TELOMERASE PATHWAY RNA EXPRESSION AS A PROGNOSTIC MARKER FOR LETHAL AND METASTATIC PROSTATE CANCER

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2016

To my family and friends

ACKNOWLEDGMENTS

I would like to thank my committee members Dr. Travis Gerke, Dr. Lusine Yaghjyan, and Dr. Catherine W. Striley who provided constructive advice throughout the thesis process. I extend my sincere appreciation to my adviser Dr. Gerke for his guidance and assistance in my academic and professional growth during my master's program. During the thesis preparation, Dr. Gerke has provided me with inspiring advice regarding statistical methods and thesis writing.

I would like to thank the Department of Epidemiology. Each member of the faculty and staff have offered invaluable knowledge and experience in Public Health.

I would like to thank my family who support and encourage me to pursue my goals. Their love makes me believe I can accomplish it all.

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By

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August 2016

Chair: Travis Gerke
Major: Epidemiology

Background: Current tools to predict the likelihood of prostate cancer metastasis and recurrence are highly sensitive but not specific, and may contribute to high rates of overtreatment for non-aggressive cancers. Telomerase activation has been implicated in tumor formation across several cancer types. We examined whether telomerase-related gene expression from tumor tissue at diagnosis could improve prediction accuracy for prostate cancer metastasis and mortality. We also sought to replicate a previously reported interaction between telomerase activity and TP53 expression on aggressive disease status.

Methods: This study is a secondary analysis of data from 545 patients who were participants in the Mayo Clinic Radical Retropubic Prostatectomy (RRP) Registry. Cases were defined as patients who experienced clinical metastasis after prostatectomy (n=212) over 16.7 median follow-up years and controls were patients who had no clinical signs of disease progression for at least 7 years. A subset of 5 genes, TERT, TERC, TEP1, DKC1 and PINX1, were selected for this investigation on the basis of their known relationship with telomerase activation. A gene expression score was derived

from these genes to assess the ability of telomerase to predict metastatic or lethal disease. The prognostic performance of each gene and a composite gene score was compared with Gleason score, and a previously identified interaction with TP53 was investigated.

Results: In both crude and Gleason-adjusted analyses, DKC1 appeared as significant predictor of metastasis. In the adjusted model, each standard deviation increase in DKC1 expression increased the odds of metastasis by a factor of 2.35 (95% CI1.31, 4.32). Genes TERC, TEP, and PINX1 did not reach significance in univariable or multivariable logistic regression models. Interactions with TP53 on metastatic disease were observed for TERT and DKC1. The odds of metastatic disease increased by a factor of 3.31 (95% CI 1.40, 8.13) for each standard deviation increase in DKC1 in individual with low TP53 expression. For intermediate TP53 expression, the odds ratio was 0.42 (95% CI: 0.20, 0.90) for each standard deviation increase in TERT.

Discussion: Telomerase gene TERT and DKC1 may be able to improve prognostic scoring in prostate cancer patients, particularly for those with TP53 deficient tumors.

CHAPTER 1
INTRODUCTION

Prostate cancer is the most common non-skin cancer and second leading cause of cancer-related death among American men [1]. The American Cancer Society estimated there will be 180,890 new cases and 26,120 deaths from prostate cancer in 2016 [2]. Changes in incidence rate will depend heavily on screening guidelines and intensity; a recent study estimated that there are approximately 45 million undiagnosed prostate cancers in the US [3]. Prognosis for prostate cancer is generally favorable: the five-year relative overall survival is 98.9% [1]. However, the five-year survival of metastatic prostate cancer is 67.3% [4]. The American Cancer Society also estimated that the US had 2.9 million prostate cancer survivors in 2014 and that this number will increase to 4.1 million by 2024 [5].

Gleason Grading System

Currently, Gleason score is considered the most accurate marker for prostate cancer prognosis [6, 7]. Gleason score is a grading system which is used by a pathologist to evaluate prostate tumor tissue from biopsy or surgery. Gleason score has been regarded as a reliable approach to help evaluate cancer aggressiveness and to offer a reference guide in therapy and survival prediction[8]. While Gleason score has become one of the most essential tools for predicting metastasis after radical prostatectomy, it lacks specificity and may not provide clear prognostication for men diagnosed with low-risk disease [9]. A study has estimated that 45.8% of new diagnoses involve low risk tumors [10]. Nonetheless, most prostate cancers are treated. Radical prostatectomy and external beam radiation therapy (EBRT) were commonly used among patients with Gleason score ≤6 cancer [11, 12]. Active surveillance may be a

more desirable therapeutic strategy for low-risk prostate cancers due Quality of Life concerns [13]. While Gleason scores range from 2 to 10, a score of 5 is the lowest grade commonly used assigned after reading the biopsy samples [7]. Due to poor correlations with prostatectomy grade and poor reproducibility, tumors with Gleason score below 4 are rarely considerable diagnosed [14]. A Gleason score of 8 or higher is very sensitive for aggressive disease, however, large amount of patients, nearly 68%, are diagnosed with Gleason score<8 [7, 10]. Thus, Gleason score alone may be insufficient to predict likely cancer outcomes. Therefore, researchers are seeking novel biomarkers to better understand cancer development and to help predict tumor metastasis. By dealing with this problem, overtreatment in low-risk cancer can be relieved [15].

Telomeres and Telomerase

Telomeres are found at the ends of chromosomes. Studies have shown that telomere shortening is associated with age related diseases, such as late stage cancer, type 2 diabetes [16] and heart disease [17]. Evidence suggests that tumor tissue has less extensive telomere shortening. By comparison, tissue specimen from normal prostate or benign prostatic hyperplasia exhibits substantial telomere shortening [18, 19]. For both normal and tumor cells, shortened telomeres can result in chromosomal damage and instability which, in turn, can lead to cell apoptosis [20, 21]. However, reactivation of telomerase makes cancer cells long lived, allowing them to continuously multiply without telomere erosion [19]. Telomerase is a reverse transcriptase that acts to repair and maintain telomeres. In most somatic cells, telomerase is inactivated or at low levels, but high levels of telomerase activities are detected in cancer tissue and early stages of oncogenic processes [22].

Telomerase Related Genes

There are several genes that have been implicated in telomerase activity in the literature. And the specific genes involved in telomerase activity may vary by tissue type. No gene expression signature of telomerase activity in the prostate exists. We selected a set of 5 genes, TERT, TERC, TEP1, DKC1 and PINX1 [23-27], to represent the telomerase pathway. TERT (telomerase reverse transcriptase) and TERC (telomerase RNA component) are major telomerase encoding genes [28, 29], though studies in most somatic cells and adult stem cells have not observed adequate expression levels of telomerase (TERT and TERC) to protect telomere length and tumor growth [30]. TEP1 (Telomerase associated protein 1) and TERT were previously associated with prostate cancer risk [31]. One of DKC1 (Dyskeratosis Congenital 1, dyskerin)'s functions is to stabilize and maintain telomerase activity. Studies show that DKC1 is highly expressed in prostate cancer cases, particularly in high-stage and recurring cases [24], and similar trends have been observed in other cancers [32]. This suggests that overexpression of DKC1 in tumor tissue might be reflective of tumor aggressiveness. PINX1 (PIN2/TERF1 Interacting, Telomerase Inhibitor 1) is a protein coding gene which is regarded as a potential telomerase inhibitor [33, 34]. Even though the mechanism by which PINX1 inhibits telomerase remains unclear, PINX1 was suggested to be a tumor suppressor due to its location on chromosome 8 near microsatellite marker D8S277 [26, 33]. Previous studies have shown that tumor suppressor TP53 is involved in telomere and telomerase monitoring [35], and that TP53 deletion in mouse models is associated with chromosome instability [36] and telomere dysfunction [37]. When the tumor suppressor TP53 is deficient in tumors, short telomeres may cause chromosomal instability [29, 37,

38]. Telomere dysfunction that leads to telomerase reactivation has been regarded as a contributing factor to cancer development [39].

Several studies have indicated that telomerase plays an important role in cancer development [22, 40]. For example, TERT mutations in glioblastoma and thyroid cancers are associated with increased age at diagnosis [41]. With the message that telomerase changes in the cancer cell occur at specific points in the oncogenic process, it is possible that telomerase could serve as a prognostic marker at diagnosis for the development of aggressive prostate cancer. Few studies have investigated this possibility in a large cohort of prostate cancer patients with long-term follow-up [22]. Table 1-1 describes the biological characteristics of the selected telomerase genes.

Purpose of the Study

There is an urgent need to improve the identification of aggressive prostate cancer beyond Gleason score. The purpose of this study was to: 1) examine the association between the expression of telomerase related genes and the risk of cancer metastasis, 2) evaluate the prognostic power of Gleason score combined with telomerase pathway gene expression; 3) examine the interacting role of tumor suppressor TP53 and telomerase expression on metastatic progression.

Table 1-1. Summary description of telomerase genes.

Gene	Type of Gene	Cytogenetic band	Biological process/Molecular function	References
TERT	Protein coding gene	5p15.33	telomere maintenance, transcription, RNA-templated	Chang et al, 2002[42]; Maida et al, 2009[43]
TERC	RNA gene	3q26.2	telomerase RNA component, involved in telomere maintenance	Marrone et al, 2007[44]
TEP1	Protien coding gene	14q11.2	telomere maintenance via recombination, RNA-dependent DNA replication	Koyanagi, Y., et al, 2000[45]; Chang et al, 2002[42]
DKC1	Protien coding gene	Xq28	telomere maintenance, rRNA processing	Heiss, N. S., et al, 1999 [46].
PINX1	Protein Coding gene	8p23.1	regulation of telomerase activity, negative regulation of telomerase activity	Banik, S. S. and C. M. Counter, 2004[47];

CHAPTER 2
METHODS

Gene Selection and Study Design

RNA microarrays have been widely used to measure gene expression profiles from a variety of cellular sources including tumor tissue samples. This study leverages data from the Affymetrix Human Exon 1.0 ST array, which provides 1.4 million probes to measure the whole gene sections for RNA. Besides TERT and TERC, the major transcriptase of telomerase, additional related genes TEP1, DKC1 and PINX1 were selected to present the telomerase activation pathway based on the literature [23-27]. We analyzed existing data from a nested-case control study that recruited prostate cancer patients from the Mayo Clinic Radical Retropubic Prostatectomy (RRP) Registry [48]. Patients from the registry provided tumor tissue specimens for molecular profiling from radical prostatectomy. Raw and normalized data from this study were accessed from the publicly available National Center for Biotechnology Information's Gene Expression Omnibus database (GSE46691) [9]. Due to the limited annotation regarding telomerase genes in the available normalized data, we re-normalized the raw CEL files from Gene Expression Omnibus (GEO) using the RMA algorithm implemented in the package "oligo" [49].

Patient Population and Analysis

During the study, 639 patients were enrolled in the Mayo Clinic RRP Tumor Registry (GSE46691) [9]. Patients received radical prostatectomy and clinical data were recorded. The patients' follow-up was completed through 2008. After ruling out low-quality samples, 545 prostate cancer patients with available RNA data were included in our study. The selecting procedure [9] is shown in Figure 2-1.

Cancer patients who experienced metastasis were assigned into the case group and patients who had no detectable clinical metastasis in 5 years of follow-up were classified into the control group.

To assess external validity of the prediction models, two-thirds of 545 patients (n=363) were randomly chosen for model training, and the remaining cases and controls (n=182) were assigned as a test set. To evaluate the interaction factor, tertiles of gene expression were selected as a cutoff points to divide samples into three groups.

Statistical Analysis

First, we computed descriptive statistics to summarize the data and report the frequencies of metastatic events among different levels of Gleason score. Logistic regression and Receiver Operating Characteristic (ROC) methods were used to measure the prognostic accuracy of the models. To avoid the multiple comparison mistake, the *P*-value of the tests were Bonferroni corrected. Statistical software R 3.2.2 with package “pROC 1.8” and “oligo version 1.32.0” was used to normalize and analyze RNA data. Changes in the area under the curve (AUC) were measured in the test data to evaluate the added prognostic value of telomerase expression beyond Gleason score. An interaction between the tumor suppressor gene P53 and telomerase expression and cancer metastasis was also evaluated in the full data set [39].

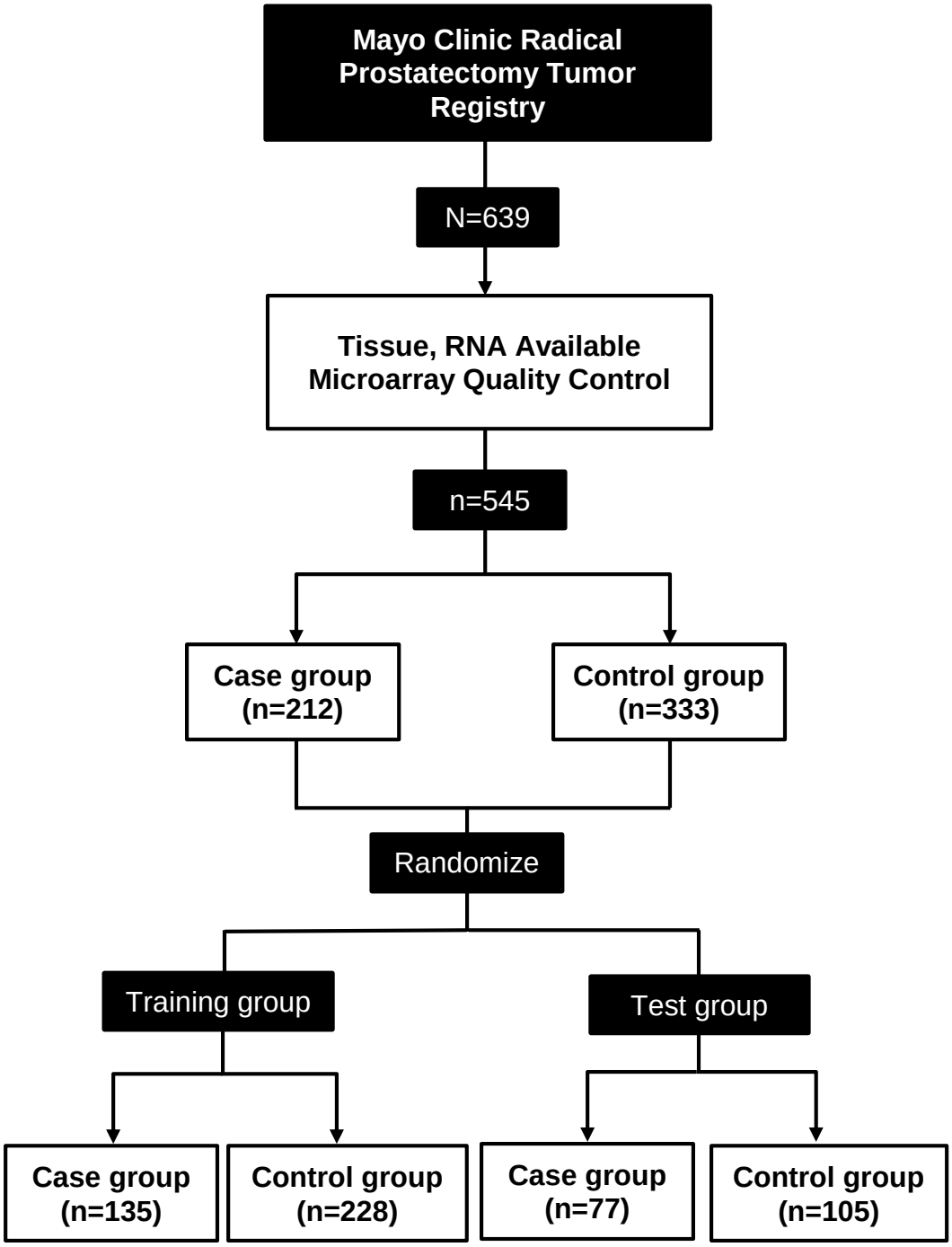

Figure 2-1. Selected diagram. Study sorted by Cases and Controls. Patients: 1987-2001.

CHAPTER 3
RESULTS

Characteristics of Study Population

There were 545 participants who underwent radical prostatectomy for the treatment of primary prostate cancer and who had tumor tissue RNA expression quantified through microarrays. Cases were defined as those patients with distant metastasis or death from prostate cancer (n=212), and controls survived at least 7 years with no evidence of biochemical recurrence (BCR) (n=333). Demographic and clinical information for this study has been described in detail elsewhere [9]; of note, the median age of the participants was 66 years, and median follow-up was16.7 years.

Table 3-1 shows the clinical diagnostic patterns of the prostate cancer patients. The results reveal that 61.6% (n=130) of cases were described as Gleason score higher than 8, 6 cases were diagnosed with grade lower than 6, and nearly 90% of the patients in Gleason score ≤ 6 group were controls. 72% (n=195) of patients in Gleason grade 7 were controls. As the previous study shows, 52 (23.7%) and 95 (37.5%) of cases had T2 and T3/4 disease respectively. For the Prostate-specific antigen (PSA) test, 106 (37.5%) cases were <10 ng/mL and 28 (21.4%) were >20 ng/mL.

Table 3-2 illustrates the distributional characteristics of training and test group after the randomized reclassification.

Trends of Telomerase Genes and TP53 Expression

Figure 3-1 illustrates the overall distribution of telomerase related gene expression levels between cases and controls. Few apparent differences appear comparing cases to controls. However, several trends appear within Gleason strata. For example, TERT and TERC are inversely related to Gleason score (Figure 3-1A and 3-1B). One-way

analysis of variance has shown the expression levels of TEP1 (Figure 3-1C) and PINX1 (Figure 3-1E) increase with Gleason score (P<0.01). For the tumor suppressor TP53 (Figure 3-1F), higher expression was observed in Gleason score≤6 relative to Gleason groups 7 and ≥8.

Telomerase Gene Performances

Table 3-3 provides the odds ratios estimated from logistic regressions for each telomerase related gene in crude and Gleason-adjusted models. In unadjusted analysis, after the Bonferroni correction we found gene DKC1 (P<0.01) is statistically significant in predicting metastasis, with an odds ratio of 2.59 (95%CI: 1.49, 4.57) for each standard deviation increase in expression. When DKC1 was categorized based on median score into high and low expression, the odds ratio was 1.74 (95%CI: 1.23, 2.47). Results for PINX1 were suggestive in the crude model, with an odds ratio of 2.11 for each standard deviation increase in PINX1 expression.

Upon adjustment for Gleason score, only the DKC1 model was statistically significant (P=0.004) with an estimated OR of 2.35 (95% CI: 1.31, 4.32) for each unit increase in expression. The significance of DKC1 may suggest that a telomerase gene could complement Gleason grading for prognosis. Of note, however, primary telomerase genes TERT and TERC were not significant predictors in either of the models.

The Interaction Between Telomerase and TP53

To evaluate the interaction of telomerase with TP53 on metastatic disease, we applied logistic regression to analyze the models from full dataset with and without the adjustment of Gleason score. Significant interactions with TP53 were observed for TERT and DKC1. Table 3-4 shows that, within high levels of TP53, intermediate and

high expression of TERT has protective odds ratios of 0.41 (95% CI: 0.19, 0.89) and 0.42 (95% CI: 0.20, 0.90), respectively. In the adjusted model, intermediate levels of TERT give an odds ratio of 0.42 (95% CI: 0.18, 0.96) when TP53 is in intermediate. For low levels of TP53, high expression of DKC1 produces an odds ratio of 2.89 (95% CI: 1.30, 6.66) in the crude model, and an OR of 3.31 (95% CI: 1. 40, 8.13) in the Gleason-adjusted model compared to low DKC1 expression. For intermediate levels of TP53, gene DKC1 remains significant with an OR of 2.475 (95% CI: 1.14, 5.37) for high expression in the crude model. Full results for the remaining genes are provided in Table 3-4.

Telomerase and Prediction of Metastasis

To assess the area under the ROC curve (AUC), we split the prostate cancer patients into training and test sets. And Figure 3-2 shows the ROC curves of each telomerase RNA genes compared with the ROC of Gleason score in the test data. The AUC value of Gleason is 0.73 (95% CI: 0.67, 0.80). AUC values for TERT (Figure 3-2A), TERC (Figure 3-2B) and TEP1 (Figure 3-2C) are 0.73, 0.71 and 0.72 when respectively added to Gleason. DKC1 (0.75) and PINX1 (0.75) are slightly better than Gleason score alone. The ROC of the integrated model, which was a linear combination of all five telomerase genes, was slightly lower than Gleason grade.

Table 3-5 illustrates the AUC of each Gleason-adjusted telomerase gene model and its comparison with the AUC of Gleason alone. In each model, each telomerase was combined with Gleason score and for the last model, all telomerase genes were linearly combined with Gleason. There are no significant differences between Gleason only model and those with telomerase added.

Table 3-1. Characteristics of metastasis and non-metastasis group

	Cases	Controls
	N (row %)	N (row %)
Pathological Stage*		
pT2N0M0	52(23.7)	167(76.3)
pT3/4N0M0	95(37.5)	158(62.5)
pTanyN+M0	45(61.6)	28(38.4)
Pathologic Gleason Score		
≤ 6	6(9.5)	57(90.5)
7	76(28)	195(72)
≥ 8	130(61.6)	81(38.4)
Pre-operative Prostate-specific Antigen*		
<10 ng/mL	106(37.5)	177(62.5)
10–20 ng/mL	31(26.7)	85(73.3)
>20 ng/mL	28(21.4)	103(78.6)
Not available	6(40)	9(60)

* the distribution data is from the paper Erho, N., et al.[9]

Table 3-2. Randomly selected training and test groups.

Gleason Category	Training group			Test group		
		Cases	Controls		Cases	Controls
	N	N (row %)	N (row %)	N	N (row %)	N (row %)
⩽ 6	47	5(10.6)	42(89.4)	16	1(6.3)	15(93.7)
7	134	79(59.0)	55(41.0)	77	51(66.2)	26(33.8)
≥ 8	182	51(28.0)	131(72.0)	89	25(28.1)	64(71.9)

Table 3-3. Unadjusted and adjusted odds ratio for telomerase RNA by Gleason Score.

	Unadjusted		Adjusted	
	OR(95%CI)	P-value	OR(95%CI)	P-value
TERT	0.34 (0.11-1.06)	0.322	0.66 (0.196-2.20)	1.000
TERC	0.68 (0.42-1.10)	0.605	0.89 (0.53-1.50)	1.000
TEP1	3.24 (0.94-11.35)	0.322	1.54 (0.41-5.89)	1.000
DKC1	2.59 (1.49-4.57)	0.004	2.35 (1.31-4.32)	0.024
PINX1	2.11 (1.20-3.73)	0.05	1.35 (0.73-2.51)	1.000

*The P-values were Bonferroni corrected.

Table 3-4. Odds ratio of interaction with TP53 of telomerase RNA in unadjusted and adjusted model.

	TP53 low		TP53 intermediate		TP53 high	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
	OR(95%CI)	OR(95%CI)	OR(95%CI)	OR(95%CI)	OR(95%CI)	OR(95%CI)
TERT						
Low	1 [Reference]	1 [Reference]	0.72 (0.35,1.46)	0.74 (0.35,1.59)	1.00 (0.49,2.03)	1.28 (0.60,2.75)
Intermediate	1.00 (0.49,2.04)	0.19 (0.55,2.57)	1.08 (0.52,2.22)	1.34 (0.61,2.94)	0.41 (0.19,0.89)*	0.42 (0.18,0.96)*
High	0.51 (0.24,1.06)	0.56 (0.25,1.22)	0.92 (0.43,1.95)	1.31 (0.58,3.01)	0.42 (0.20,0.90)*	0.47 (0.21,1.07)
TERC						
Low	1 [Reference]	1 [Reference]	0.84 (0.40,1.78)	0.83 (0.37,1.84)	0.69 (0.35,1.37)	0.79 (0.38,1.63)
Intermediate	0.68 (0.34,1.38)	0.79 (0.37,1.68)	0.91 (0.42,1.93)	1.30 (0.57,2.97)	0.61 (0.28,1.31)	0.84 (0.37,1.93)
High	0.70 (0.34,1.45)	0.84 (0.38,1.82)	0.67 (0.31,1.45)	0.94 (0.41,2.19)	0.71 (0.34,1.46)	0.93 (0.42,2.03)
DKC1						
Low	1 [Reference]	1 [Reference]	1.03 (0.42,2.50)	1.24 (0.48,3.23)	1.13 (0.52,2.53)	1.42 (0.61,3.37)
Intermediate	1.86 (0.85,4.21)	1.76 (0.76,4.20)	1.44 (0.66,3.16)	1.84 (0.78,4.33)	1.09 (0.51,2.36)	1.19 (0.52,2.74)
High	2.89 (1.30,6.66)*	3.31 (1.40,8.13)*	2.48 (1.14,5.37)*	1.90 (0.83,4.37)	1.29 (0.63,2.63)	1.28 (0.59,2.78)
TEP1						
Low	1 [Reference]	1 [Reference]	0.75 (0.33,1.72)	0.95 (0.39,2.34)	0.49 (0.22,1.06)	0.65 (0.28,1.50)
Intermediate	0.81 (0.37,1.79)	0.85 (0.36,2.01)	1.46 (0.70,3.04)	1.52 (0.69,3.39)	1.94 (0.92,4.10)	1.62 (0.72,3.62)
High	1.14 (0.53,2.49)	1.10 (0.48,2.55)	0.94 (0.44,2.02)	0.70 (0.31,1.62)	1.43 (0.68,3.01)	1.38 (0.62,3.08)
PINX1						
Low	1 [Reference]	1 [Reference]	1.57 (0.62,4.16)	1.39 (0.50,3.97)	0.99 (0.34,2.92)	1.17 (0.37,3.79)
Intermediate	2.01 (0.71,5.95)	1.83 (0.59,5.89)	1.57 (0.25,9.74)	6.81 (1.60,28.91)*	3.01 (0.86,10.53)	2.98 (0.76,11.60)
High	1.28(0.49,3.46)	1.14 (0.40,3.31)	0.75 (0.22,2.55)	0.74 (0.20,2.83)	0.90 (0.27,2.96)	0.56 (0.15,2.04)

* $p < .05$; ** $p < 0.01$; *** $p < .001$ based on multinomial logistic regression results.

Table 3-5. AUC of Gleason score and telomerase related gene.

	AUC (95% CI)	*P*-value
Gleason score	0.7346 (0.6667-0.8025)	—
TERT + Gleason score	0.7349 (0.6605-0.8094)	0.981
TERC + Gleason score	0.7132 (0.636-0.7903)	0.159
TEP1 + Gleason score	0.7223 (0.6461-0.7985)	0.449
DKC1 + Gleason score	0.7491 (0.6767-0.8216)	0.317
PINX1 + Gleason score	0.7478 (0.6748-0.8208)	0.399
All telomerase Adjusted	0.7284 (0.6532-0.8036)	0.701

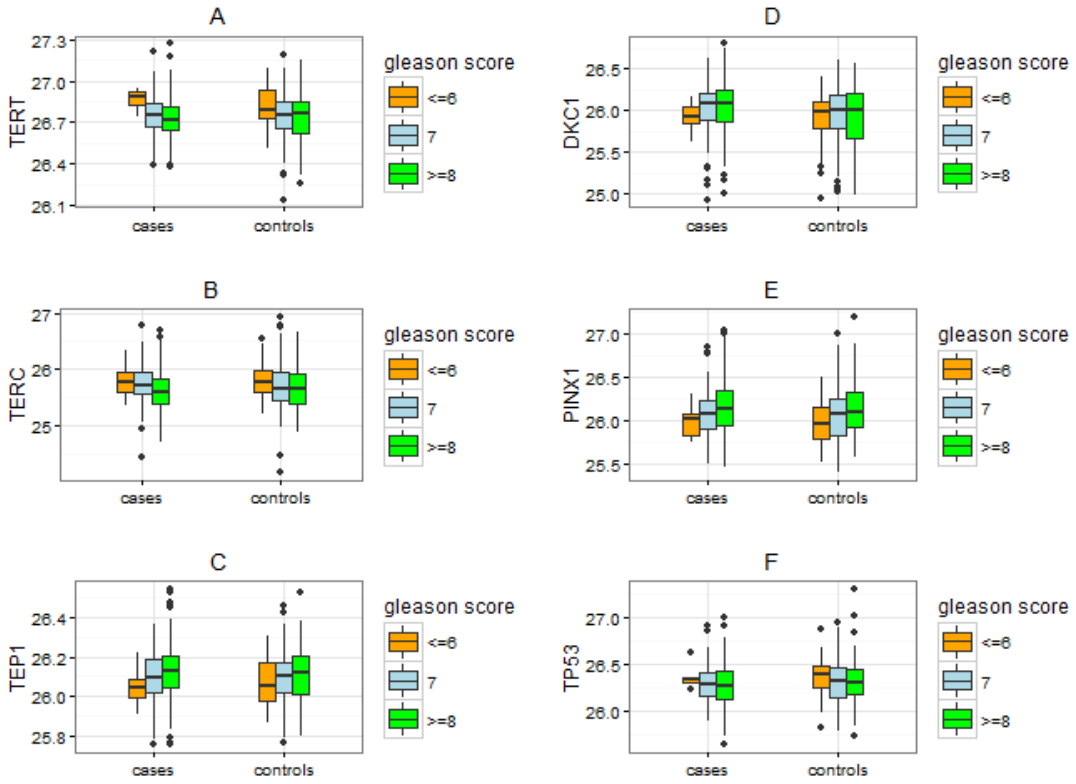

Figure 3-1. RNA expression distribution of telomerase genes and TP53 between case and control groups.

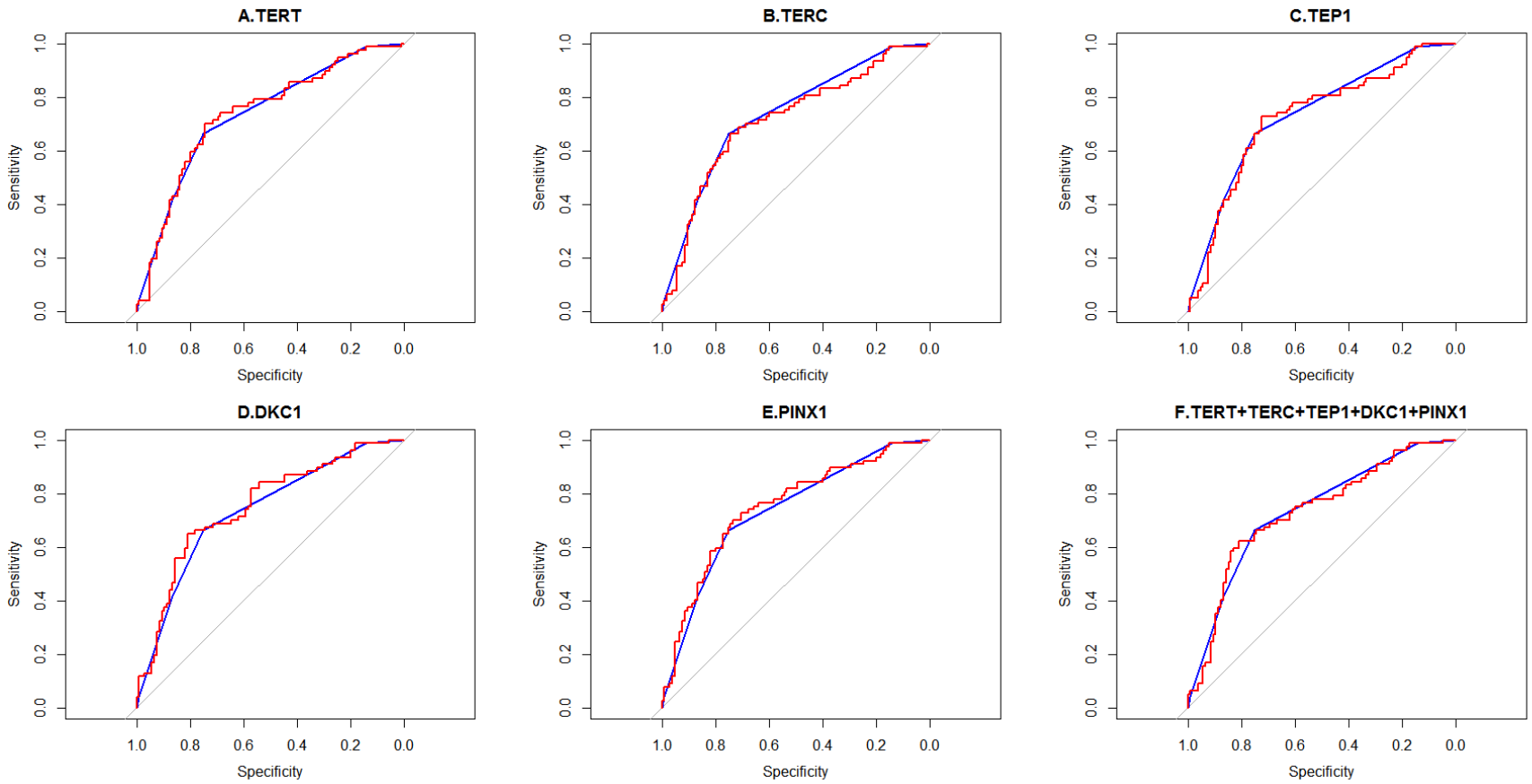

Figure 3-2. ROC curve of Gleason Score and Telomerase genes for prostate cancer metastasis. (a) ROC curve of TERT adjusted (AUC =0.7349) vs. Gleason (AUR curve =0.7346); (b) ROC of TERC adjusted (AUR curve=0.7132) vs. Gleason; (c) ROC of TEP1 adjusted (AUR curve=0.7223) vs. Gleason; (d) ROC of DKC1 adjusted (AUR curve=0.7491) vs. Gleason; (e) ROC of PINX1 adjusted (AUR curve=0.7478) vs. Gleason; and(f) ROC of all telomerase adjusted (AUR curve=0.7284) vs. Gleason.

CHAPTER 4
DISCUSSION

The purpose of this study was to assess the hypothesis that telomerase gene expression in primary prostate cancer has the capability to predict the development of clinical cancer metastasis. The secondary purpose was to examine the previously reported interaction between the tumor suppressor TP53 and telomerase-related gene expression. In addition to tests of association through logistic regressions, we evaluated whether telomerase markers could improve the discrimination of Gleason score by evaluating changes in AUC. Our findings are 1) most selected telomerase genes did not significantly improve the prediction of primary prostate cancer metastasis; 2) high DKC1 expression was a significantly predicted prostate cancer progression; and 3) DKC1's effect on metastasis was enhanced under low TP53 expression.

The TP53-telomerase interaction we observed is consistent with previous animal studies [27, 37, 39]. When TP53 was knocked out in prostate tumors, it revealed an increase in telomere structural instability and telomerase reactivation [39]. The present study showed that, in lower levels of TP53 expression, high expression of telomerase related genes like DKC1 were correlated with adverse outcomes. And under highly expressed TP53, the effects of other telomerase genes on outcome were insignificant.

Our finding indicates that TERT may play as a protective role in lethal and metastatic prostate cancer. From the expression trends, TERT is decreased with cancer progression. We hypothesize that, at prostate cancer initiation, TERT is highly expressed in tumor cell. However, expression is reduced by tumor multiplication. Moreover TP53's interaction may have inhibitory effects on TERT expression following tumorigenesis. This hypothesis is aligned with previous studies in vivo, which proved

that the reactivation of TERT occurs in most human tumors [50] and that TERT expression directed by β-Catenin was one of the hallmarks of oncogenesis [22]. The insignificance of TERT in human data may indicate that TERT's molecular interactions with other genes or factors in the human environment are complex. In the present study, TERT expression did not differ by Gleason score. Beyond the explanation that this may result from the complex molecular environmental factors that monitor telomere expression, it may also imply that TERT serves as a mechanism of mediation in cancer progression.

DKC1 was observed to deliver significant prognostic value in this study. This result is consistent with the Sieron's study which reported DKC1 overexpression in prostate cancer [24], particularly in high grade and recurrent cancer. Thus, DKC1 overexpression has potential capability to assist predicting metastasis as a biomarker. Moreover, as a gene to maintain telomere stability, the mechanism of DKC1 in cancer cell implies potential as a therapeutic marker [51].

We found that even though Gleason score is the most accurate prognostic marker in use, the AUC of 0.735 shows that as a predictive model, the Gleason score leaves room for improvement. Several previous studies have aimed to discover gene expression biomarkers in order to fulfill the need to predict aggressive prostate cancer development [9, 52-54]. Since Gleason score is graded based on the pathologic patterns of cancer tissue, gene expression and protein activities are likely to affect the medical prognosis of prostate cancer. Therefore, gene expression signatures may measure much of the same information as Gleason score grading.

One considerable strength of the present study is the large sample size and long follow-up period. Since most previous studies which focused on the discovery of telomerase were animal-based, a complete human analysis may be a robust and externally reliable source for testing the association of telomerase expression.

Limitations

This study has several limitations. First, the original study does not offer sufficient demographic and clinical information regarding the recruited patients for further control and stratification in our analysis. Such stratification could have examined the interactions of genes as well as the predicted power of cancer diagnosis. Second, the original study did not provide the patients' clinical therapy records during the following-up time period after they had been received radical prostatectomy. The clinical and medication therapies other than prostatectomy which have not been recorded in the data may be confounders to affect the tumor's RNA expression and the outcome of aggressive cancer recurrence. Nevertheless, the sensitivity of therapy reaction of prostate cancer is not as high as other type of cancers, such as breast cancer [55], so the effect of adjuvant therapies may be minimal. Moreover, the patients were all treated with radical prostatectomy although the grade of biomedical indicators may not necessarily need to receive the therapy. Patients' telomerase expression may be impacted by other unknown RNA networks.

Implications

A primary finding of the present study concerns the significant prognostic value of DKC1 for prostate cancer progression. Further research may characterize the activity of DKC1 in the prostate in order to better improve the accuracy of prostate cancer prognosis and clarify its potential clinical role. Moreover, telomerase gene expression

might not enough to represent the telomerase activities in cancer development. A protein measurement to discover the roles of telomerase in cancer metastasis would be a valuable complementary effort to examine the hypothesis.

In summary, the present study discovered an association between telomerase gene expression and cancer metastasis. We provided evidence that gene DKC1 may be associated with early metastasis and demonstrated its interaction with the tumor suppressor TP53. It provided a progressive step to discover the development of predicting prostate cancer recurrence, yet more studies are needed to focus on the sensitivity and specificity improvement in prediction of prostate cancer metastasis, and telomerases' influences of risk and protective factors.

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BIOGRAPHICAL SKETCH

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