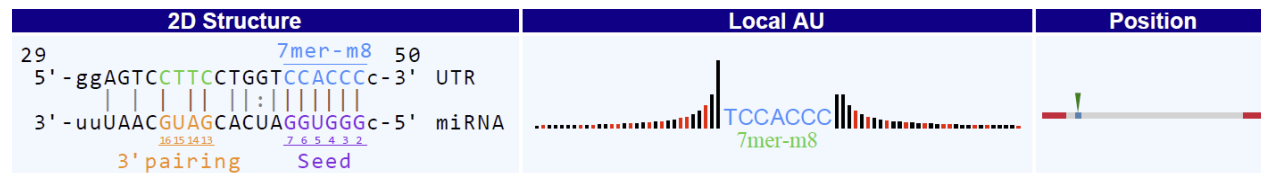


Fig. 3 Binding sites in hsa\_circRNA\_104595 for hsa-miR-412-3p and has-miR-363-5p.

## MicroRNA binding sites in hsa\_circRNA\_002143

### hsa-miR-663a

2D Structure	Local AU	Position
<p>Imperfect match 4078</p> <p>5' -gcGGTCCACGGGCCGCTGCCa -3' UTR</p> <p>3' -cGCCAGG-GCGCCGCGGGGCGGa-5' miRNA</p> <p>3' pairing Seed</p>	<p>CCTGCC</p> <p>Imperfect</p>	
<p>6mer 4429</p> <p>5' -cgcGACCCCGGGGA C CCGCCg-3' UTR</p> <p>3' -cgcCAGGGCGCCGCGGGGCGGa-5' miRNA</p> <p>3' pairing Seed</p>	<p>CCCGCC</p> <p>6mer</p>	
<p>6mer 4556</p> <p>5' -ggGGAGCCGGGACCGT C CCGCCc-3' UTR</p> <p>3' -cgcCAGGGCGCC--GCGGGGCGGa-5' miRNA</p> <p>3' pairing Seed</p>	<p>CCCGCC</p> <p>6mer</p>	
<p>Offset 6mer 5555</p> <p>5' -tcctTCTCGTCCGC C CCGCGg-3' UTR</p> <p>3' -cgccAGGGCGCCGCGGGGCGGa-5' miRNA</p> <p>3' pairing Seed</p>	<p>CCCGCG</p> <p>Offset 6mer</p>	
<p>7mer-m8 5584</p> <p>5' -ctcGTCTCTCTCT C CCGCCc-3' UTR</p> <p>3' -cgcCAGGGCGCCGCGGGGCGGa-5' miRNA</p> <p>3' pairing Seed</p>	<p>CCCGCC</p> <p>7mer-m8</p>	

Fig. 4 Binding sites in hsa\_circRNA\_002143 for has-miR-663a. Only top 5 sites are listed here.

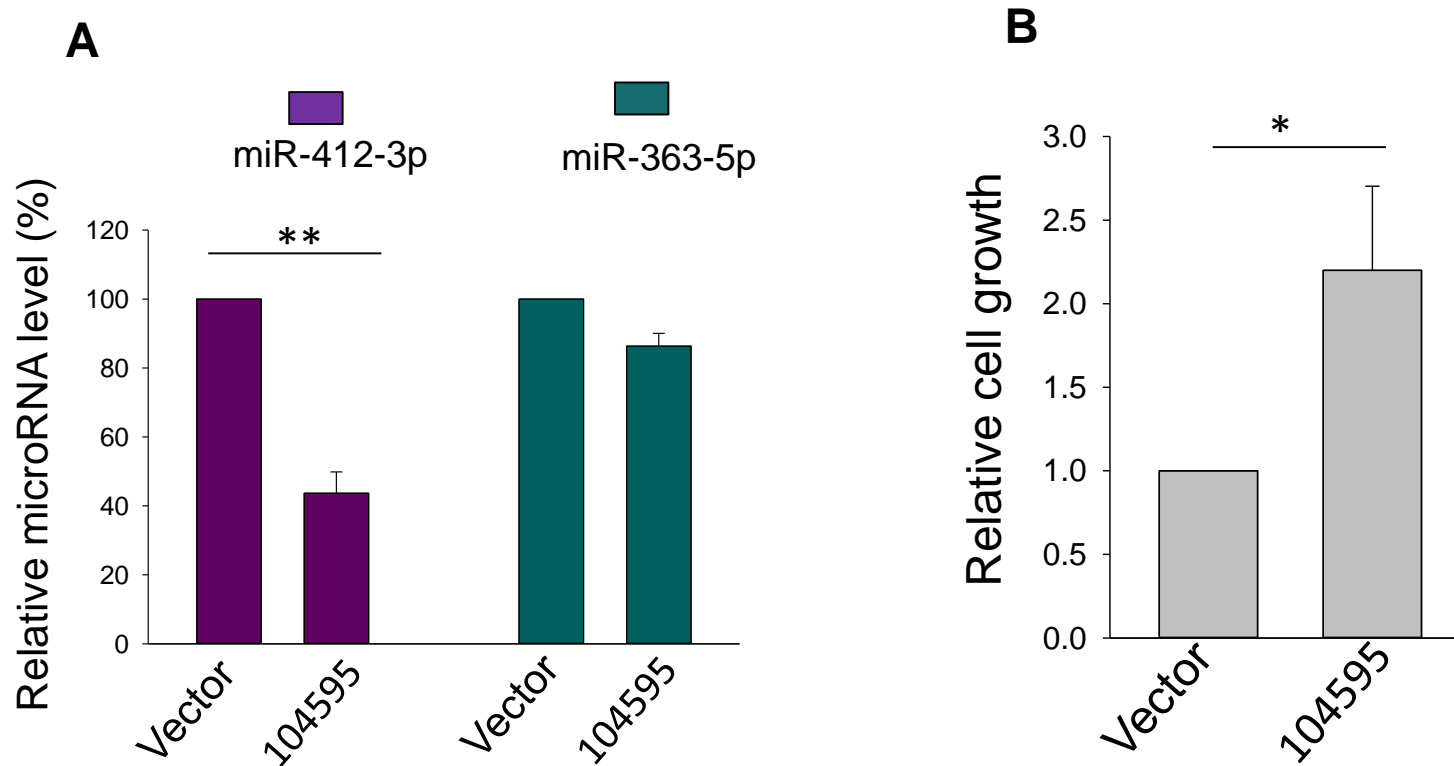


Fig. 5 Effect of overexpression of hsa\_circRNA\_104595 on microRNA expression and tumor cell growth. A, Hsa\_circRNA\_104595 downregulates miR-412-3p. LNCaP cells were transfected with hsa\_circRNA\_104595 or vector control. Total RNA was isolated for qRT-PCR analysis. B, Hsa\_circRNA\_104595 promotes tumor cell growth, as determined by MTT assay.

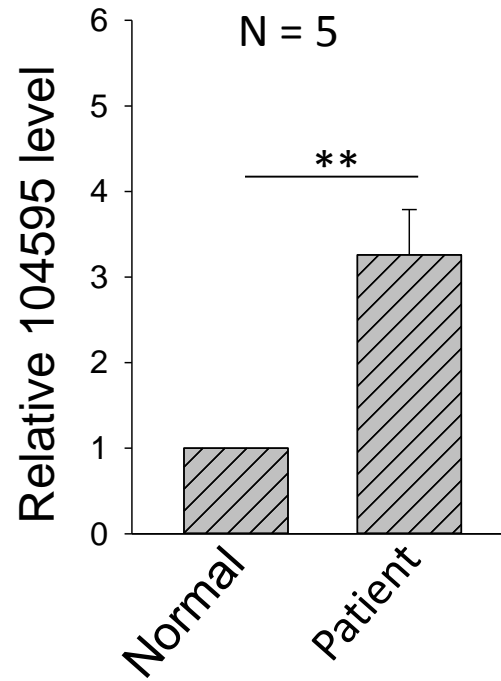


Fig. 6 Upregulation of hsa\_circRNA\_104595 in serum samples of prostate cancer patients, as detected by qRT-PCR.