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ORIGINAL ARTICLE

Association of CAA and TATC Insertion/Deletion Genetic Polymorphisms in *RTN4* 3′-UTR with Hepatocellular Carcinoma Risk

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Abstract Evidence from recent researchers suggested that *RTN4* is a multifunctional gene, including tumor suppression, apoptosis, vascular remodeling, and inhibition of axonal regeneration. The CAA and TATC insertion/deletion polymorphisms (CAA/TATC polymorphisms) of *RTN4* 3″-untranslated regions (UTRs) have been linked to cervical squamous cell carcinoma (CSCC), uterine leiomyomas (UL) and non-small cell lung cancer (NSCLC). However, the association between these two polymorphisms sites with Hepatocellular Carcinoma (HCC) risk was not carry out before. A total of 284 HCC patients and 484 control subjects were recruited for this study. The *RTN4* CAA/TATC insertion/deletion genotypes were determined using polymerase chain reaction (PCR) assay. The ID/DD genotypes of CAA were significantly associated with an increased risk of HCC compared with the II genotype (ID vs. II: OR = 1.50, 95% CI: 1.10–2.04; DD vs. II: OR = 2.00, 95%CI: 1.15–3.46). Meanwhile, the frequency of D allele of CAA was significantly related with an increased risk of HCC compared with the I allele (D vs. I: OR = 1.39, 95% CI: 1.12–1.73). The ID genotypes of TATC was significantly associated with an increased risk of HCC compared with the DD genotype (ID vs. DD: OR = 1.70, 95% CI: 1.23–2.33). The present study provided evidence that *RTN4* CAA/TATC polymorphisms were associated with HCC development in Chinese Han population.

Keywords Hepatocellular carcinoma · *RTN4* · Nogo · Polymorphisms

Introduction

HCC is one of the most common malignancies with the fourth highest incidence rate in the world. More than a half million people have been diagnosed with HCC in the world every year [1]. HCC is the second leading cause of cancer deaths in

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China, and nearly half of all new cases of liver cancer (50.5%) and related deaths (51.4%) worldwide, are estimated to occur in China [2, 3]. Many causes for HCC have been proposed, and it's clear that a hereditary predisposition to HCC development exists [4–7]. And genetics of HCC pathogenesis is complex and largely unknown [8].

RTN4 gene, mapped to chromosome 2p12–14, plays an important role in the apoptosis and inhibition of tumor, vascular remodeling, inhibition of axonal regeneration [9]. A tremendous amount of researchers have been focusing on two insertion/deletion polymorphisms sites [CAA (rs34917480), (TATC (rs71682890)] in the 3′- UTR of *RTN4*, and confirmed the associations with diseases such as CSCC [10], UL [11] and NSCLC [12], schizophrenia [13], dilated cardiomyopathy (DCM) [14], congenital heart disease [15]. However, the association of *RTN4* CAA/TATC polymorphisms with HCC remained unclear. Therefore, a hospital based case-control study was conducted to explore whether these two genetic variations are associated with HCC risk in Chinese Han people.

Material and Methods

Study Population

Our study conform the ethics committee guidelines and all the participants provided written informed consent. Peripheral blood was taken from each participant and stored at −20 °C with EDTA anticoagulating agent until use. The case-control study enrolled 768 subjects including 284 unrelated HCC patients (243 men and 41 women) from the West China Hospital of Sichuan University between 2008 and October 2010. Clinical characteristics were abstracted from the participants medical records regarding the individual's gender, age, family history of HCC, and the state of hepatitis B surface antigen (HBs Ag) which indicates whether to be infected by HBV or not. 484 healthy individuals (394 men and 90 women) from a routine health survey as controls. The diagnosis of HCC was confirmed by tissue pathology examination. The control group inclusive criteria were no evidence of any personal or family history of cancer or other serious diseases, especially any disease in liver, such as infected by hepatitis virus, alcoholic hepatitis, or cirrhosis were excluded from the study. Patients with other cancers or family history of cancer were also excluded. The demographics of the patients and controls enrolled in this study were presented in Table 1.

Genotyping

Genomic DNA of each individual was extracted from 200 μl EDTA-anticoagulated peripheral blood samples according to the instruction manual by DNA isolation kit from Bioteke (Peking, China).

The PCR assay was used to genotype the CAA/TATC polymorphisms of *RTN4*. The sequence of primers and condition for amplification was according to the previously published study [11]. PCR products were analyzed directly by vertical nondenaturing polyacrylamide gel electrophoresis and visualized by silver staining. The results were confirmed by two persons each time. To confirm the accuracy of the method used, different genotypes of PCR products were analyzed by direct sequencing, and the results were 100% agreed.

Statistical Analysis

All data analyses were carried out using SPSS for Windows software package version 13.0 (SPSS Inc., Chicago, IL). Demographic and clinical data of both groups were compared by the chi-square test. Allele and genotype frequencies of *RTN4* CAA/TATC polymorphisms were obtained by using Modified-Powerstates standard edition software. Hardy–Weinberg equilibrium was evaluated by the $\chi 2$ test. Odds ratio (OR) and 95% confidence intervals (CI) were used to evaluate the effects of any difference between genotypes or alleles. Statistical significance was assumed at $P < 0.05$ level.

Results

The genotypes of the *RTN4* CAA/TATC polymorphisms were successfully gained in all 768 subjects. The genotype and allele frequency distributions of the two polymorphisms in the control group met the requirements of the Hardy-Weinberg equilibrium (Table 2). As shown in Table 2, the ID/DD genotypes of CAA were significantly associated with an increased risk of HCC compared with the II genotype (CAA ID vs. II: OR = 1.50, 95% CI: 1.10–2.04, $p = 0.01$; DD vs. II: OR = 2.00, 95%CI: 1.15–3.46, $p = 0.01$). At the

Table 1 Clinical characteristics of the HCC patients and controls

Characteristics	HCC patients $n = 284$ (%)	Controls $n = 484$ (%)
Gender		
Male	243(85.6)	394(81.4)
Female	41(14.4)	90(18.6)
Age (years)		
Mean ± SD	51.8 ± 12.7	48.2 ± 15.8
HBV serological markers		
HBs Ag(+)	215(75.7)	
HBs Ag(−)	69(24.3)	
Family history of HCC		
Yes	26(9.2)	
No	258(90.8)	

n the number of individuals

same time, the frequency of D allele of CAA was significantly associated with an increased risk of HCC compared with the I allele (CAA D vs. I: OR = 1.39, 95% CI: 1.12–1.73, p = 0.003). Meanwhile, compared with the DD genotype, the ID genotypes of TATC was significantly increased risk of HCC (ID vs. DD: OR = 1.70, 95% CI: 1.23–2.33, p = 0.01).

Discussion

HCC is clinically silent for most of its course, and the majority of patients present with advanced disease that has little chance of effective treatment. Despite the recent 20 years in the pathogenesis of the HCC involved in epigenetic changes have been greatly developed, genetic pathogenesis of HCC is complex and still largely unknown yet [8]. The present study sheds light on the potential association between HCC and *RTN4* CAA/TATC polymorphisms. Our results identified that the *RTN4* CAA polymorphisms (ID/DD genotypes and D allele) and the TATC polymorphisms (ID genotype) were significantly associated with an increased risk of HCC.

RTN4 gene contains eight introns and nine exons, and located on chromosome 2p12–14 [16]. Derived from differential splicing and varied promoter usage, the *RTN4* produces 3 major isoforms, named neurite growth inhibitor (Nogo)-A, Nogo-B and Nogo-C [16, 17]. Nogo-A, mainly express in the central nervous system, which has been identified as an inhibitor of axonal regeneration. Nogo-B is found in most tissues, while Nogo-C is highly express in skeletal muscles [17, 18]. Recent investigations have indicated that the Nogo protein induces apoptosis in various tissue or cell lines, such as HCC tissue [19], Human HCC cell line(SMMC-7721) [19] and hepatic stellate cell [20], human glioma cell line [21], other cancer cell lines (SaOS-2, HT-1080, MeWo, CGL4) [22], and cardiomyocytes [23]. Suggesting that *RTN4* may act a role in suppressing tumor development [24]. The 3′-UTRs of eukaryotic mRNA, which is among noncodingregions, have been shown to be involved in regulating mRNA stability, cellular and subcellular localization, and translation efficiency [25–27]. Former research revealed mutations in 3′-UTR was associated with neuroblastoma, myotonic dystrophy and a-thalassemia [28]. The CAA/TATC polymorphisms are located at 4068–4071 and 4548–4554 in 3′-UTR of *RNT4* mRNA, respectively [27]. Novak G. et.al reported that *RNT4* CAA/TATC polymorphisms induce abnormal regulation of *RTN4* expression [29]. Recently, numbers of case-control studies were conducted to investigate the association between CAA/TATC polymorphisms and cancer risk [11, 12, 30]. De-Yi L et al. conducted the case-control study including 411 NSCLC patients and 471 unrelated healthy controls. They found the D allele and ID/DD genotypes of *RNT4* CAA polymorphisms distributions were significantly different between cases and controls. Therefore, they conclude that the *RNT4* CAA polymorphisms contribute to NSCLC risk in Chinese population [12]. This result was consistent with K Zhang et al.'s study, which including 286 UL patients and 450 control subjects, and they declared the DD genotypes carriers had significantly increased association of UL risk when compared with other genotypes [11]. Shi S et al. determined the genotypes of the *RNT4* CAA/TATC polymorphisms in 336 CSCC patients and 450 unrelated control subjects, but they didn't find any difference allele frequencies between patients and control subjects. While stratified analysis results revealed both CAA/TATC polymorphisms were associated with high clinical stage, and the CAA polymorphisms was also associated with positive parametrial invasion [30]. In the present study, significantly increased HCC risk was found to be associated with CAA polymorphisms (ID/DD genotypes and D allele) and TATC polymorphisms (ID genotype). The data indicated that *RTN4* CAA/TATC polymorphisms may be involved in the development of HCC.

There were a few limitations in this study. The relatively small sample size may cause instability to the result. And the information of environmental exposure was not detailed. Further studies with a larger size of samples and the genetic and environmental interaction analysis could help to confirm the exact significance of the association between these polymorphisms and the susceptibility of HCC.

In conclusion, the present study demonstrated that CAA/TATC polymorphisms in *RTN4* were linked to increased HCC risk. Suggesting the *RTN4* CAA/TATC polymorphisms may participate in the development and progression of HCC. Nevertheless, larger sample size and genetic and environmental interaction studies will be needed to clarify the findings in the future.

Table 2 Genotype and allele frequencies of RNT4 CAA/TATA polymorphisms between the HCC patients and controls

Polymorphism	HCC(284)	Control(484)	CI(95%)	p
CAA				
II	107(37.7)	235(48.6)	Ref	
ID	148(52.1)	217(44.8)	1.50(1.10–2.04)	**0.01**
DD	29(10.2)	32(6.6)	2.00(1.15–3.46)	**0.01**
I	362(63.7)	687(71.0)	Ref	
D	206(36.3)	281(29.0)	1.39(1.12–1.73)	**0.003**
TATC				
DD	96(33.8)	215(44.4)	Ref	
ID	152(53.5)	201(41.5)	1.70(1.23–2.33)	**0.01**
II	36(12.7)	68(14.1)	1.19(0.74–1.90)	0.54
D	344(60.6)	631(65.2)	Ref	
I	224(39.4)	337(34.8)	1.22(0.98–1.51)	0.07

Boldfaced values indicate a significant difference at the 5% level

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Compliance with Ethical Standards

Conflict of Interest Statement The authors have declared that no conflict of interest exists.

References

1. Hoyos S, Escobar J, Cardona D, Guzmán C, Mena Á, Osorio G, Pérez C, Restrepo JC, Correa G (2015) Factors associated with recurrence and survival in liver transplant patients with HCC - a single center retrospective study. Ann Hepatol Off J Mex Assoc 14(1):58–63
2. Hong Y, Long J, Li H, Chen S, Liu Q, Zhang B, He X, Wang Y, Li H, Li Y (2015) An analysis of Immunoreactive signatures in early stage hepatocellular carcinoma. Ebiomedicine 39(6):438–446
3. Mu LN, Cao W, Zhang ZF, Cai L, Jiang QW, You NC, Goldstein BY, Wei GR, Chen CW, Lu QY (2007) Methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms and the risk of primary hepatocellular carcinoma (HCC) in a Chinese population. Cancer Causes Control 18(6):665–675
4. Wang ZC, Liu LZ, Liu XY, Hu JJ, Wu YN, Shi JY, Yang LX, Duan M, Wang XY, Zhou J, Fan J, Gao Q (2015) Genetic polymorphisms of the multidrug resistance 1 gene MDR1 and the risk of hepatocellular carcinoma. Tumour Biol: J Int Soc Oncodevelopmental Biol Med 36(9):7007–7015. doi:10.1007/s13277-015-3407-1
5. Toyoda H, Kumada T, Tada T, Kiriyama S, Tanikawa M, Hisanaga Y, Kanamori A, Kitabatake S, Ito T (2015) Risk factors of hepatocellular carcinoma development in non-cirrhotic patients with sustained virologic response for chronic hepatitis C virus infection. J Gastroenterol Hepatol 30 (7):1183–1189. doi:10.1111/jgh.12915
6. Thompson KJ, Humphries JR, Niemeyer DJ, Sindram D, McKillop IH (2015) The effect of alcohol on Sirt1 expression and function in animal and human models of hepatocellular carcinoma (HCC). Adv Exp Med Biol 815:361–373. doi:10.1007/978-3-319-09614-8_21
7. Kazuaki N, Michiie S, Susumu Y, Satoru T, Setsuo H (2005) Akt phosphorylation is a risk factor for early disease recurrence and poor prognosis in hepatocellular carcinoma. Cancer 103(2):307–312
8. Herceg Z, Paliwal A (2011) Epigenetic mechanisms in hepatocellular carcinoma: how environmental factors influence the epigenome. Mutat Res/Fundam Mol Mech Mutagen 727(3):55–61
9. Yang J, Yu L, Bi AD, Zhao SY (2000) Assignment of the human reticulon 4 gene (RTN4) to chromosome 2p14– > 2p13 by radiation hybrid mapping. Cytogenet Cell Genet 88(1–2):101–102
10. Shi S, Zhou B, Wang Y, Chen Y, Zhang K, Wang K, Quan Y, Song Y, Rao L, Zhang L (2012) Genetic variation in RTN4 3'-UTR and susceptibility to cervical squamous cell carcinoma. DNA Cell Biol 31(6):1088–1094
11. Zhang K, Bai P, Shi S, Zhou B, Wang Y, Song Y, Rao L, Zhang L (2013) Association of genetic variations in RTN4 3'-UTR with risk of uterine leiomyomas. Pathol Oncol Res Por 19(3):475–479
12. De-Yi L, Xu-Hua M, Ying-Hui Z, Xiao-Long Y, Wei-Ping W, Ya-Biao Z, Juan-Juan X, Ping Z, Jian-Guo W, Neetika A (2014) RTN4 3'-UTR insertion/deletion polymorphism and susceptibility to non-small cell lung cancer in Chinese Han population. Asian Pac J Cancer Prev Apjcp 15(13):5249–5252
13. Novak G, Kim D, Seeman P, Tallerico T (2002) Schizophrenia and nogo: elevated mRNA in cortex, and high prevalence of a homozygous CAA insert. Brain Res Mol Brain Res 107(2):183–189
14. Zhou B, Rao L, Li Y, Gao L, Li C, Chen Y, Xue H, Liang W, Lv M, Song Y (2009) The association between dilated cardiomyopathy and RTN4 3'UTR insertion/deletion polymorphisms. Clin Chimica Acta; Int J Clin Chem 400(1–2):21–24
15. Chen Y, Zhou B, Li H, Peng Y, Wang Y, Rao L (2010) Analysis of RTN4 3'UTR insertion/deletion polymorphisms in ventricular septal defect in a Chinese Han population. DNA Cell Biol 30(5):323–327
16. Oertle T, Huber CPH, Schwab ME (2003) Genomic structure and functional characterisation of the promoters of human and mouse nogo/rtn4. J Mol Biol 325(2):299–323
17. Yates D (2012) Neural development: nogo to synapse formation. Nat Rev Neurosci 13(4):221–221
18. Liu X, Wang Y, Yong Z, Wei Z, Xu X, Niinobe M, Yoshikawa K, Lu C, Cheng H (2009) Nogo-a inhibits necdin-accelerated neurite outgrowth by retaining necdin in the cytoplasm. Mol Cell Neurosci 41(1):51–61
19. Chen YC, Dong-Dong LU, Cao XR, Zhang XR (2005) RTN4-C Gene expression in He patocellular carcinoma and its influence on SMMC7721 cell growth and apoptosis. Acta Genet Sin 32(9):891–897
20. Tashiro K, Satoh A, Utsumi T, Chung C, Iwakiri Y (2013) Absence of nogo-B (reticulon 4B) facilitates hepatic stellate cell apoptosis and diminishes hepatic fibrosis in mice - the American journal of pathology. Am J Pathol 182(3):786–795
21. Xiao Jun WU, Cheng LY, Rui CH, Xiang CJ, Huang CG, Rong YZ, Wei PJ, Luo C, Han HG, Lou MQ (2008) Nogo-B expression in human brain gliomas and its relationship with tumor pathological grades. Chin J Cancer Biother 15(6):566–569
22. Li Q, Qi B, Oka K, Shimakage M, Yoshioka N, Inoue H, Hakura A, Kodama K, Stanbridge EJ, Yutsudo M (2001) Link of a new type of apoptosis-inducing gene ASY/nogo-B to human cancer. Oncogene 20(30):3929–3936. doi:10.1038/sj.onc.1204536
23. Sarkey JP, Chu M, Mcshane M, Bovo E, Mou YA, Zima AV, Tombe PPD, Kartje GL, Martin JL (2011) Nogo-a knockdown inhibits hypoxia/Reoxygenation-induced activation of mitochondrial-dependent apoptosis in cardiomyocytes. Biophys J 100(3):1044–1055
24. Watari A, Yutsudo M (2003) Multi-functional gene ASY/nogo/RTN-X/RTN4: apoptosis, tumor suppression, and inhibition of neuronal regeneration. Apoptosis 8(1):5–9
25. Mendell JT, Dietz HC (2001) When the message goes awry: disease-producing mutations that influence mRNA content and performance. Cell 107(4):411–414
26. Kuersten S, Goodwin EB (2003) The power of the 3' UTR: translational control and development. Nat Rev Genet 4(8):626–637. doi:10.1038/nrg1125
27. Li H, Chen Y, Zhou B, Peng Y, Bai W, Rao L (2012) RNT4 3'-UTR insertion/deletion polymorphisms are not associated with atrial septal defect in Chinese Han population: a brief communication. DNA Cell Biol 31(6):1121–1124. doi:10.1089/dna.2011.1386
28. Conne B, Stutz A, Vassalli JD (2000) The 3' untranslated region of messenger RNA: a molecular 'hotspot' for pathology? Nat Med 6(6):637–641. doi:10.1038/76211
29. Novak G, Tallerico T (2006) Nogo a, B and C expression in schizophrenia, depression and bipolar frontal cortex, and correlation of nogo expression with CAA/TATC polymorphism in 3'-UTR. Brain Res 1120(1):161–171
30. Shaoqing S, Bin Z, Kui Z, Lin Z (2013) Association between two genetic variants of CD226 gene and cervical squamous cell carcinoma: a case–control study. Gene 519(1):159–163