

Award Number: W81XWH-12-1-0113

TITLE: Genetic and epigenetic biomarkers for recurrent prostate cancer after radiotherapy

PRINCIPAL INVESTIGATOR: Jong Park

CONTRACTING ORGANIZATION: H. Lee Moffitt Cancer Center and Research Institute
Tampa, FL 33612-9416

REPORT DATE: May 2013

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE May 2013		2. REPORT TYPE Annual		3. DATES COVERED 1 May 2012 – 30 April 2013	
4. TITLE AND SUBTITLE Genetic and epigenetic biomarkers for recurrent prostate cancer after radiotherapy				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-12-1-0113	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Jong Park E-Mail: jong.park@moffitt.org				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) H. Lee Moffitt Cancer Center and Research Institute Tampa, FL 33612-9416				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 It is not clear why some prostate cancers are aggressive and progress to metastasis. Accumulating evidence suggests that the angiogenesis pathway may play a critical role. The significance of angiogenesis in prostate cancer is demonstrated by its correlation with Gleason score, clinical stage, progression, metastasis and survival. However, relatively few studies have assessed the role of genes involved in angiogenesis in recurrence of prostate cancer after radiotherapy. On the basis of strong biological rationale, we propose to comprehensively study this pathway in a well-characterized cohort of prostate cancer cases. Our hypothesis is that genetic and epigenetic individual variation in angiogenesis genes is associated with recurrence of prostate cancer after radiotherapy. We will test this hypothesis with a systematic evaluation of the 82 key genes in the angiogenesis pathway with recurrence of prostate cancer. The ultimate goal of this study is to identify biomarkers that can be used at the time of diagnosis to predict risk of recurrence and improve clinical treatment decision making.					
15. SUBJECT TERMS Prostate cancer, recurrence, angiogenesis, radiotherapy.					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	7
Reportable Outcomes.....	7
Conclusion.....	7
References.....	7
Appendices.....	7

Introduction

In some patients with prostate cancer, the disease progresses relatively slowly. However, some cases grow aggressively and metastasize through the bloodstream and lymphatic system to other parts of the body. The most important clinical challenge for prostate cancer is to determine which of these two clinical forms a patient is presenting with. This information is critically important given the significant morbidity associated with treatment interventions and could eventually help distinguish men who need intensive treatment from those who may be better served by watchful waiting.

Among several treatment options, the primary treatment options for initial therapy for localized prostate cancer are radical prostatectomy or radiotherapy. Radical prostatectomy is considered an appropriate therapy for patients who have a life expectancy of 10 years or more and no serious co-morbid conditions. Among prostate cancer patients who had a radical prostatectomy, -30% will have recurrence [7]. The side effects of prostatectomy are urinary incontinence, erectile dysfunction, and typical post-operative complications.

Radiation therapy (RT) shows several distinct advantages over radical prostatectomy. RT avoids complications from surgery as well as risks associated with anesthesia. Moreover, this therapy includes a low risk of urinary incontinence. Major disadvantage of external beam RT include a treatment course of 8-9 weeks. -50% of patients have some temporary bladder or bowel symptoms during treatment. There is a risk of protracted rectal symptoms from radiation proctitis, and the risk of erectile dysfunction increases over time. Brachytherapy, another type of RT, involves placing radioactive sources into the prostate tissue. Disadvantages of this treatment include the risk of acute urinary retention.

Currently, the level of PSA, clinical stage and the Gleason score are used to estimate prognosis and inform treatment modalities (1). Although they are extremely useful, additional biomarkers are needed to better predict the outcome of prostate cancer. It is not clear why some prostate cancers are aggressive and progress to metastasis.

Accumulating evidence suggests that the angiogenesis pathway may play a critical role. The significance of angiogenesis in prostate cancer is demonstrated by its correlation with Gleason score, clinical stage, progression, metastasis and survival (2-4). However, relatively few studies have assessed the role of genes involved in angiogenesis in recurrence of prostate cancer after radiotherapy. Research to identify the specific genes and genetic variations relevant to angiogenesis risk among prostate cancer patients with radiation therapy remain largely unexplored. Part of the reason why previous results have been inconclusive may be that a major source of genetic regulation has been ignored: gene silencing through epigenetics. On the basis of strong biological rationale, we propose to comprehensively study this pathway in a well-characterized cohort of prostate cancer cases. Our hypothesis is that genetic and epigenetic individual variation in angiogenesis genes is associated with recurrence of prostate cancer after radiotherapy.

We will test this hypothesis with a systematic evaluation of the key genes in the angiogenesis pathway with recurrence of prostate cancer. The ultimate goal of this study is to identify biomarkers that can be used at the time of diagnosis to predict risk of recurrence and improve clinical treatment decision making.

Body

Task 1: Since we had an IRB approval for this project, we identified and confirmed 1499 prostate cancer patients, including 262 confirmed recurrent cases, who had a radiation therapy as a primary treatment between 1987 and 2003 at Moffitt Cancer Center. 346 patients were excluded from the

study because they were not treated with radiation therapy as a primary option. Clinical and demographic information of these patients were collected (Table 1).

Table 1. Characteristics of prostate cancer patients, who had a radiation therapy

Variables		Recurrent N (%)	Non-Recurrent N (%)
Stage	1	51 (20)	177 (16)
	2	138 (55)	812 (73)
	3	42 (18)	90 (8)
	4	20 (7)	32 (3)
Age at diagnosis		65.7 ± 7.3	66.7 ± 7.2
Race	White	255 (97)	1165 (95)
	Black	7 (3)	57 (5)
Death	Alive	118 (82)	808 (65)
	Dead	144 (18)	164 (35)
Total		262	1234

Task 2: To evaluate role of genetic variations in progression of prostate cancer, we used public data base and extensive literature search to identify candidate SNPs from 82 angiogenesis related genes which show significant differential expression in prostate tumor tissue, and selected 1,500 SNPs in these 82 genes using the binning algorithm based on linkage disequilibrium (Table 2).

In addition, ~200 DNA samples were prepared for genotyping. We will prepare 200 additional DNA samples as proposed.

Table 2. Angiogenesis related genes that are expressed in prostate tissues

Gene	Pro/Anti-angiogenesis	# of tagSNPs	# of cSNPs	Total # of SNPs
ANG	pro	17	0	17
ANGPT1	pro	127	0	127
ANGPT2	pro	90	0	90
CEACAM1	pro	4	2	6
COL18A1	anti	54	0	54
EGF	pro	26	7	34
EPHB4	pro	9	0	9
ERBB2	pro	6	0	6
F2	pro	7	0	7
FGF1	pro	65	0	65
FGF2	pro	37	0	37
FGFR4	anti	2	0	2
FLT1	anti	99	0	99
FN1	anti	41	4	45
HGF	pro	22	0	22

HSPG2	anti	39	22	62
IFNA1	anti	4	2	6
IGF1	pro	26	0	26
IGF1R	anti	194	0	194
IL10	anti	13	0	13
IL18	pro	9	0	9
IL6	pro	6	2	7
IL8	pro	2	0	2
JAG1	pro	30	0	30
KDR	pro	39	0	39
KLK3	anti	11	0	11
MMP11	pro	4	0	4
MMP14	pro	13	2	15
MMP15	pro	4	0	4
MMP16	pro	112	0	112
MMP2	pro	15	0	15
MMP7	pro	7	2	9
MMP9	pro	9	6	15
MUC1	pro	0	2	2
NPY	pro	11	0	11
PDGFRB	pro	45	2	47
PECAM1	pro	17	0	17
PGF	pro	6	0	6
PLAU	anti	4	2	6
PRL	anti	9	0	9
PROK2	pro	9	0	9
SLC5A8	anti	19	0	19
TF	pro	26	2	28
TFPI	anti	30	0	30
TGFB1	pro	9	4	13
THBS1	anti	7	0	7
THSD1	anti	4	0	4
TIMP1	anti	0	4	4
TIMP2	anti	41	0	41
TIMP3	anti	45	0	45
TNF	pro	2	0	2
VEGF	pro	6	0	6
Total		1436	64	1500

Task 3: We will evaluate the genetic variation effects in the candidate genes on recurrence after we obtain genotyping results.

Key Research Accomplishments

1. We constructed cohorts for prostate cancer patients who had radiation therapy between 1987-2003.
2. 262 confirmed recurrent cases were identified.
3. We prepared ~200 DNA samples for genotyping.

Reportable Outcomes

Evaluation of genetic profile in prostate cancer progression will be reported in next progress report.

Conclusion

Not available

References

1. Datan, Z.a.R., J, *Nomograms as a tool in predicting prostate cancer prognosis*. European Urology Supplements, 2009. **8**: p. 721-724.
2. Crawford, E.D., *Epidemiology of prostate cancer*. Urology, 2003. **62**(6 Suppl 1): p. 3-12.
3. Kai, L., et al., *Targeting prostate cancer angiogenesis through metastasis-associated protein 1 (MTA1)*. Prostate, 2010. **71**(3): p. 268-80.
4. Mucci, L.A., et al., *Prospective study of prostate tumor angiogenesis and cancer-specific mortality in the health professionals follow-up study*. J Clin Oncol, 2009. **27**(33): p. 5627-33.

Appendices

None