RESEARCH COMMUNICATION

No Association Between the USP7 Gene Polymorphisms and Colorectal Cancer in the Chinese Han Population

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Abstract

Colorectal cancer (CRC), now the third most common cancer across the world, is known to aggregate in families. USP7 is a very important protein with an important role in regulating the p53 pathway, which is critical for genomic stability and tumor suppression. We here genotyped eight SNPs within the USP7 gene and conducted a case-control study in 312 CRC patients and 270 healthy subjects in the Chinese Han population. No significant associations were found for any single SNP and CRC risk. Our data eliminate USP7 as a potential candidate gene towards for CRC in the Han Chinese population.

Keywords: Association - USP7 gene - colorectal cancer - Chinese Han population

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Introduction

Ubiquitin specific protease 7 (USP7), also known as herpes virus-associated ubiquitin specific protease (HAUSP), belongs to the ubiquitin specific peptidase class of deubiquitination enzymes (Everett et al., 1997; Hu et al., 2002). It plays an important role in regulating the p53 pathway, which is critical for genomic stability and tumor suppression (Li et al., 2002).

p53, one of the most important tumor suppressor proteins, plays an essential role in regulating cell cycle, senescence and apoptosis in response to many types of stress. The level of P53 protein is critical for normal cellular homeostasis. In normal cells, P53 protein is maintained at very low levels due to regulation by the ubiquitin-proteolysis system. The ubiquitination and subsequent degradation of P53 are largely controlled by mdm2 (Ashcroft and Vousden, 1999). By contrast, ubituitin specific protease 7 deubiquitinates the P53 protein and directly stabilizes P53 (Li et al., 2002).

In addition, USP7 regulates Mdm2 through deubiquitination and MdmX is also known to be regulated by USP7, while under normal circumstance MdmX and Mdm2 down-regulate the activity of P53 (Meulmeester et al., 2005). USP7-mediated effects in the P53-Mdm2 axis are thus highly complex and non-linear. A number of studies have confirmed that up-regulation of USP7 induces P53 stabilization (Li et al., 2002). However, it has also been found that knock-down or deletion of USP7 induces profound stabilization of P53 (Cummins et al., 2004) and it may be that the balance between the deubiquitination of the different targets of HAUSP determines the steady-state level of P53. In a human colon carcinoma xenograft model, Becker et al demonstrated that up and down regulation of HAUSP both stabilize endogenous P53 levels and inhibit cell proliferation (Becker et al., 2008). USP7 might therefore play an important role in carcinogenesis. Based on this, USP7 interference has been proposed as a rational therapeutic strategy for activating wild type P53 in tumors.

Apart from its role in the P53 pathway, USP7 is also known to be involved in other pathways affecting cell proliferation and apoptosis. Directly or indirectly, USP7 controls the cellular levels of many proteins such as transcription factor FoxO4 (van der Horst et al., 2006), histone H2B, regulator protein Daxx (Tang et al., 2010), DNA methyltransferase Dnmt1 (Qin et al., 2010), and many other important proteins (Kessler et al., 2007; de Bie et al., 2010; Maertens et al., 2010).

Colorectal cancer is the third most common cancer and the fourth-leading cause of cancer death worldwide. It is known to aggregate in families, with the disease

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Table 1 Allele and Genotype Distribution in Colorectal Cancer Patients and Controls.

SNP ID	Genotype frequency			P value*	Allele frequency		X^2	P value*	Odds Ratio (95%CI)
rs2304467	CC	CG	GG		C	G			
CRC	86(0.305)	139(0.493)	57(0.202)	0.978	311(0.551)	253(0.449)	0.037	0.848	1.02(0.81~1.30)
NZ	79(0.300)	129(0.490)	55(0.209)		287(0.546)	239(0.454)			
rs7195624	GG	GT	TT		G	T			
CRC	127(0.446)	128(0.449)	30(0.105)	0.779	382(0.670)	188(0.330)	0.212	0.645	0.94(0.73~1.21)
NZ	124(0.473)	110(0.420)	28(0.107)		358(0.683)	166(0.317)			
rs2447911	AA	AC	CC		A	C			
CRC	146(0.541)	101(0.374)	23(0.085)	0.556	393(0.728)	147(0.272)	0.837	0.360	1.13(0.87~1.48)
NZ	127(0.494)	107(0.416)	23(0.089)		361(0.702)	153(0.298)			
rs7192405	CC	CT	TT		C	T			
CRC	1(0.003)	23(0.079)	266(0.917)	0.748	25(0.043)	555(0.957)	0.556	0.456	1.24(0.71~2.16)
NZ	1(0.004)	25(0.098)	230(0.898)		27(0.053)	485(0.947)			
rs1657066	AA	AC	CC		A	C			
CRC	144(0.503)	117(0.409)	25(0.087)	0.967	405(0.708)	167(0.292)	0.061	0.805	1.03(0.79~1.34)
NZ	131(0.512)	104(0.406)	21(0.082)		366(0.715)	146(0.285)			
rs4985012	AA	AG	GG		A	G			
CRC	111(0.487)	88(0.386)	29(0.127)	0.782	310(0.680)	146(0.320)	0.368	0.544	0.92(0.70~1.20)
NZ	117(0.455)	106(0.412)	34(0.132)		340(0.661)	174(0.339)			
rs2304466	AA	AG	GG		A	G			
CRC	128(0.451)	129(0.454)	27(0.095)	0.584	385(0.678)	183(0.322)	0.066	0.797	0.97(0.75~1.25)
NZ	120(0.460)	110(0.421)	31(0.119)		350(0.670)	172(0.330)			
rs1058329	CC	CG	GG		C	G			
CRC	254(0.885)	31(0.108)	2(0.007)	0.869	539(0.939)	35(0.061)	0.169	0.681	0.90(0.54~1.50)
NZ	228(0.898)	24(0.094)	2(0.008)		480(0.945)	28(0.055)			

*Pearson's p value; CRC, colorectal cancer; NZ, normal control

being two-to-three times more common among the first degree-relatives of cases than in those of population controls. Twins studies have indicated that in ~35% of all colorectal cancer cases, the disease was inherited (Lichtenstein et al., 2000). While about 5% of CRC can be ascribed to highly penetrant germline mutations of APC, MLH1 or MSH2, the majority of the excess familial risk remains unaccounted for. Genes in p53-dependent pathways are typical candidate genes for colorectal cancer association analysis. A number of studies have examined the association between P53/Mdm2 and colorectal cancer (Fang et al., 2010; Tang et al., 2010), but very few have focused on USP7 polymorphisms.

Here we investigated the possibility that USP7 gene polymorphisms might have an effect on colorectal cancer. To test for association with the disease, we genotyped eight SNPs (rs2304467, rs7195624, rs2447911, rs7192015, rs1657066, rs4985012, rs2304467, rs1058329) of the USP7 gene in colorectal cancer patients and normal controls in the Chinese Han population.

Materials and Methods

Subjects

In total, 312 sporadic colorectal cancer patients (178 males and 134 females, age: 61.23 ± 14.03 years) and 270 healthy controls (145 males and 125 females, age: 43.53 ± 7.94 years) were recruited for the case-control study. All the CRC patients had undergone curative resection between 1999 and 2007 at the surgical department of the Shanghai First People's Hospital or the Shanxi People's Hospital, China. The pathologic tumor staging was performed according to Duke's criteria. All subjects gave informed consent for the genetic analysis, which was reviewed and approved by the ethics committee of the Human Genetics Center in Shanghai. DNA was extracted using standard methods with phenol/chloroform purification.

Genotyping

We genotyped eight genetic polymorphisms, namely rs2304467, rs7195624, rs2447911, rs7192015, rs1657066, rs4985012, rs2304467, rs1058329 from the dbSNP (http: //www.ncbi.nlm.nih.gov/SNP) to cover a ~57.8kb region of USP7. All eight markers are intronic SNPs. We genotyped these SNPs using the TaqMan® assay method and the ABI 7900 DNA detection system (Applied Biosystems, Foster City, California). All probes and primers were designed by the Assay-on-Design service of Applied Biosystems. The standard PCR was performed using the TaqMan® Universal PCR Master Mix (Applied Biosystems) reagent.

Statistical analysis

We analyzed Hardy-Weinberg equilibrium, allelic and genotypic distributions on http://analysis.bio-x.cn/myAnalysis. php (Shi and He 2005), a user-friendly platform which integrates analysis tools appropriate to association studies. A Monte Carlo simulation strategy and the χ^2 test were used to compare the discrepancies of allele and genotype frequencies between CRC patients and controls. We estimated linkage disequilibrium (LD) with D' as the standardized measurement for all possible pairs of SNP loci. The haploview program was used to estimate haplotype frequencies. Power calculations were performed using the G*Power program (Faul et al., 2007). All the p values in the study were two-tailed and the significance level was set at p=0.05. Results are also expressed in terms of the odds ratio (OR) and 95% confidence interval (CI), which were calculated on the website http://www.

Table 2 Estimation of Linkage Disequilibrium Between the 8 SNPs

9r2\D'	rs2304467	rs7195624	rs2447911	rs7192015	rs1657066	rs4985012	rs2304466	rs1058329
rs2304467	-	0.927	0.901	0.825	0.905	0.903	0.932	0.932
rs7195624	0.506	-	0.92	0.982	0.922	0.969	0.748	0.694
rs2447911	0.387	0.704	-	0.199	0.951	0.958	0.701	0.346
rs7192015	0.04	0.023	0.001	-	0.117	0.338	0.829	0.99
rs1657066	0.409	0.71	0.895	0	-	0.988	0.728	0.531
rs4985012	0.514	0.861	0.73	0.003	0.767	-	0.835	0.715
rs2304466	0.507	0.557	0.398	0.07	0.439	0.639	-	0.852
rs1058329	0.057	0.057	0.003	0.002	0.007	0.051	0.086	-

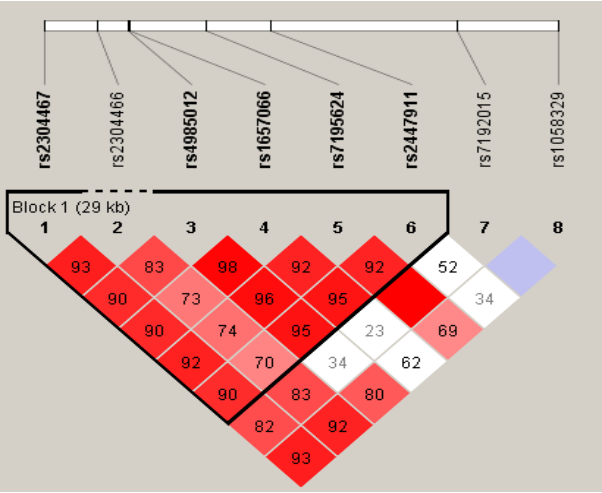

Figure 1. Haplotypes Estimated by the Haploview Program. Block 1 consists of rs2304467, rs7195624, rs2447911, rs1657066 and rs4985012

hutchon.net/ConfidOR.htm. The significant level of power calculation was set α=0.05 with odds ratio set at 1.25.

Results

A total of 312 colorectal cancer patients and 270 normal controls were studied in our experiment. Genotype distributions for the eight SNPs showed no significant deviations from Hardy-Weinberg equilibrium in either CRC or controls. The allele and genotype frequencies of the SNPs are listed in Table 1. No significant association was found between any of the eight SNPs and colorectal cancer. In the power calculations using the G*Power 3 program, our sample size had a greater than 90% power to detect a significant ($a < 0.05$) association for alleles, genotypes and haplotypes when an effect size index of 0.1 (corresponding to a "weak" gene effect) was used.

Discussion

In this study, we genotyped eight polymorphisms, and all genotypes were in Hardy-Weinberg equilibrium. No significant SNP or haplotype associations were found. The SNPs selected for the study are mostly tag-SNPs from the human hapMap data. The statistical power of our data was considered sufficient to detect any association between the patients and controls. Dividing the groups by gender did not reveal any significant differences in alleles or genotype distribution (data not show).

LD between each pair of the eight SNPs is presented in Table 2. They are derived from data of the total sample.

Table 3 Estimated Haplotype Frequencies and Association Significance

Haplotype*	frequences case	frequences control	chi2	p value	odd ratio (95% CI)
CGAAA	0.553	0.524	0.526	0.468	1.105[0.843~1.448]
GGAAA	0.118	0.128	0.278	0.598	0.898[0.601~1.341]
GTAAG	0.04	0.038	0.017	0.895591	1.046[0.531~2.062]
GTCCG	0.251	0.263	0.221	0.637932	0.930[0.687~1.259]

*Haplotype consist rs2304467, rs7195624, rs2447911, rs1657066, rs4985012

Five of the SNPs were in strong LD and therefore a five-variant haplotype analysis was carried out. Only 4 out of 19 possible haplotypes exhibited more than 3% frequency in both cases and controls but no significant haplotype difference was observed (Table 3).

The human USP7 gene is 71.4kb in size, consisting of 31 exons. The eight SNPs investigated in this study span the region around most of the exons, covering about 57.8 kb, but up and down regulated regions were not included. Though we found no association, the possibility could not be ruled out that other polymorphisms in the gene are involved in the pathogenesis of colorectal cancer.

Genetic studies of cancer have identified many candidates as tumor susceptibility genes. However, the effect of high penetrance genes only accounts for a small fraction of common cancers (Pasche and Yi, 2010). The high-risk familial variants of APC, MMR, MUTYH, SMAD4, BMPR1A and STK11/LKB1 identified in colorectal cancer explain < 6% of CRC cases (Aaltonen et al., 2007). Some of the unexplained familial risk is presumably due to mutations in as yet unidentified genes, but polygenic mechanisms are likely to account for the larger proportion of familial cancer risks (Houlston and Peto, 2004). USP7-mediated effects in the P53-Mdm2 axis are highly complex and non-linear and may contribute to cancer etiology by interacting with other candidate genes. We genotyped polymorphisms in USP7 only and the effect of one gene might not be significant enough to be detected.

Colorectal cancer (CRC) is a multifactorial disease with both environmental and genetic factors contributing to its development. It has been thought to be less common amongst Asians compared with Caucasians. However, the incidence and mortality rate of CRC has been rising in a number of Asian countries in the last few decades (Hyodo et al., 2010). The effect of environmental, lifestyle and dietary factors in CRC incidence within the Chinese Han population are still unclear. More studies need to be done to identify CRC risk factors.

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