

The Role of Functional Polymorphisms of Cyclooxygenase 2 in Renal Cell Carcinoma

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Abstract. Renal cell carcinoma (RCC) accounts for about 3% of all cancer-related mortalities worldwide and the risk factors for the development of RCC have not yet been fully elucidated. Mounting evidence shows that overexpression of cyclo-oxygenase 2 (COX2) is commonly found in malignant tumors, including RCC. However, the contribution of genotypic variations of COX2 to RCC has not been studied. We hypothesized that variants of the COX2 gene are associated with risk of susceptibility to RCC in Taiwan. In this hospital-based case-control study, 92 patients with RCC and 580 age- and gender-matched cancer-free controls were recruited and the associations of COX2 A-1195G, G-765C, T+8473C, intron 1, intron 5, and intron 6 polymorphisms with RCC risk were examined in this Taiwanese population. The results showed that compared to the wild-type GG genotype, the CG genotype for COX2 G-765C was significantly associated with a lower risk of RCC (odds ratio=0.34, 95% confidence interval=0.15-0.80, $p=0.0082$). For other polymorphic sites, no obvious associations were found. There was also an obvious association of COX2 G765C genotype with reduced RCC risk among those without family cancer history ($p=0.0331$). These evidence indicated that COX2 G-765C genotype involved in the etiology of RCC and may serve as a novel genetic marker for susceptibility of RCC.

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Key Words: COX2, genotype, polymorphism, renal cell carcinoma, Taiwan.

Renal cell carcinoma (RCC) has a worldwide incidence of more than 270,000 new cases and is responsible for 100,000 deaths annually (1). The incidence of RCC has been increasing worldwide (2). After Japan, Taiwan has the second-highest prevalence of end-stage renal disease in the world (2). Epidemiological investigations have shown that cigarette smoking, hypertension, obesity, occupational exposures, diet, and family history of cancer are associated with RCC (3-5). However, only few exposed individuals actually develop RCC during their lifetime, suggesting that genomic factor may be involved in the etiology of RCC. For urologists, RCC behavior is unpredictable, and tumor stage and grade are not satisfactory parameters for determining the prognosis of patients with RCC.

Cyclooxygenase-2 (COX2) is an inducible enzyme for the conversion of arachidonic acid to prostanoid, prostaglandin and thromboxane (6). Typically, COX2 is often undetectable in normal tissues, whereas overexpression of COX2 has been observed in neoplastic cells of canine (7) and human RCC (8-10). It is reported that overexpression of COX2 contributes to carcinogenesis via increasing cell proliferation, suppressing apoptosis, enhancing invasiveness, and inducing chronic activation of immune responses and angiogenesis (11, 12). In several animal and clinical studies, COX2-specific inhibitors were found to have both preventive and therapeutic effects as anticancer drugs for breast, bladder, lung and pancreatic cancer (13-16). However, the association of COX2 genotypes with RCC has never been investigated as far as we are aware of.

According to the central dogma, subtle genetic variants of the COX2 gene may affect the quantity of COX2 protein through altered self-regulated transcriptional activity, or alternative splices resulting from polymorphic variations at the promoter region or introns, respectively (17). To clarify the hypothesis that the polymorphic variants at promoter or intron regions of COX2 may be associated with the risk of RCC, we analyzed the genotypes for six single nucleotide

Table I. Distributions of the frequencies of selected characteristics among renal cell carcinoma (RCC) cases and healthy controls.

Characteristics	Cases (n=92)		Controls (n=580)		p-Value
	N	%	N	%	
Age (years) (mean±SD)	58.8±11.7		58.3±11.5		0.8971
<60	47	51.1%	307	52.9%	0.8223
>60	45	48.9%	273	47.1%	
Gender					
Male	59	64.1%	371	64.0%	1.0000
Female	33	35.9%	209	36.0%	
Smoking status					
Smokers	41	44.6%	220	37.9%	0.2499
Non-smokers	51	55.4%	360	62.1%	
Alcohol drinking status					
Drinkers	37	40.2%	209	36.0%	0.4848
Non-drinkers	55	59.8%	371	64.0%	
Hypertension					
Yes	61	66.3%	302	52.1%	0.0130
No	31	33.7%	278	47.9%	
Diabetes					
Yes	21	22.8%	104	17.9%	0.2523
No	71	77.2%	476	82.1%	
Family cancer history					
Yes	6	6.5%	17	2.9%	0.1125
No	86	93.5%	563	97.1%	

polymorphisms of *COX2*, including A-1195G (rs689466), G-765C (rs20417), T+8473C (rs5275), intron 1 (rs2745557), intron 5 (rs16825748), and intron 6 (rs2066826), in a Taiwanese population. To the best of our knowledge, this is the first study to evaluate the association between *COX2* genotype and RCC susceptibility in Taiwan.

Materials and Methods

Study population. The hospital-based case-control study recruited 92 RCC patients and 580 cancer-free controls matched by age and sex, and none of the participants were related to each other by any biological relationship. RCC in all patients was diagnosed and histopathologically confirmed, and no patient had a prior history of other cancer. All the age- and gender-matched cancer-free controls were genetically unrelated to the RCC patients and had no individual history of cancer. A further exclusion criterion for the controls were symptoms suggestive of RCC, such as hematuria. Each patient donated 3-5 ml venous blood after providing their written informed consent. The study was approved by the Institutional Review Board of China Medical University. The details of the characteristics for all the participants are summarized and compared in Table I.

Genotyping protocol. The total genomic DNA of each participant was extracted from leukocytes from peripheral blood and stored as previously described (18-20). Pairs of polymerase chain reaction (PCR) primer sequences and restriction enzyme for each DNA product of *COX2* genotyping are all listed in Table II. The PCR

cycling conditions were: one cycle at 94°C for 5 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 10 min. The PCR products were cut by appropriate restriction enzymes and the reaction mixture was incubated for 2 h at 37°C. Then 10 µl of product was loaded into a 3% agarose gel for electrophoresis.

Statistical analyses. To ensure that the controls included were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype frequencies of *COX2* single nucleotide polymorphisms in the controls from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's Chi-square test or Fisher's exact test (when the expected number in any cell was less than five) was used to compare the distribution of the *COX2* genotypes between cases and controls. The associations between the *COX2* polymorphisms and RCC risk were estimated by computing odds ratios (ORs) and their 95% confidence intervals (CIs) from unconditional logistic regression analysis with adjustment for possible confounders. A value of $p < 0.05$ was considered statistically significant, and all statistical tests were two-sided.

Results

Comparison of basic characteristics between the case and control groups. The frequency distributions of the characteristics for the controls and cases are summarized in Table I. There were no differences between the case and

Table II. *Primer sequences and restriction enzymes used for cyclooxygenase 2 (COX2) genotyping and analysis.*

Polymorphism	Primer sequences (5' to 3')	Restriction enzyme	Polymorphic type	DNA fragment size (bp)
A-1195G (rs689466)	F: CCCTGAGCACTACCCATGAT R: GCCCTTCATAGGAGATACTGG	<i>Hha I</i>	A	273
G-765C (rs20417)	F: TATTATGAGGAGAATTTACCTTTCGC R: GCTAAGTTGCTTTCAACAGAAGAAT	<i>Pvu II</i>	G	220+53
T+8473C (rs5275)	F: GTTTGAAATTTTAAAGTACTTTTGAT R: TTTCAAATTATTGTTTCATTGC	<i>Bcl I</i>	C	100
intron 1 (rs2745557)	F: GAGGTGAGAGTGTCTCAGAT R: CTCTCGGTTAGCGACCAATT	<i>Taq I</i>	G	74+26
intron 5 (rs16825748)	F: GCGGCATAATCATGGTACAA R: CAGCACTTCACGCATCAGTT	<i>BsrG I</i>	T	147
intron 6 (rs2066826)	F: ACTCTGGCTAGACAGCGTAA R: GCCAGATTGTGGCATAATC	<i>Aci I</i>	A	124+23
			G	439
			A	353+76
			A	417
			A	314+103
			A	327
			G	233+94

F and R indicate forward and reverse primers, respectively.

control groups regarding age, gender, smoking or alcohol drinking status, diabetes or family history of cancer ($p>0.05$). However, there were more individuals with hypertension (66.3%) among the RCC cases than the controls (52.1%), and the difference was statistically significant ($p=0.0130$).

Association of COX2 genotypes and RCC risk. The genotypic distributions of the COX2 polymorphisms for the cases and controls are presented and compared in Table III. The distributions of the genotypes of all the polymorphisms of COX2 did not significantly differ between the two groups ($p>0.05$) except that of G-765C ($p=0.0082$) (Table III). The OR for those carrying the CG genotype at COX2 G-765C was 0.34 (95% CI=0.15-0.80) compared to those carrying the GG wild-type genotype.

The frequencies of the alleles for COX2 polymorphisms in controls and patients with RCC are shown in Table IV. Neither of the alleles of any of the COX2 polymorphisms were found to be associated with RCC ($p>0.05$) except that of G-765C ($p=0.0113$). The percentages of allele C were 3.3% and 8.4% among RCC patients and controls, and the OR for those carrying the C allele at COX2 G-765C was 0.37 (95% CI=0.16-0.85) compared to those carrying the wild-type G allele. These data indicate that individuals carrying the variant C allele at promoter G-765C may have a lower risk of RCC.

Interaction of COX2 G-765C genotype with individual characteristics. We stratified the controls and RCC cases according to their characteristics and evaluated the interactions of COX2 G-765C genotype and these characteristics on the risk of RCC (Table V). As shown in Table V, the association between COX2 G-765C genotype and RCC risk did not vary by age, gender, smoking, alcohol

Table III. *Distribution of cyclooxygenase 2 (COX2) genotypic frequencies among the renal cell carcinoma (RCC) cases and controls.*

	RCC Cases (%)	Controls (%)	Crude OR (95% CI)	p-Value
A-1195G (rs689466)				
AA	26 (28.3)	155 (26.7)	1.00 (reference)	
AG	44 (47.8)	281 (48.5)	0.83 (0.55-1.57)	0.7898
GG	22 (23.9)	144 (24.8)	0.91 (0.49-1.68)	0.8765
<i>P</i> _{trend}				0.9499
G-765C (rs20417)				
GG	86 (93.5)	482 (83.1)	1.00 (reference)	
CG	6 (6.5)	98 (16.9)	0.34 (0.15-0.80)	0.0082
T+8473C (rs5275)				
TT	64 (69.6)	381 (65.7)	1.00 (reference)	
CT	28 (30.4)	199 (34.3)	0.84 (0.52-1.35)	0.5531
Intron 1 (rs2745557)				
GG	68 (73.9)	452 (77.9)	1.00 (reference)	
AG	21 (22.8)	116 (20.0)	1.20 (0.71-2.04)	0.4851
AA	3 (3.3)	12 (2.1)	1.66 (0.46-6.04)	0.4341
<i>P</i> _{trend}				0.6120
Intron 5 (rs16825748)				
TT	90 (97.8)	569 (98.1)	1.00 (reference)	
AT	2 (2.2)	11 (1.9)	1.15 (0.25-5.27)	0.6952
Intron 6 (rs2066826)				
GG	80 (87.0)	519 (89.5)	1.00 (reference)	
AG	10 (10.9)	51 (8.8)	1.27 (0.62-2.61)	0.5556
AA	2 (2.1)	10 (1.7)	1.30 (0.28-6.03)	0.6687
<i>P</i> _{trend}				0.7695

OR: Odds ratio; CI: confidence interval.

drinking, hypertension status or diabetes stratification. However, the association of GC genotype with reduced risk of RCC appeared to be stronger in the subgroup without a family history of cancer (OR=0.24, 95% CI=0.06-1.02; $p=0.0331$).

Table IV. Distributions of cyclo-oxygenase 2 (COX2) allelic frequencies among the renal cell carcinoma (RCC) cases and controls.

Allele	RCC cases	%	Controls	%	Crude OR (95% CI)	p-Value
A-1195G (rs689466)						
Allele A	96	52.2%	591	50.9%	1.00 (reference)	
Allele G	88	47.8%	569	49.1%	0.95 (0.70-1.30)	0.8119
G-765C (rs20417)						
Allele G	178	96.7%	1062	91.6%	1.00 (reference)	
Allele C	6	3.3%	98	8.4%	0.37 (0.16-0.85)	0.0113
T+8473C (rs5275)						
Allele T	156	84.8%	961	82.8%	1.00 (reference)	
Allele C	28	15.2%	199	17.2%	0.87 (0.56-1.33)	0.5964
Intron 1 (rs2745557)						
Allele G	157	85.3%	1020	87.9%	1.00 (reference)	
Allele A	27	14.7%	140	12.1%	1.25 (0.80-1.96)	0.3356
Intron 5 (rs16825748)						
Allele T	182	98.9%	1149	99.1%	1.00 (reference)	
Allele A	2	1.1%	11	0.9%	1.15 (0.25-5.22)	0.6955
Intron 6 (rs2066826)						
Allele G	170	92.4%	1089	93.9%	1.00 (reference)	
Allele A	14	7.6%	71	6.1%	1.26 (0.70-2.29)	0.4171

OR: Odds ratio; CI: confidence interval.

Discussion

RCC is a highly heterogeneous tumor type, and the cancer cells do not respond well to radiotherapy or chemotherapy, but partially to targeted therapies. Although it seems targeted therapies for advanced RCC are promising, specific prognostic biomarkers for RCC are still lacking and are urgently needed (21-23). In the present study, the association of COX2 genotype and RCC risk was examined in Taiwan, where the prevalence of end-stage renal disease is second-highest in the world after Japan. After performing the genotyping and analysis, we found that individuals carrying the CG genotype were at lower risk of RCC compared with those carrying the GG genotype for COX2 G-765C. Regarding the other five polymorphic sites, A-1195G, T+8473C, intron 1, intron 5, and intron 6, no association was found (Tables III and IV). In addition, we also investigated the interactions of COX2 G-765C genotype with personal characteristics on RCC risk, finding that the protective effect of the GC genotype for COX2 G-765C was stronger among those without a family history of cancer (Table V). To the best of our knowledge, this is the first study of the role of COX2 genotype in RCC in Taiwan.

The single nucleotide polymorphism COX2 G-765C is a functional one at the promoter region, determining the transcriptional activity of COX2. Many epidemiological studies have demonstrated the genotypes of COX2 G-765C to be associated with altered risk for human cancer, such as gastric (24), colorectal (25), prostate (26, 27) and bladder (28)

cancer, and childhood leukemia (29). However, some other studies showed that COX2 G-765C genotype was not associated with cancer risk (30, 31). The above evidence could be interpreted to suggest that the immunoregulating gene and protein may play different roles in different types of carcinogenesis. However, the contribution of COX2 genotype to RCC risk has never been studied. From the viewpoint of protein level, several studies focused on the COX2 expression levels in RCC cells. In 2004, Chen *et al.* reported that COX2 is overexpressed in the OS-RC-2 RCC cell line and may play an important role in tumorigenesis. It is noticeable that regarding other RCC cell lines, SMKT-R4 and ACHN, COX2 was not overexpressed (32). Similar findings were reported by Mungan's (8) and Miyata's (10) groups. In 2008, Dirim *et al.* reported that COX2, proliferating cell nuclear antigen (PCNA) and vascular endothelial growth factor (VEGF) were observed primarily in the cytoplasm of RCC tumor cells and about half of 99 samples examined showed immunoreactivity for COX2 (33). In 2010, Kankuri-Tammilehto *et al.* reported that higher COX2 expression may be associated with longer metastasis-related survival (34).

The present study has some limitations. Firstly, our sample size is only moderate, which may restrict the reliability and feasibility of stratification and interaction analyses. For instance, the interaction findings such as stronger association of COX2 G-765C genotype with reduced RCC risk in the subgroup without family history should be validated with a larger population. Secondly, more clinical and behavioral information, such as occupational exposure, daily diet and

Table V. Stratification analysis between cyclooxygenase 2 (COX2) G-765C (rs20417) polymorphism and renal cell carcinoma (RCC) risk among cases and controls.

Variable	Cases/controls	COX2 G-765C (rs20417) genotype				OR (95% CI)	p-Value
		GG		CG			
		n	%	n	%		
Age, years							
<60	47/307	44/274	93.6/89.2	3/33	6.3/10.7	0.57 (0.17-1.93)	0.4468
>60	45/273	42/251	93.3/91.9	3/22	6.7/8.1	0.81 (0.23-2.84)	1.0000
Gender							
Male	59/371	55/333	93.2/89.8	4/38	6.8/10.2	0.64 (0.22-1.86)	0.4879
Female	33/209	31/192	93.9/91.9	2/17	6.1/8.1	0.73 (0.16-3.31)	1.0000
Smoking status							
Smoker	41/220	36/183	87.8/83.1	5/37	12.2/16.8	0.69 (0.25-1.87)	0.6434
Non-smoker	51/360	50/342	98.0/95.0	1/18	2.0/5.0	0.38 (0.05-2.91)	0.4901
Alcohol status							
Drinker	37/209	33/173	89.2/82.8	4/36	10.8/17.2	0.58 (0.19-1.75)	0.4688
Non-drinker	55/371	53/352	96.4/94.9	2/19	3.6/5.1	0.70 (0.16-3.09)	1.0000
Hypertension							
Yes	61/302	58/269	95.1/89.1	3/33	4.9/10.9	0.42 (0.13-1.42)	0.2374
No	31/278	28/256	90.3/92.1	3/22	9.7/7.9	1.25 (0.35-4.43)	0.7267
Diabetes							
Yes	21/104	18/90	85.7/86.5	3/14	14.3/13.5	1.07 (0.28-4.12)	0.1393
No	71/476	68/435	95.8/91.4	3/41	4.2/8.6	0.47 (0.14-1.55)	0.2491
Family history of cancer							
Yes	6/17	2/12	33.3/70.6	4/5	66.7/29.4	4.80 (0.65-35.20)	0.1616
No	86/563	84/513	97.7/91.1	2/50	2.3/8.9	0.24 (0.06-1.02)	0.0331

OR: Odds ratio; CI: confidence interval.

physical exercise habits, metastasis and survival may strengthen our capacity of performing further analysis of risk factors. Lastly, transcriptional (mRNA) and translational (protein) studies, especially the comparison of tissues from those with the CG and GG genotypes of *COX2* G-765C, should be validated in both tumor tissues and normal adjacent tissues.

In conclusion, the present study indicates that the functional *COX2* G-765C polymorphism is associated with susceptibility to RCC in Taiwan, and this polymorphic site may serve both as a novel biomarker for RCC and as a potential target for anticancer drug development. Functional assays are warranted to reveal the role of *COX2* G-765C in RCC carcinogenesis.

Acknowledgements

This study was supported by research grants from the Terry Fox Cancer Research Foundation and Taiwan Ministry of Health and Welfare Clinical Trial and Research Center of Excellence (MOHW103-TDU-B-212-113002). The assistance from Tsai-Ping Ho in data collection together with that from Yi-Kai Huang, and Ding-Hao Sun in genotyping was highly appreciated by the Authors.

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Received June 18, 2014

Revised July 29, 2014

Accepted July 30, 2014