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Award Number: W81XWH-13-1-0461

TITLE : Targeting Tumor Oct4 to Deplete Prostate Tumor and Metastasis Initiating Cells

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REPORT DATE: October 2014

TYPE OF REPORT: Annual

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

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE 1 Oct 2014		2. REPORT TYPE Annual		3. DATES COVERED Sept 30 2013 - Sept 29 2014	
4. TITLE AND SUBTITLE Targeting Tumor Oct4 to Deplete Prostate Tumor and Metastasis Initiating Cells				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-13-1-0461	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Daotai Nie				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Southern Illinois University Springfield, IL 62794 dnie@siumed.edu				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) US Army Medical Research and Command Fort Detrick Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT X Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Identification of genes driving prostate carcinogenesis will lead to new cancer treatment. The human chromosome 8q24.21 region has been linked with increased risk for prostatic carcinoma but the how this region contributes to prostate carcinogenesis is unknown. We cloned a candidate gene, <i>POU5F1B</i> (also called <i>POU5F1P1</i>), in this gene desert of 1.2Mb between <i>FAM84B</i> and the <i>c-MYC</i> oncogene. <i>POU5F1B</i> is a pseudogene of embryonic Oct4 (<i>POU5F1</i>). A recent study found that tumor Oct4 found in prostate cancer cells is due to the gene expression of <i>POU5F1B</i> , not embryonic Oct4 (<i>POU5F1</i>). In a dataset of 171 patients, it was found that tumor Oct4 was significantly increased in primary tumors and markedly increased in metastatic tumors, when compared to normal prostate or adjacent normal tissues. Based on the analyses and our preliminary data, we think, tumor Oct4, expressed from <i>POU5F1B</i> in the prostate cancer susceptibility loci 8q24, is a driver of prostate tumor formation and progression, and therefore, this driver is a novel target of intervention to eliminate prostate cancer. We propose to further determine the roles of tumor Oct4 in prostate tumor formation and metastasis. We hope we can validate whether tumor Oct4 can be targeted to inhibit prostate cancer progression and metastasis. In addition, we will map out the regions critical for Oct4 to promote prostate carcinogenesis so that we can target this region to develop therapeutics for cancer treatment in the future					
15. SUBJECT TERMS OCT4, cancer stem cells, prostate cancer, metastasis, tumor formation					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES 12	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (include area code)

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Introduction

Background: Genome-wide association studies (GWAS) have linked human chromosome 8q24.21 region with increased risk for prostatic carcinoma but the how this region contributes to prostate carcinogenesis is unknown. In this gene desert of 1.2Mb between *FAM84B* and the *c-MYC* oncogene, *POU5F1B* (also called POU5F1P1) is a candidate gene with coding capacity. It is a pseudogene of embryonic Oct4 (POU5F1). A recent study found that tumor Oct4 found in prostate cancer cells is due to the gene expression of POU5F1P1 (Hugo name: POU5F1B), not embryonic Oct4 (POU5F1). Our *in silico* analysis found a significant increase in Oct4 (*POU5F1B*) in primary tumors and a marked increase in metastatic tumors, when compared to normal prostate or adjacent normal tissues. Tumor Oct4 expression was higher in tumorigenic prostate cancer cells than in nontumorigenic RWPE-1 cells. Depletion of tumor Oct4 in prostate cancer cells reduced their tumorigenic potential. We cloned tumor Oct4 and found that increased expression of tumor Oct4 in prostate cancer cells stimulated tumor cell motility. Further a significant divergence was found between tumor Oct4 and embryonic Oct4 in regulating Wnt/ - caenin signaling. It is our hypothesis that tumor Oct4, expressed from POU5F1B in the prostate cancer susceptibility loci 8q24, is a driver of prostate tumor formation and progression, and therefore, this driver is a novel target of intervention to eliminate prostate cancer.

Objective: The objective is to determine whether tumor Oct4 promotes tumor formation and metastasis, to determine whether tumor Oct4 can be targeted to treat prostate cancer progression, and to elucidate the mechanism involved for tumor Oct4 to promote prostate carcinogenesis.

Specific Aims: 1) Investigate whether tumor Oct4 promotes prostate tumor initiation and metastasis.

2) Determine whether tumor Oct4 can be targeted to reduce prostate tumor formation, progression, and metastasis.

3) Elucidate the mechanism involved for tumor Oct4 in promoting prostate carcinogenesis.

BODY OF REPORT

Scientific portion:

Task 1. Investigate whether tumor Oct4 promotes prostate tumor initiation and metastasis (Month 1 – 18).

We have cloned the coding region of *POU5F1B* from prostate cancer PC3 cells into a lentiviral expression vector pCDH. Sequencing revealed the presence of SNPs in the coding region of *POU5F1B* in PC3 cells when compared to the reference sequence (NM_001159542.1) but the SNPs did not alter the deduced amino acid sequence of tumor Oct4 (*POU5F1B*) (**Table 1**).

PCDH-myc-Pou5F1B Clones	Sequenced range	Blast with POU5F1B ORF (NM_001159542.1)	
		Nucleotide	Amino Acid
2	1-297, 284-1080	684(CAG---CAA) 712(TCG---TCC)	no change
3	1-513 285-1080	684(CAG---CAA) 712(TCG---TCC)	no change
4	1-272 285-1080	684(CAG---CAA) 712(TCG---TCC)	no change

Table 1. Single nucleotide polymorphism in the coding region of *POU5F1B* cloned from prostate cancer PC-3 cells. Note the SNPs did not lead to a change in the amino acid sequence in tumor Oct4 (*POU5F1B*).

The utility of the construct to express tumor Oct4 was confirmed Western blot using the Myc tag (**Figure 1A**) and also by an Oct4 antibody (**Figure 1B**). It should be noticed that *POU5F1B* and *POU5F1* (wild type Oct4) have 95% homology in amino acid sequence (Panagopoulos et al., 2008) and therefore, many commercial antibodies against Oct4 cannot differentiate the tumor Oct4 encoded by *POU5F1B* from those by *POU5F1*. Since *POU5F1* is not expressed in prostate cancer cells (in contrast to embryonic stem cells), the Oct4 detected by immunohistochemistry or Western blot is due to the expression of its pseudogene, *POU5F1B* (Kastler et al.).

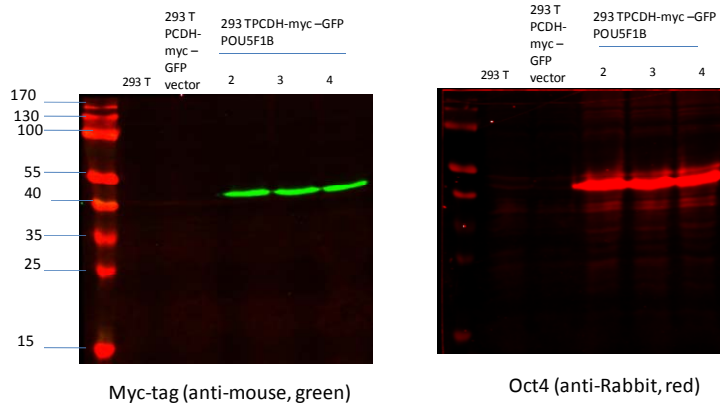


Figure 1. Validation of the lentiviral pCDH POU5F1B expression constructs. POU5F1B was expressed as Myc tag under the promoter of EF1. Left panel, Western blot with Myc tag antibody. Right panel, Western blot with an antibody against Oct4.

At the time of our study, these SNP risk associations have not been published. Complete analysis of these risk alleles with POU5F1B or Oct4 protein expression and transactivation activity has not been conducted yet, so the full extent of this association and functional consequences of these risk alleles is still not known. Nevertheless, we speculated that these SNPs contributed to the tumor functions of POU5F1B and examined the functional consequence of PC3 POU5F1B (which carries the SNPs present in all examined prostate cancer cell lines, rs6998254 and rs7002225) overexpression in prostate cancer cells. We cloned POU5F1B from cDNA of PC3 cells into a pCDH-myc lentiviral expression construct. Sequencing of our cloned insert confirmed the presence of the SNPs in PC3 POU5F1B, rs6998254 and rs7002225, and our PC3 POU5F1B protein product differs from "normal" POU5F1B at one amino acid residue. (Fig.2A) (Breyer et al., 2014).

A

POU5F1	226	LVQARKRKRTSIENRVRGNNLENLFL	250
POU5F1B	226	LMQARKRKRTSIENRVRGNNLENLFL	250
PC3 POU5F1B	226	LMQARKRKRTSIQNRVRGNLENLFL	250

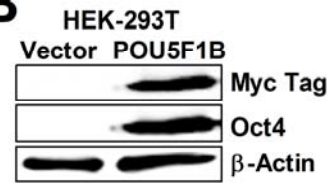
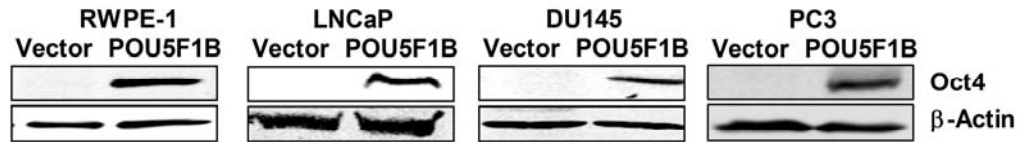
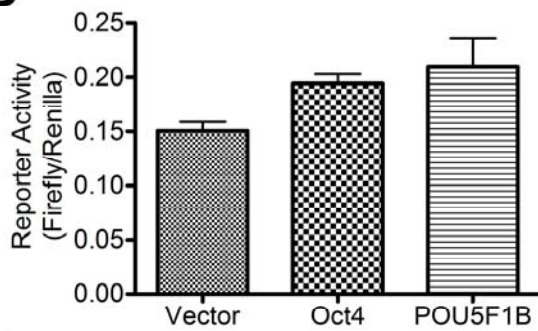
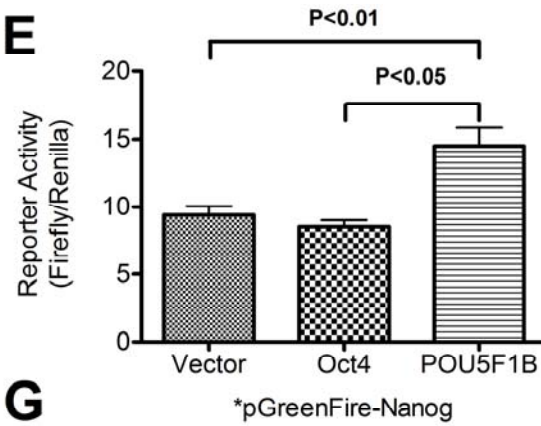
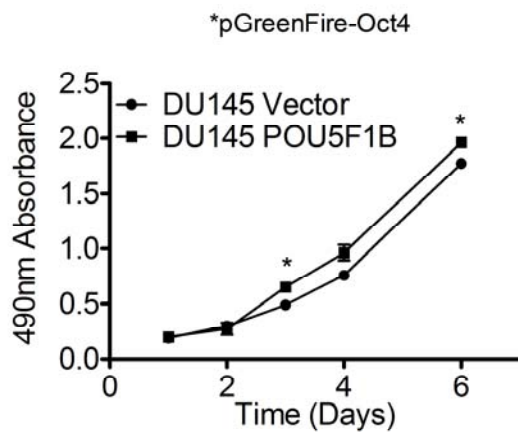
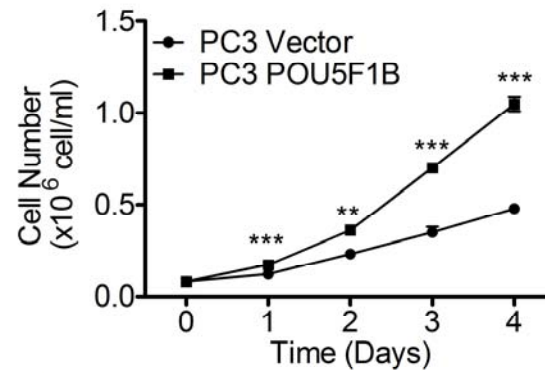
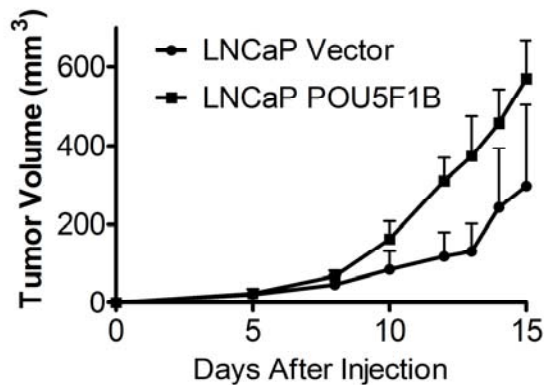
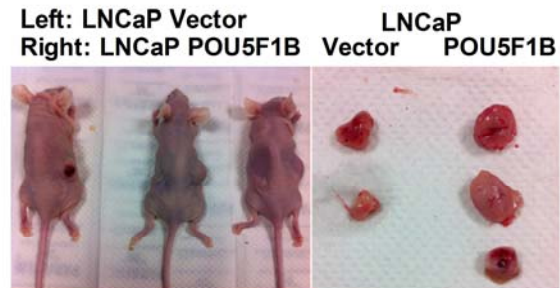
B**C****D****E****F****G****H****I**

Figure 2. POU5F1B overexpression promotes increased prostate cancer cell proliferation and tumor growth. **A.** POU5F1B cloned from PC3 cells carry two prostate cancer risk associated SNPs, rs6998254 and rs7002225. rs7002225 leads to the E238Q missense mutation in the homeodomain. **B.** Western blot confirmation of the pCDH-Myc-POU5F1B construct in HEK-293T cells. **C.** Western blot confirmation of POU5F1B protein overexpression in prostate cancer cell lines. **D.** POU5F1B exerts gene transactivation activity through Oct4 response elements. **E.** POU5F1B can promote nanog transactivation activity. **F-G.** POU5F1B overexpression promotes cell proliferation in prostate cancer cells. **H.** POU5F1B overexpression promotes LNCaP tumor xenograph growth in male Nu/J mice. Tumor volume = $0.5 \times (\text{Length}) \times (\text{Width}) \times (\text{Width})$. n=3. **I.** Representative images of mice carrying LNCaP-Vector and LNCaP-POU5F1B tumors 15 days after inoculation. * denotes $P \leq 0.05$, ** denotes $P \leq 0.01$, and *** denotes $P \leq 0.001$.

POU5F1B expression has been detected in various cancers by several independent research groups (Hayashi et al., 2013; Kastler et al., 2010; Suo et al., 2005), and Panagopoulos *et al.* showed that a putative POU5F1B protein is localized in the nucleus, acts as a transcriptional activator, and regulates the expression in a similar way to the Oct4A (Panagopoulos et al., 2008). In our study, we confirmed, via western blots and immunocytochemistry, that our POU5F1B expression construct encodes protein in all tested cell lines: HEK-293T, RWPE1, LNCaP, DU145, and PC3 (Fig.2B-C).

Using the pGreenFire reporter constructs for OCT4 and NANOG, we further confirmed that POU5F1B exerts transactivation activity. In our studies, the transactivation activity of POU5F1B is similar to that of Oct4A in HEK-293T cells, confirming that POU5F1B proteins functions as a transcription factor. Furthermore, POU5F1B produces a significantly greater increase in Nanog transactivation activity than Oct4A (Fig.2D-E), indicating important divergence and gains of functions between POU5F1B and Oct4.

Our transcription reporter data, however, is in conflict with the findings published by Panagopoulos *et al.* In their study, using a POU5F1B transcript that does not carry prostate cancer risk associated SNPs, reported that POU5F1B displays decreased transactivation activity compared to Oct4A and that POU5F1B does not enhance Nanog transactivation activity

(Panagopoulos et al., 2008). We used PC3 POU5F1B that harbors a missense mutation to the homeobox DNA binding domain. We believe that the conflict in reported POU5F1B is the result of inherent functional differences between native and tumor-associated POU5F1B variants. Furthermore, we speculate that the two prostate cancer risk associated missense SNPs, rs6998061 and rs7002225, reported by Breyer *et al.* 1.) exert very important consequences to POU5F1B DNA binding and gene transactivation/repression functions and 2.) presence of at least one of these risk-associated alleles may be required for POU5F1B driven tumor incidence and progression. The risk-associated allele rs7002225 is examined in our study, but rs6998061 has not. Further studies should scrutinize the functional differences between these POU5F1B variants.

GWAS studies and the evidence of POU5F1B transcription in cancers suggest POU5F1B is important for tumorigenesis, but the functional effects of this protein in cancer cells are still unclear. Recently, Hayashi *et al.* showed that overexpression of POU5F1B in gastric cancer cells increased cell growth *in vitro* as well as both tumorigenicity and tumor growth *in vivo*. They also found POU5F1B could promote angiogenesis and inhibit apoptosis (Hayashi et al., 2013). Consistent with their observation, our study found that overexpression of POU5F1B increase cell proliferation (Fig. 2F-G) and tumor growth (Fig. 2H-I) in prostate cancer cell lines. Furthermore, we found that overexpression of POU5F1B does not produce tumorigenic transformation in RWPE-1 cells (Data Not Shown), indicating that POU5F1B alone is not sufficient for malignant transformation.

Task 2: Determine whether tumor Oct4 can be targeted to reduce prostate tumor formation, progression, and metastasis (Month 12 -30).

The studies have been planned and will be initiated soon.

Task 3: Elucidate the mechanism involved for tumor Oct4 in promoting prostate carcinogenesis (Month 18 – 36).

The studies are in the planning stage.

KEY RESEARCH ACCOMPLISHMENT and REPORTABLE OUTCOMES

Presentations:

Hongmei Jiang, Man-Tzu Wang and Daotai Nie. The Role of POU5F1B in Prostate Cancer. AACR 2013 Annual Meeting, Washington, DC, 2013.

Hongmei Jiang, Man-Tzu Wang, and Daotai Nie. The Role of POU5F1B in Prostate Cancer. Simmons Cancer Institute 2013 Research Symposium, Springfield, IL, October 2013.

Abstracts published:

Hongmei Jiang, Man-Tzu Wang and Daotai Nie. The Role of POU5F1B in Prostate Cancer. Proc. Amer. Assoc. Cancer Res. 52 Late breaking: LB-281, 2013.

Review article published:

Man-Tzu Wang, Hongmei Jiang, Debasish Boral and Daotai Nie. Cancer Stem Cells in Resistance to Cytotoxic Drugs: Implications in Chemotherapy. B. Bonavida (ed.), Molecular Mechanisms of Tumor Cell Resistance to Chemotherapy, Resistance to Targeted Anti-Cancer Therapeutics 1, DOI: 10.1007/978-1-4614-7070-0_8, Springer Science+Business Media New York 2013.

Research articles published:

The manuscript is in the process of revisions.

Conclusions and significance (So what?):

The studies will determine whether tumor Oct4 is a novel driver of prostate carcinogenesis. The research will validate tumor Oct4 as a target of intervention to eliminate tumorigenic and metastatic cells, providing a new avenue to reduce or eliminate prostate cancer.

APPENDICES

N/A

SUPPORTING DATA

Embedded in the reporting body

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