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Polymorphism in protein tyrosine phosphatase receptor delta is associated with the risk of clear cell renal cell carcinoma

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ABSTRACT

Clear cell renal cell carcinoma (ccRCC) is a common urological malignancy. Our previous study has indicated that the protein tyrosine phosphatase receptor type delta (*PTPRD*) gene may play a role. To determine the effect of *PTPRD* genetic polymorphisms on ccRCC occurrence and progression, a total of 377 ccRCC cases and 754 matched controls were enrolled in the study. DNA sequencing and genotyping, and immunohistochemistry were conducted to test the associations of genotypes with ccRCC risk and PTPRD expression level in somatic tissues. The C allele of *PTPRD* rs2279776 was associated with a higher risk of ccRCC (per allele OR = 1.23, $P = 0.03$). Patients without distant metastasis at the time of surgery were followed for a median of 33.1 months. Overall survival was not different between different rs2279776 genotype groups ($P = 0.30$). The C allele was associated with a higher percentage of negative immunostaining in adjacent normal renal tissues ($P = 0.02$). *PTPRD* rs2279776 SNP may be a novel genetic risk factor of ccRCC.

1. Introduction

Renal cell carcinoma (RCC) is a common urological malignancy, and the incidence has increased over the past 20 years (Siegel et al., 2012). Clear cell renal cell carcinoma (ccRCC), which originates from proximal tubular epithelial cells, is the most common subtype of RCC and accounts for 70% to 80% of all kidney cancers (Motzer et al., 1996). The current curative therapy of RCC is surgical resection. However, for patients with metastatic ccRCC, the local recurrence or distant metastasis rate is high after curative nephrectomy (Leibovich et al., 2003). A large proportion (30%–50%) of patients with clinically localized disease will subsequently develop metastases. Although anti-vascular epithelial growth factor (VEGF) agents like sorafenib and sunitinib have been proved to improve progression-free survival (PFS) in both first-line and second-line settings of metastatic ccRCC patients (Coppin et al., 2011; Rini et al., 2011), little progress has been made to improve the median survival for disseminated ccRCC. Hence, there is an urgent need to develop more effective treatment strategies for ccRCC.

Previous researches have demonstrated some genetic factors to be associated with ccRCC (Alimov et al., 2004; Chen et al., 2009; Grady et al., 2001; Hirata et al., 2005; Kaku et al., 2004; Perego et al., 2008). The von Hippel–Lindau disease tumor suppressor (*VHL*) gene is well studied in ccRCC, and it is thought to be an early event in the ccRCC pathway (Kondo et al., 2002). Up to 57% and 19% of sporadic ccRCC have intragenic mutation and aberrant hypermethylation of VHL, respectively (Gnarra et al., 1994; Herman et al., 1994; Shuin et al., 1994). In addition, 90% of ccRCC has loss of heterozygosity (LOH) of the *VHL* gene locus (3p25) (Brauch et al., 2000; Gnarra et al., 1994). Research also reported that the fragile histidine triad (*FHIT*) gene is associated with ccRCC (Sukosd et al., 2003). A better understanding of ccRCC genetics is important for prevention, early detection and development of innovative treatment.

Our previous study has observed that D9S168 (9p23–22) had the highest frequency of microsatellite alteration in the ccRCC specimens, and D9S168 alterations were frequent in high-stage tumors. We further detected that the D9S168 alteration, which was located at the 5′-untranslated region (5′UTR) of a gene that encodes protein tyrosine phosphatase receptor delta (PTPRD), was significantly associated with low expression of PTPRD at the messenger RNA level. Immunohistochemistry revealed that the PTPRD expression was strong in normal kidney proximal tubular epithelial cells, and was greatly downregulated in ccRCC (Li et al., 2011). These suggest genetic variations in the *PTPRD* gene may affect an individual's susceptibility to ccRCC

Abbreviations: ccRCC, clear cell renal cell carcinoma; FHIT, fragile histidine triad; HWE, Hardy–Weinberg equilibrium; LOH, loss of heterozygosity; MGB, minor groove binder; OR, odds ratio; PFS, progression-free survival; PTPRD, protein tyrosine phosphatase receptor type delta; qPCR, quantitative PCR; RCC, renal cell carcinoma; SMMU, Second Military Medical University; VEGF, vascular epithelial growth factor; VHL, von Hippel–Lindau; 5′UTR, 5′-untranslated region; 95% CI, 95% confidence interval.

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and its prognosis. So far, there have been no studies looking at the association between *PTPRD* polymorphisms and ccRCC. Therefore, we evaluated the effects of *PTPRD* genetic polymorphisms on the occurrence and survival of ccRCC, and further looked into the function of genetic variants using immunohistochemistry.

2. Materials and methods

2.1. Study population

The study participants were recruited at the Second Military Medical University (SMMU) affiliated Changhai Hospital and Changzheng Hospital. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institutional review board at the University. All participants provided written informed consent.

Briefly, cases were newly diagnosed sporadic ccRCC patients who underwent nephrectomy at the Department of Urology of the two hospitals between December 1998 and December 2011. Eligibility criteria included: 1) non-missing sex, 2) histologically confirmed ccRCC cases, 3) underwent curative nephrectomy, and 4) no metastasis during the procedure. We did not include patients who had received radiotherapy and/or immunotherapy before or after surgical treatment. The control series is selected from participants who went for regular physical check-up in Changhai Hospital between December 2006 and December 2011. Participants were excluded if they had any types of cancer. Two controls for each case were randomly selected from possible controls by matching on sex and age at baseline (in 2-year intervals). In total, 377 ccRCC patients and 754 controls were selected. All participants were of ethnic Chinese origin.

2.2. Follow-up after curative nephrectomy

Clinical data and histopathologic data were obtained from the clinical and pathologic records. The follow-up in the present study was started 6 months after the surgery. Follow-up was performed by examinations every 3 months on an outpatient bases and/or by telephone calls according to our standard epidemiologic procedure. Finally, 190 ccRCC patients who did not have metastasis at the time of curative nephrectomy completed the follow-up.

2.3. PTPRD SNPs and genotyping

SNPs were selected based on their locations at known and putative functional domains in the coding regions and 5′UTR of this gene. A total of six SNPs in the exons (rs2279776, rs34704234, rs3215089, rs2133788, and rs12351899) and near the 5′UTR (rs73398255) were selected (Table 1). Data of the SNPs of Chinese population were either monomorphic (rs2133788 and rs12351899) or no frequency data (rs34704234, rs3215089, and rs73398255) in the International HapMap Project (www.hapmap.org). We then amplified and sequenced genomic DNA fragments covering the six SNPs from 30 randomly selected healthy controls to evaluate the genetic variations of the six SNPs in our study population. Genomic DNA was extracted from the blood samples using QIAquick PCR purification kits (QIAGEN, Germany). Primers chosen for amplification of the six SNPs were designed using Primer 3.0 software (Supplementary Table 1). The amplicons were directly sequenced using ABI PRISM BigDye sequencing kits and an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA) in both forward and reverse directions.

Genotyping of rs2279776 in 377 cases and 754 controls was performed using fluorescent-probe real-time quantitative PCR (qPCR) in a LightCycler™480 (Roche, Basel, Switzerland). Primers and probes (Minor Groove Binder [MGB]) were designed by GeneCore Bio Technologies (Shanghai, China). The sequences of primers and probes are forward primer, 5′-CCAAATGTTTCGGGGAGAGA-3′; reverse primer, 5′-CTGGTTGAATAACACTCCTTGTTTTC-3′; probe-1, FAM-TTCCTATA GCC ATCTAT-MGB; probe-2, HEX-TTCCTATACCCATCTAT-MGB. Genotyping was carried out by laboratory technicians who were blinded to case–control status. All the samples were successfully genotyped. For genotyping quality control, 5% samples were randomly selected and directly sequenced, and 100% identical results were obtained.

2.4. Immunohistochemistry

PTPRD expression in 61 available paraffin-embedded surgical specimens containing adjacent normal renal tissues was examined using immunohistochemistry. The procedure and protocol of immunohistochemistry were described previously (Li et al., 2011; Wu et al., 2010). The affinity-purified goat polyclonal antibody PTP-δ (C-20)/sc-1118, a primary antibody against the carboxyl terminus of human PTPRD/PTPδ, was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Briefly, the de-paraffinized sections were incubated with 20% goat serum for 30 min to block non-specific binding and then were incubated with 1:50 diluted primary antibodies for 30 min in room temperature. All samples were analyzed by 3 observers (Y.Y., X.T., and G.C.) who were blinded to the clinical information. Immunostaining was scored as negative and positive for analysis. There was close agreement (>90%) among the 3 investigators. Disagreements were resolved by consensus.

2.5. Statistical analyses

Patient characteristics in the cases and controls were compared by Student's t-test for continuous variables and Chi-square test or Fisher's exact test for discrete variables. Genotype and allele frequencies were calculated. Observed genotype frequencies in controls and cases were separately tested for deviation from Hardy–Weinberg equilibrium (HWE) with the exact test. Logistic regressions were used to calculate the odds ratios (ORs) and 95% confidence intervals (95% CIs). Additive

Table 1
PTPRD (Chr. 9p23–p24.3) SNP information.

SNP	Nucleotide	Function	Allele	HapMap MAF	Chr position*	Protein residue change
rs2279776	C4396G	Exon27, synonymous change (Gly1418Gly)	G→C	0.378	8389364	G [Gly]⇒G [Gly]
rs34704234	–	DIV:deletion/insertion variation, Frameshift	G→deletion	NA	8338984:8338985	P [Pro]⇒PH [Pro]
rs3215098	–	Frameshift	ATTCCTGAACTGTAACTTACC /ATTCTTGAACTGTAACTTACC→deletion	NA	8331582:8331583	S [Ser]⇒R*VTVQEC [Arg] S [Ser]⇒R*VTVQEC [Arg]
rs2133788	–	Missense	A→T	0	8376656	D [Asp]⇒V [Val]
rs12351899	–	Synonymous change	T→C	0	8341185	N [Asn]⇒N [Asn]
rs73398255	–	Near Gene-5	G→C	NA	10614368	NA

Abbreviations: MAF, minor allele frequency; NA, not available.
Database of single nucleotide polymorphisms (dbSNP). Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. (dbSNP Build ID: {build ID}). Available from: http://www.ncbi.nlm.nih.gov/SNP/.
* dbSNP Chromosome Report, GRCh37.p5 sequence.