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
12902 Magnolia Dr.
Tampa FL
33612-9416

REPORT DATE: July 2015

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
July 2015	Final	1May2012 - 30Apr2015

4. TITLE AND SUBTITLE
Genetic and epigenetic biomarkers for recurrent prostate cancer after radiotherapy

5a. CONTRACT NUMBER
W81XWH-12-1-0113

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Jong Y. 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

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

Some prostate cancers are very aggressive and progress to metastasis. Accumulating evidence suggests that the angiogenesis pathway may play a critical role for this aggressiveness. 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
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	8	19b. TELEPHONE NUMBER *(include area code)*

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2015 Introduction

The goal of this study is to identify genetic and epigenetic biomarkers that can be used at the time of diagnosis to predict risk of recurrence and improve clinical treatment decision making. Although most prostate cancer progresses relatively slowly, some cases progress aggressively and metastasize to other parts of the body. The current clinical challenge for prostate cancer is to identify which of these two clinical forms a patient is presenting with. This information is critically important given the significant morbidity induced from treatment interventions and could be used to identify patients who need to treat aggressively from those who may not need to be treated.

One of the primary treatments for localized prostate cancer is radiotherapy. Although radiation therapy (RT) shows several distinct advantages over surgical treatment, such as no complications from surgery, and a low risk of urinary incontinence, RT treatment takes longer and many patients have some temporary bladder or bowel symptoms during treatment. Further, there is a risk of protracted rectal symptoms from radiation proctitis, and the risk of erectile dysfunction increases over time. The current best predictor for recurrence for local prostate cancer cases are PSA, clinical stage and Gleason score [1-3]. However, their accuracy is at best 70%. Therefore, additional biomarkers are needed to better predict the outcome of prostate cancer.

The angiogenesis pathway is well-known for progression of cancer. Most indicators for prostate cancer progression, Gleason score, metastasis, recurrence, and survival, are associated with angiogenesis [4-9]. Only a few studies have assessed the role of angiogenesis genes in recurrence of prostate cancer after radiotherapy. There are inconsistent reports on association between genetic variations in VEGF and recurrence [10, 11]. Therefore, we proposed to investigate genetic and epigenetic variations, a major part of gene regulation, of angiogenesis genes in progression of prostate cancer.

Table 1. Characteristics of prostate cancer patients who treated by radiation therapy

Variables	value	N (%)
Treatment	Radiation	713
Gleason score	2-6	358 (51.1)
	7	274 (39.2)
	8-10	68 (9.7)
Age at diagnosis	years	60.7 ± 6.7
Race	White	665 (93.3)
	Black	29 (4.1)
	Others	19 (2.6)
Recurrence	No	478 (68.3)
	Yes	235 (31.7)
PSA	≤4	36 (10.3)
	4-10	223 (64.1)
	≥10	89 (25.6)
Stage	1-2	245 (77.3)
	3-4	72 (22.7)
Family History	Y	104 (30.9)
	N	233 (69.1)

Body

Task 1: Since we initiated our project, we identified 713 prostate cancer patients, who had a radiation therapy as a primary treatment between 1987 and 2012 at Moffitt Cancer Center. Clinical and demographic information of these patients, including recurrence information were collected from either cancer registry or electronic/paper medical charts (Table 1). As expected, mean age at diagnosis is ~61 years old and family history of prostate cancer is higher than one in general population (0.31 vs. 0.15). Aggressive types of prostate cancer based on Gleason score (8-10), PSA level (>10ng/ml) and clinical stage (3 and 4) are 10-

25%. Recurrence rate after therapy is ~32%. Majority patients at Moffitt Cancer Center are Caucasians.

Task 2a: To evaluate a role of genetic variations in recurrences of prostate cancer, ~3,184 genetic polymorphisms from 527 angiogenesis genes were selected by extensive literature, and expression data, which show significant differential expression between prostate tumor tissue and normal tissues. Since we started our study, genotyping was performed on randomly selected 400 cases (200 recurrent and 200 non-recurrent cases) using illumine Goldengate SNPchip and the data were analyzed to evaluate a role of these polymorphisms in recurrence of prostate cancer. The representative results are presented in Table 2. With p value $\leq$0.001, we identified 14 SNPs in 11 angiogenesis genes. ANGPT1, FGF12, FGFR2, are IL16 are well known angiogenesis genes, which were involved in various cancers, including prostate cancer. Each SNP significantly influenced risk for progression of prostate cancer. For statistical analysis, the primary endpoint was recurrent free survival (RFS), was defined as the interval from the date of therapy to the date of disease recurrence or death or was censored on the date of the last contact. The associations of genetic variations with recurrence after radiologic therapy and RFS were investigated in univariate analyses using Kaplan-Meier estimates and log-rank tests. Cox proportional-hazards regression models were used in multivariate analyses and were adjusted for age. The statistical software package SAS/STAT 12.3 (SAS Institute, Cary, NC) was used for all analyses. Two sided tests were used for all analyses. P values<0.005 were significant and were not adjusted for multiple testing.

Table 2: SNPs associated with recurrence of prostate cancer (p<0.001).

SNP	Gene	p value	HR	Minor	MAF
rs10955455	ANGPT1	0.000	0.62 (0.49-0.78)	C	0.39
rs1654680	ANGPT1	0.000	1.68 (1.30-2.17)	A	0.07
rs2514864	ANGPT1	0.000	1.87 (1.36-2.56)	A	0.33
rs684	ITGB2	0.000	2.04 (1.40-2.97)	A	0.25
rs198605	P2RY5	0.000	1.51 (1.22-1.88)	C	0.08
rs12745851	DDX20	0.000	1.52 (1.21-1.92)	A	0.18
rs859	IL16	0.001	1.37 (1.15-1.64)	G	0.25
rs1460924	FGF12	0.001	1.49 (1.19-1.87)	A	0.24
rs1047100	FGFR2	0.001	1.48 (1.18-1.87)	A	0.22
rs1131445	IL16	0.001	1.33 (1.13-1.58)	G	0.33
rs3209148	FGF12	0.001	1.58 (1.21-2.08)	A	0.44
rs4778641	IL16	0.001	1.33 (1.12-1.58)	G	0.37
rs1960669	FGF2	0.001	2.69 (1.47-4.92)	A	0.13
rs3759509	LECT1	0.001	0.67 (0.53-0.86)	A	0.44

As expected, mean age at diagnosis is ~61 years old and family history of prostate cancer is higher than one in general population (0.31 vs. 0.15). Aggressive types of prostate cancer based on Gleason score (8-10), PSA level (>10ng/ml) and clinical stage (3 and 4) are 10-25%. Recurrence rate after therapy is ~32%. Majority patients at Moffitt Cancer Center are Caucasians.

Task 2b: We recently performed an epigenetic study with a relatively newly developed Illumina Infinium HumanMetylation 450K Beadchip microarray, high density beadchip, for this study. This array includes 485,577 CpG sites and covers CpGs in 99% of genes and 96% of CpG islands in the human genome. These assays were run by the Moffitt Cancer Center, Molecular Genomics Core. We profiled the epigenetic landscape of 60 prostate tumors using bisulfite treated tumor DNA samples extracted from 30 recurrent and 30 non-recurrent cases (Table 3).

We identified 20 differentially methylated CpG sites in 17 genes between recurrent and non-recurrent tumor tissues, with a false discovery rate (FDR) [12] q-value less than 0.05 and a mean methylation

difference greater than 0.1 between the two groups. Data were analyzed by Biostatistics and Bioinformatics Core facilities based on methods by current literatures including Chen *et al.* [13]. The representative results are presented in Table 3. None of genes identified in Task 2a, was differentially methylated in recurrent cases. We identified various genes, such as tumor suppressor gene (EHD2), alcohol dehydrogenase (ALDH16A1), transporters (SLC44A4, SLC35E2), kinase (NOL9), mitochondrial protein (MRPS27), and protein binding (SNCAIP).

Table 3: Differentially methylated CpG sites associated with recurrence of prostate cancer (p<0.000001)

Chr	location of CpG	Gene	Mean Difference	p value
6	Body	C6orf134	-0.12	0.00000
19	Body	ALDH16A1	-0.11	0.00000
1	Body	NOL9	-0.10	0.00000
9	Body	TRUB2	-0.11	0.00000
6	Body	LOC285830	-0.10	0.00000
1	Body	RPS10P7	-0.11	0.00000
5	Body	SNCAIP	0.30	0.00000
6	Body	SLC44A4	-0.10	0.00000
19	5'UTR	EHD2	-0.11	0.00000
5	Body	MRPS27	-0.11	0.00000
17	Body	SAMD14	-0.11	0.00000
7	Body	C7orf46	0.11	0.00000
15	Body	MYEF2	-0.11	0.00000
X	Body	AIFM1	-0.10	0.00000
10	Body	TNKS2	-0.12	0.00000
3	3'UTR	TPRG1	0.10	0.00000
6	Body	SH3BGRL2	-0.11	0.00000
5	Body	PLEKHG4B	-0.10	0.00000
1	3'UTR	SLC35E2	-0.18	0.00000
6	5'UTR	FAM184A	-0.11	0.00000

Twenty differentially methylated CpG sites in 17 genes between recurrent and non-recurrent tumor tissues, with a false discovery rate (FDR) q-value less than 0.05 and a mean methylation difference greater than 0.1 between the two recurrent and non-recurrent groups. We identified various genes, such as tumor suppressor gene (EHD2), alcohol dehydrogenase (ALDH16A1), transporters (SLC44A4, SLC35E2), kinase (NOL9), mitochondrial protein (MRPS27), and protein binding (SNCAIP).

Task 3: We evaluated SNP, DNA methylation, and combined effects on recurrences. As we proposed, we constructed Receiver Operating Characteristic curves and compared the areas under the curve between a model with clinical variables that currently accepted as clinical nomogram and our model which will also include SNP and DNA methylated biomarkers (Fig. 1)

As expected, nomogram based on clinical variables showed 77% (95%CI, 0.65-0.88), p<0.001 prediction accuracy. Accuracy based on our biomarkers, SNP and DNA methylation are 72%, (95% 0.62-0.81) p<0.001 and 95% (95% 0.94-0.996) p<0.001 respectively. Epigenetic biomarkers contribute significantly on accurate prediction on recurrence. Combined model with all variables, clinical, SNP and DNA methylation showed 96% accuracy based on our population. These data are promising but we need to validate these results in larger

Key Research Accomplishments in study period

1. Since our study initiated, we constructed cohorts (n=713) for prostate cancer patients who had radiation therapy between 1987-2012 (Table 1).
2. Randomly selected 400 DNA samples were genotyped with 3,000 SNPs selected from genes involved in angiogenesis pathway. We identified SNPs significantly associated with recurrence of prostate cancer. (Table 2)

3. In epigenome wide scale analysis, we identified differentially methylated genes in recurrent cases (Table 3).
4. After evaluation of accuracy of different prediction models, we found DNA methylation contribute significantly on accuracy of prediction model (Fig. 1)

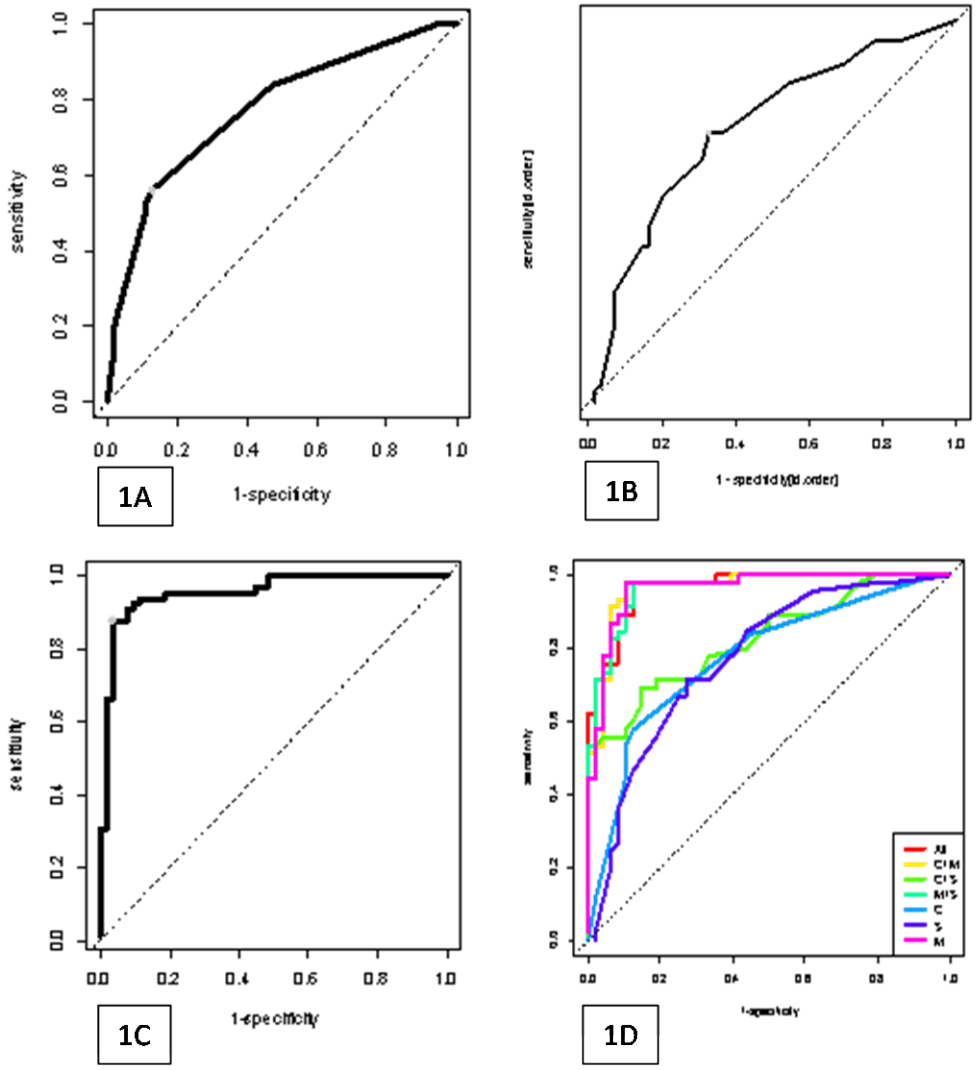

Fig.1: ROC curves based on different variables: 1A: clinical variables only. AUC 0.77 (95%CI, 0.65-0.88), p<0.001. 1B: SNP only. AUC 0.72 (95% 0.62-0.81) p<0.001. 1C: DNA methylation markers only. AUC 0.95 (95% 0.94-0.996) p<0.001. 1D: combined variables. Epigenetic biomarkers contribute significantly on accurate prediction on recurrence.

Reportable

Outcomes

We identified SNPs, DNA methylation which are significantly associated with recurrence. Our biomarkers significantly enhance accuracy of nomogram, which is currently use in clinical setting

Conclusion

Biomarkers for recurrence, especially epigenetic biomarkers are promising candidates to investigate further for translation study.

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Acronym.

radiation therapy (RT)
prostate specific antigen (PSA)
single neuclotide polymorphism (SNP)
recurrent free survival (RFS)
false discovery rate (FDR),

Appendices

None