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Association of endothelin-1 gene single-nucleotide polymorphisms and haplotypes with risk of hormone refractory prostate cancer

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Androgen deprivation is often the treatment of choice for patients with a new diagnosis of metastatic or locally advanced prostate cancer (CaP). However, most CaP patients showing a first response to androgen deprivation will progress to a hormone refractory phase of the disease (HRPC) with a much poorer prognosis. Accumulating evidence suggests that endothelin-1 (ET-1) plays an important role in CaP progression. Single-nucleotide polymorphisms (SNPs) of the ET-1 gene reportedly have been associated with cancer progression and chemoresistance. In the present study, we explored the association of SNPs and haplotypes of the ET-1 gene with the risk of HRPC. We genotyped three SNPs (rs1800541, rs2070699 and rs5370) in the ET-1 gene in a case-control study; 234 CaP patients who developed HRPC within six years after androgen deprivation therapy was used as HRPC cases, and 234 age- and primary therapy-matched CaP patients who had not developed HRPC within six years after androgen deprivation therapy were used as non-HRPC controls. Our results revealed that the G allele at rs1800541 and the G allele at rs2070699 were respectively associated with reduced and increased risk of HRPC at borderline statistical significance (p=0.047 and p=0.058, respectively). With adjustment for potential confounders including body mass index, initial Gleason score at diagnosis of CaP, and post-treatment nadir serum PSA level, we found that rs1800541-rs2070699 TG haplotype was significantly associated with increased risk of HRPC (p=0.033; adjusted OR, 2.10; 95% CI, 1.37-5.04). In conclusion, this study provides the first evidence that a 2-SNP haplotype of the ET-1 gene is associated with increased risk of HRPC, which adds new insights into early identification of CaP patients who are likely to develop HRPC in a later stage of the disease.

1. Introduction

Prostate cancer (CaP) is one of the most commonly diagnosed malignancies and leading causes of cancer death in men (Kesarwani et al. 2011). CaP is mostly an endocrine-dependent tumor. Thus, hormonal therapy is still a first-choice treatment, which is quite effective with rapid biochemical responses, as evaluated by declines in levels of the serum marker, prostate-specific antigen (PSA) (Ahmed et al. 2013). Androgen deprivation is often the treatment of choice for patients with a new diagnosis of metastatic or locally advanced CaP. In the metastatic setting, androgen deprivation is associated with a response rate of 80-90%, and overall survival estimates range from 24 to 36 months (Ahmed et al. 2013). However, most CaP patients showing a first response to androgen deprivation will progress to a hormone refractory phase of the disease (HRPC) with a much poorer prognosis (Chi et al. 2009).

Endothelin-1 (ET-1) was initially identified as a potent vasoconstrictor from endothelial cells (Nelson et al. 2003). Previous studies have shown that CaP tissues produce ET-1 (Pirtskhalaishvili and Nelson 2000); the ET-1 level is evidently higher in advanced, metastatic CaP tissues than in primary lesions (Nelson et al. 2000). ET-1 plays an important role in CaP progression (Sun et al. 2006). SNPs of the ET-1 gene reportedly have been associated with cancer progression and chemoresistance (Zang et al. 2013; Zhou et al. 2014). Previous studies also have suggested that single-nucleotide polymorphisms (SNPs) may contribute to the neoplastic initiation and progression of CaP (Kesarwani et al. 2011). In the present study, we for the first time explored the association of ET-1 SNPs and haplotypes with the risk of HRPC in a case-control study, using 234 pairs of age- and primary therapy-matched patients.

2. Investigations and results

In this case-control study, 234 CaP patients who developed HRPC within six years after androgen deprivation therapy were used as HRPC cases, and 234 age- and primary therapy-matched CaP patients who had not developed HRPC within six years after androgen deprivation therapy were used as non-HRPC controls. All patients had received androgen deprivation therapy either as the primary therapy or as an adjunctive therapy to prostatectomy or radiation therapy. As shown in Table 1, there were no significant differences between the HRPC and the non-HRPC groups in age, primary therapy, post-treatment nadir serum PSA level, and BMI and initial Gleason score at diagnosis of CaP.

As shown in Table 2, both allele frequencies at rs5370 were not significantly associated with the risk of HRPC (p=0.711); the G allele at rs1800541 was associated with reduced risk of HRPC at borderline statistical significance (p=0.047), while the G allele at rs2070699 was associated with increased risk of HRPC at borderline statistical significance (p=0.058). Pairwise LD tests indicated that rs1800541 and rs2070699 were in strong LD (Table 3). According to the genotyping data in cases and controls, 2-SNP haplotypes for rs1800541 and rs2070699 were reconstructed. As shown in Table 4, a 2-SNP haplotype TG showed a significant association with increased risk of HRPC after adjustment for body mass index, initial Gleason score at diagnosis of CaP, and post-treatment nadir serum PSA level (p=0.033; adjusted OR, 2.10; 95% CI, 1.37-5.04).

Table 1: Characteristics of HRPC and non-HRPC patients

Characteristic	HRPC (n=234)	Non-HRPC (n=234)	*p*
Age (years)	65.9±7.6	66.3±7.8	0.57
Age range (years)	52-77	52-77	N/A
BMI at diagnosis of CaP (kg/m^2)	24.6±5.3	25.5±5.7	0.08
Primary therapy n(%)	162 (69.2)	162 (69.2)	1.00
Prostatectomy	45 (19.2)	45 (19.2)	
Radiation therapy			
Androgen deprivation therapy	27 (11.6)	27 (11.6)	
Initial Gleason score at diagnosis of CaP n(%)			0.07
6	20 (8.5)	24 (10.3)	
7	132 (56.4)	125 (53.4)	
8	22 (9.4)	39 (16.7)	
9	60 (25.7)	46 (19.6)	
Post-treatment nadir PSA (ng/mL)	35.2±12.4	36.7±14.7	0.26

For continuous variables, all values were expressed as mean±SD and compared with student t tests. For categorical variables, all values were expressed as n (%) and compared with Chi-square tests. CaP, prostate cancer; PSA, prostate-specific antigen; HRPC, hormone refractory prostate cancer; BMI, body mass index.

Table 2: Genotype and allele frequencies of endothelin-1 SNPs in HRPC and non-HRPC patients

	HRPC/Non-HRPC n (%)	OR (95% CI)	*p*
rs1800541			
Genotype			
GG	14 (6.0)/24 (10.3)	0.10 (0.03-0.29)	
TG	121 (51.7)/131 (56.0)	0.21 (0.14-0.32)	
TT	99 (42.3)/79 (33.7)	1.00	0.072
Allele			
G	149 (31.8)/179 (38.2)	0.76 (0.58-1.00)	
T	319 (68.2)/289 (61.8)	1.00	0.047
rs2070699			
Genotype			
GG	60 (25.6)/46 (19.7)	1.64 (0.96-2.80)	
TG	125 (53.4)/123 (52.5)	1.33 (0.85-2.08)	
TT	49 (21.0)/65 (27.8)	1.00	0.128
Allele			
G	245 (52.4)/215 (45.9)	1.26 (0.98-1.63)	
T	223 (47.6)/253 (54.1)	1.00	0.058
rs5370			
Genotype			
TT	20 (8.5)/24 (10.3)	0.63 (0.43-1.56)	
GT	81 (34.6)/79 (33.7)	1.00 (0.68-1.50)	
GG	133 (56.9)/131 (56.0)	1.00	0.817
Allele			
T	121 (25.9)/127 (27.1)	0.94 (0.70-1.25)	
G	347 (74.1)/341 (72.9)	1.00	0.711

HRPC, hormone refractory prostate cancer; OR, odds ratio.

Table 3: D' and r^2 between *endothelin-1* SNPs rs1800541 and rs2070699 in HRPC and non-HRPC patients

SNP Pair		D' Cases/Controls	r^2 Cases/Controls
rs1800541	rs2070699	0.967/0.951	0.041/0.050

Values of *D'* and *r*^{2} were calculated with the Haploview program. HRPC, hormone refractory prostate cancer.

3. Discussion

Androgen deprivation is often the treatment of choice for patients with a new diagnosis of metastatic or locally advanced CaP (Hellerstedt and Pienta 2002). However, advanced CaP brought to remission with androgen deprivation therapy often relapses, leading to hormone refractory tumors, for which only experimental treatments exist (Sun et al. 2006). Previous studies have suggested that ET-1 is an important promoter of CaP progression (Sun et al. 2006). In this study, we have shown that a 2-SNP TG haplotype at polymorphic sites rs1800541 and rs2070699 in the ET-1 gene are associated with increased risk of HRPC.

All three ET-1 gene SNPs (rs1800541, rs2070699, and rs5370) analyzed in this study have been involved in previous studies exploring the association of ET-1 SNPs with cancer progression and chemoresistance. Zang et al. 2013 genotyped rs1800541, rs2070699 and rs5370 in a case-control study and found that The G allele at rs1800541 was associated with reduced risk of pulmonary metastatic osteosarcoma. By genotyping the same set of SNPs, Zhou et al. (2014) reported that the G allele at rs1800541 and the G allele at rs2070699 were associated with reduced and increased risk of chemoresistant osteosarcoma, respectively; in addition, rs1800541-rs2070699 TG and GT haplotypes were respectively associated with increased and reduced risk of chemoresistant osteosarcoma. In agreement with the previous studies, we found that the G allele at rs1800541 and the G allele at rs2070699 were associated with reduced risk and increased risk of HRPC respectively, albeit both at borderline statistical significance (p=0.047 and p=0.058, respectively). With adjustment for potential confounders including body mass index, initial Gleason score at diagnosis of CaP, and post-treatment nadir serum PSA, we found that the rs1800541-rs2070699 TG haplotype was significantly associated with increased risk of HRPC, which is in line with the report by Zhou et al. (2014).

ET-1 is implicated in multiple functions during angiogenesis, cell invasion, cell proliferation, and apoptosis that affect tumor progression (Qiao et al. 2015). Zhou et al. 2014 showed that the rs1800541-rs2070699 TG haplotype could increase the expression of ET-1 in primary osteosarcoma cells, providing a mechanistic explanation for the association between the TG haplotype and osteosarcoma progression and chemoresistance. ET-1 has been shown to promote cell proliferation and survival against apoptotic stress in both androgen-dependent and -independent (hormone refractory) CaP cells (Sun et al. 2006). In the present study, we have found that the rs1800541-rs2070699 TG haplotype is associated with increased risk of HRPC. However, the underlying mechanism(s) is still unclear, which will be explored in our future studies.

The strength of the present study is that we used a relatively large sample size of age- and primary therapy-matched HRPC and non-HRPC patients with six years of follow-up after androgen deprivation therapy (n=468 in total; case, n=234; control, n=234). The sample size ensured adequate statistical power of our find-

Table 4: Frequencies of Estimated 2-SNP Haplotypes of *endothelin-1* SNPs rs5370 and rs2070699 in HRPC and non-HRPC patients

	rs5370 - rs2070699 2-SNP Haplotype			
	TT	TG	GT	GG
HRPC (%)/Non-HRPC (%)	32.5/33.4	35.7/28.4	15.1/20.7	16.7/17.5
Crude OR (95% CI)[a]	0.96 (0.65-1.42)	1.45 (0.98-2.14)	0.68 (0.42-1.10)	0.94 (0.58-1.52)
p	0.922	0.076	0.146	0.902
Adjusted OR (95% CI)[b]	0.89 (0.49-1.64)	2.10 (1.37-5.04)	0.45 (0.20-1.26)	0.85 (0.46-1.69)
p	0.705	0.033	0.119	0.691

[a]Calculated with the SHEsis program. [b]Adjusted for body mass index and initial Gleason score at diagnosis of prostate cancer and post-treatment nadir serum PSA level. PSA, prostate-specific antigen; HRPC, hormone refractory prostate cancer.

Table 5: Targeted endothelin-1 SNPs in this study

Reference SNP ID (rs)	Chromosome Position	Region in Gene	Alleles[a]
rs1800541	12397205	Promoter	T:G
rs2070699	12400758	Intron	T:G
rs5370	12404241	Lys>Asn	G:T

Non-hormone refractory prostate cancer (HRPC) patients were used as controls for HRPC patients in this study. [a]Alleles are presented as major:minor allele.

ings after adjustment for multiple potential confounders. Patients with HRPC often have distant metastatic disease, particularly bone metastasis, which results in significant morbidity and a decline in quality of life (Ahmed et al. 2013). Thus, early identification of patients who are susceptible to developing HRPC may lead to adjustment of CaP treatment and better outcomes.

There are some limitations of this study: (1) We only enrolled Chinese Han patients in this study to minimize background noise. Nevertheless, as Chinese Han accounts for 90% of the population in China and 19% of global population (Huang et al. 2011), our findings have significant potential impact. (2) We only followed up the patients for six years after androgen deprivation therapy; some non-HRPC may turn into HRPC after six years. Nevertheless, 222 (94.9%) of the patients in the case group developed HRPC within five years after androgen deprivation therapy; therefore, the probability of developing HRPC after the sixth year should be well under 5%, which will not add significant alteration to our current findings. (3) This case-control study is to explore the association of ET-1 SNPs and haplotypes with the risk of developing HRPC from CaP patients but not healthy people. Thus, we used non-HRPC CaP patients as the control rather than healthy controls. We will explore whether ET-1 SNPs and haplotypes could contribute to the risk of developing CaP in a case-healthy control study in the future.

In conclusion, this study provides the first evidence that a 2-SNP haplotype of the ET-1 gene is associated with increased risk of HRPC, which adds new insights into early identification of CaP patients who are likely to develop HRPC in a later stage of the disease.

4. Experimental

4.1. Patients

From March 2010 to January 2016, blood samples were collected from 234 Chinese Han CaP patients who developed HRPC within six years after androgen deprivation therapy; 222 (94.9%) of them developed HRPC within five years after androgen deprivation therapy. A total of 234 age- and primary therapy-matched Chinese Han CaP patients who had not developed HRPC within six years after androgen deprivation therapy were recruited as controls. CaP diagnoses were based on biopsy. All patients had received androgen deprivation therapy either as the primary therapy or as an adjunctive therapy to prostatectomy or radiation therapy. HRPC was defined as an increase in the serum PSA level over the baseline level in ≥2 consecutive samples obtained ≥7 days apart despite receiving androgen deprivation therapy. Baseline characteristics of all subjects are summarized in Table 1. Body mass index (BMI) and initial Gleason scores at diagnosis of CaP and post-treatment nadir serum PSA levels were assessed retrospectively. This study was approved by the Ethics Committee of Shanghai First People's Hospital, Shanghai Jiao Tong University. Written informed consent was obtained from all patients before the collection of blood samples.

4.2. SNP selection and genotyping

Three SNPs in the ET-1 gene, including rs1800541 in the promoter region, rs2070699 in the intron, and rs5370 in the coding region were selected as targets of this study (Table 5). All three SNPs had been involved in previous studies on the association of ET-1 SNPs with cancer progression and chemoresistance (Zang et al. 2013; Zhou et al. 2014). Genomic DNA was isolated from white blood cells using the phenol/chloroform method and was stored in 400 ml of TE (10 mM Tris/HCl and 1 mM EDTA (pH 8.0). As previously described, SNPs were genotyped using SNPlex assays (Applied Biosystems, Foster City, CA, USA) based on oligonucleotide ligation assay for capillary electrophoresis on an ABI 3700 DNA Analyzer (Applied Biosystems) (Zhao et al. 2010a; Zhao et al. 2010b).

4.3. Linkage disequilibrium (LD) and haplotype analysis

Pairwise measures of LD measured by Lewontin coefficient (D¢) and squared correlation coefficient (r^2) between the SNPs genotyped were calculated with the Haploview program (Barrett et al. 2005). Frequencies of individual haplotypes were estimated from genotype data using the SHEsis program, which implement a Full-Precise-Iteration algorithm for reconstructing haplotypes (Shi and He 2005). Logistic regression analysis was done using SAS PROC LOGISTIC to estimate the odds ratios (OR) and 95% confidence intervals (95% CI) of haplotypes.

4.4. Statistical analysis

All continuous variable values were expressed as Mean±SD. Comparison of means between two groups was performed with student t tests. Categorical variables were compared with Chi-square tests. Genotype and allele frequencies between cases and controls were compared with Chi-square tests. Logistic regression was performed to assess OR and 95% CI. All the statistical analyses were performed with SAS 9.1.3. Statistical significance level was set at $p<0.05$.

Conflicts of interest: None declared.

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