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Research Paper

FUT2 genetic variants as predictors of tumor development with hepatocellular carcinoma

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Abstract

Lewis antigens related to the ABO blood group are fucosylated oligosaccharides and are synthesized by specific glycosyltransferases (FUTs). FUTs are involved in various biological processes including cell adhesion and tumor progression. The fucosyltransferase-2 gene (*FUT2*) encodes alpha (1,2) fucosyltransferase, which is responsible for the addition of the alpha (1,2)-linkage of fucose to glycans. Aberrant fucosylation occurs frequently during the development and progression of hepatocellular carcinoma (HCC). However, the association of *FUT2* polymorphisms with HCC development has not been studied. Therefore, we aimed to investigate the association of *FUT2* polymorphisms with demographic, etiological, and clinical characteristics and with susceptibility to HCC. In this study, a total of 339 patients and 720 controls were recruited. The genotypes of *FUT2* at four single-nucleotide polymorphisms (SNPs; rs281377, rs1047781, rs601338, and rs602662) were detected by real-time polymerase chain reaction from these samples. Compared with the wild-type genotype at SNP rs1047781, which is homozygous for nucleotides AA, at least one polymorphic T allele (AT or TT) displayed significant association with clinical stage (p = 0.048) and tumor size (p = 0.022). Our study strongly implicates the polymorphic locus rs1047781 of *FUT2* as being associated with HCC development.

Key words: Fucosyltransferase-2; Hepatocellular carcinoma; Single-nucleotide polymorphism

Introduction

Hepatocellular carcinoma (HCC) is one of the most lethal and prevalent cancers worldwide. HCC occurs frequently among Asian and African populations because of the endemic hepatitis B and C virus infections [1]. Recent evidence has shown that the expression of cancer-specific carbohydrate antigens (CACAs) is associated with malignant transformation [2]. Aberrant fucosylation of glycosphingolipids occurs frequently during the development and progress of HCC [3]. In a previous study, we found that several CACAs are better predictive and more sensitive biomarkers than alpha-fetoprotein (AFP) for HCC, including disialosyl galactosyl globoside (DSGG), fucosyl GM1, and Gb2

based on an analysis with a glycan array [4]. Additionally, hepatitis B virus X protein (HBX) suppresses the expression of microRNA 15b (miR-15b), which directly targets fucosyltransferase 2 (FUT2) and leads to an increase in Globo H expression. Globo H is a cancer-associated carbohydrate antigen and it can synthesis by *FUT2*, this glycolipid highly expressed in various types of cancers, including breast cancer, liver cancer and prostate cancer cells. Overexpression of miR-15b effectively suppresses tumor growth in a mouse xenograft model of HCC [5].

FUT2 is the alpha (1,2) fucosyltransferase responsible for the synthesis of Lewis type 2 (Galβ1,4-GlcNAc) and type 1 (Galβ1,3-GlcNAc) antigen precursors and Globo H. Increasing alpha (1,2) fucosyltransferase activity changes the glycolipid composition and the cellular properties, including cell-to-cell adhesion and drug resistance, of ovarian cancer cells or tumor tissue [6]. *FUT2*, which is 9,980 base pairs in length, codes for the alpha (1,2) fucosyltransferase [7]. Inactivating mutations in *FUT2* reduce susceptibility to *Helicobacter pylori* infection by mediating *H. pylori* adhesion to gastric mucosa [8, 9]. Two *FUT2* mutants (739G to A, and 839T to C) are almost inactive and responsible for some non-secretor status [10].

Single-nucleotide polymorphisms (SNPs) in *FUT2* have been reported that are associated with the levels of vitamin B12 [11-13]. The vitamin B12 can reduce the DNA damage and decrease the cancer incidence rate. One SNP (rs1047781) of *FUT2* is the susceptible locus for recurrence of colorectal cancer in individuals from southern China [14, 15]. The association of rs1047781 with cancer antigen 19-9 (CA19-9) levels and carcinoembryonic antigen (CEA) concentration in esophageal squamous cell cancer and pancreatic cancer also was confirmed by genome-wide association studies [16]. Moreover, our previously study also shown that interactions of FUT2 polymorphisms with betel quid chewing habits maybe altering oral cancer susceptibility [17]. However, the effects of *FUT2* polymorphisms are still unclear in HCC. In the present study, we aimed to investigate the association of *FUT2* polymorphisms with HCC. We analyzed four SNPs (rs281377, rs1047781, rs601338, and rs602662) in exon 2 region of *FUT2* gene for associations with demographic, etiological, and clinical characteristics and with susceptibility to HCC.

Materials and Methods

Study subjects and specimen collection

This hospital-based case-control study recruited 339 patients with HCC between 2010 and 2015 from the Chung Shan Medical University Hospital in Taichung, Taiwan, to serve as the case group. The diagnosis of HCC was made according to the criteria specified in the national guidelines for HCC. HCC patients were clinically staged at the time of diagnosis according to the tumor/node/metastasis staging system of the American Joint Committee on Cancer (2002). For control group, all 720 control individuals were recruited at the same hospital and these control individuals had neither self-reported history of cancer of any sites. The patients' clinic pathological characteristics, including clinical staging, lymph node metastasis, and histopathologic grading levels, were verified by chart review. Whole-blood specimens collected from the controls and HCC patients were placed in tubes containing EDTA, immediately centrifuged, and stored at −80°C. Study protocols were approved by the institutional review of the Taichung Chung Shan Medical University Hospital. All methods were carried out in accordance with the approved guidelines. All subjects provided written informed consent before participating in the study.

Selection of *FUT2* polymorphisms

Four SNPs in *FUT2* (NM_000511) were selected from the International HapMap Project data for this study. We included the synonymous SNP rs281377 and non-synonymous SNPs rs1047781, rs601338, and rs602662, all of which are located in the exon region of *FUT2* gene.

FUT2 Genotyping

Allelic discrimination of the *FUT2* polymorphisms rs281377, rs1047781, rs601338, and rs602662 was assessed using an ABI StepOne Real-Time PCR System (Applied Biosystems), SDS v3.0 software (Applied Biosystems), and the TaqMan assay [17-19]. The final volume for each reaction was 5 µL, containing 2.5 µL TaqMan Genotyping Master Mix, 0.125 µL TaqMan probes mix, and 10 ng genomic DNA. The reaction conditions included an initial denaturation step at 95°C for 10 min followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min.

Statistical analysis

A Mann-Whitney U-test was used to compare differences in age and demographic characteristics between controls groups and HCC patients. The odds ratios (ORs) with 95% confidence intervals (CIs) were estimated by logistic regression models. The adjusted odds ratios (AORs) with 95% CIs of the association between genotype frequencies and HCC risk as well as clinical pathological characteristics were estimated by multiple logistic regression models after controlling for other covariates. Values of $p < 0.05$

were considered significant. The data were analyzed using SAS statistical software (Version 9.1, 2005; SAS Institute Inc., Cary, NC).

Table 1. Demographic characteristics and clinical parameters for 720 controls and 339 patients with HCC.

Variable	Controls (N = 720)	HCC patients (N = 339)	*p*-value
Age (yr)	**Mean ± S.D.**	**Mean ± S.D.**	
	52.26 ± 10.08	62.99 ± 11.65	<0.001*
Gender	**n (%)**	**n (%)**	
Male	590 (81.9%)	242 (71.4%)	
Female	130 (18.1%)	97 (28.6%)	<0.001*
Alcohol consumption			
No	598 (83.1%)	214 (63.1%)	
Yes	122 (16.9%)	125 (36.9%)	<0.001*
Tobacco consumption			
No	413 (57.4%)	202 (59.6%)	
Yes	307 (42.6%)	137 (40.4%)	0.493
HCC stage			
I or II		222 (65.5%)	
III or IV		117 (34.5%)	
Tumor T status			
≤T2		225 (66.4%)	
>T2		114 (33.6%)	
Lymph node status			
N0		328 (96.8%)	
N1 + N2		11 (3.2%)	
Metastasis			
M0		321 (94.7%)	
M1		18 (5.3%)	
Vascular invasion			
No		278 (82.0%)	
Yes		61 (18.0%)	

Mann-Whitney U-test was used between controls and patients with HCC. *$p < 0.05$.

Results

For this case-cohort study, 720 healthy controls and 339 patients with HCC were recruited. According to our analysis of the demographic characteristics and the etiological and clinical characteristics of HCC among these individuals (Table 1), we found that age ($p < 0.001$), gender ($p < 0.001$), and alcohol consumption ($p < 0.001$) were significantly associated with HCC risk. HCC was more common in individuals over 60 years old, in males, and in those who have a history of alcohol consumption. To decrease the possible interference of several environmental factors, the AORs and their 95% CIs were estimated after controlling for the risk related to age, gender, alcohol consumption, and tobacco use in each comparison by multiple logistic regression models. The distribution frequency of *FUT2* genotypes in both controls and HCC patients is shown in Table 2. Alleles with the highest distribution frequency were as follows: homozygous T/T for rs281377, heterozygous A/T for rs1047781, and homozygous G/G for both rs601338 and rs602662. There was no significant difference with respect to rs281377, rs1047781, rs601338, and rs602662 polymorphisms of *FUT2* between healthy controls and patients with HCC.

Table 2. Distribution frequency of *FUT2* genotypes in 720 controls and 339 patients with HCC.

Variable	Controls (N = 720) n (%)	Patients (N = 339) n (%)	OR (95% CI)	AOR (95% CI)
rs281377				
TT	534 (74.2%)	259 (76.4%)	1.00	1.00
TC	174 (24.2%)	75 (22.1%)	0.889 (0.653–1.210)	1.069 (0.722–1.582)
CC	12 (1.6%)	5 (1.5%)	0.859 (0.299–2.464)	0.502 (0.123–2.047)
TC + CC	186 (25.8%)	80 (23.6%)	0.887 (0.656–1.198)	1.018 (0.693–1.494)
rs1047781				
AA	217 (30.2%)	95 (28.0%)	1.00	1.00
AT	363 (50.4%)	169 (49.9%)	1.063 (0.786–1.439)	1.020 (0.697–1.493)
TT	140 (19.4%)	75 (22.1%)	1.224 (0.845–1.771)	1.106 (0.690–1.771)
AT + TT	503 (69.8%)	244 (72.0%)	1.108 (0.833–1.474)	1.044 (0.730–1.495)
rs601338				
GG	715 (99.3%)	336 (99.1%)	1.00	1.00
GA	5 (0.7%)	3 (0.9%)	1.277 (0.303–5.374)	0.694 (0.076–6.361)
AA	0 (0%)	0 (0%)	–	–
GA + AA	5 (0.7%)	3 (0.9%)	1.277 (0.303–5.374)	0.694 (0.076–6.361)
rs602662				
GG	715 (99.3%)	336 (99.1%)	1.00	1.00
GA	5 (0.7%)	3 (0.9%)	1.277 (0.303–5.374)	0.694 (0.076–6.361)
AA	0 (0%)	0 (0%)	–	–
GA + AA	5 (0.7%)	3 (0.9%)	1.277 (0.303–5.374)	0.694 (0.076–6.361)

The odds ratios (ORs) and their 95% confidence intervals (CIs) were estimated by logistic regression models. The adjusted odds ratios (AORs) and their 95% CIs were estimated by multiple logistic regression models after controlling for age, gender, alcohol consumption, and tobacco use.

We further investigated the effects of polymorphic genotypes of *FUT2* (rs281377 and rs1047781) on the clinical status of HCC (Table 3 and Table 4). Based on the genotypic frequencies of rs281377 and rs1047781, only *FUT2* rs1047781 showed a significant association with clinical stage ($p = 0.048$), tumor size ($p = 0.022$), and the absence of anti-HCV antibodies ($p = 0.037$). In the routine blood tests carried out in conjunction with a HCC diagnosis, including alpha-fetoprotein (AFP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), we demonstrated that at least one polymorphic T allele of rs1047781 displayed a high association with the ratio of AST/ALT ($p = 0.037$) as compared with the wild-type genotype (Table 5).

Table 3. Odds ratio (OR) and 95% confidence interval (CI) of clinical status and *FUT2* rs281377 genotypic frequencies in 339 HCC patients.

Variable	Genotypic frequencies			
	TT (N = 259)	TC + CC (N = 80)	OR (95% CI)	*p*-value
HCC Stage				
I or II	171 (66.0%)	51 (63.8%)	1.00	0.709
III or IV	88 (34.0%)	29 (36.2%)	1.105 (0.655–1.865)	
Tumor size				
≤T2	174 (67.2%)	51 (63.8%)	1.00	0.570
>T2	85 (32.8%)	29 (36.2%)	1.164 (0.689–1.967)	
Lymph node metastasis				
No	253 (97.7%)	75 (93.8%)	1.00	0.083
Yes	6 (2.3%)	5 (6.2%)	2.811 (0.835–9.469)	
Distant metastasis				
No	246 (95.0%)	75 (93.8%)	1.00	0.668
Yes	13 (5.0%)	5 (6.2%)	1.262 (0.436–3.653)	
Vascular invasion				
No	215 (83.0%)	63 (78.8%)	1.00	0.386
Yes	44 (17.0%)	17 (21.2%)	1.319 (0.705–2.466)	
Child-Pugh grade				
A	197 (76.1%)	61 (76.3%)	1.00	0.972
B or C	62 (23.9%)	19 (23.7%)	0.990 (0.549–1.783)	
HBsAg				
Negative	151 (58.3%)	47 (58.8%)	1.00	0.943
Positive	108 (41.7%)	33 (41.2%)	0.982 (0.590–1.633)	
Anti-HCV				
Negative	140 (54.1%)	37 (46.3%)	1.00	0.222
Positive	119 (45.9%)	43 (53.7%)	1.367 (0.827–2.216)	
Liver cirrhosis				
Negative	56 (21.6%)	11 (13.8%)	1.00	0.122
Positive	203 (78.4%)	69 (86.2%)	1.730 (0.858–3.491)	

The ORs and their 95% CIs were estimated based on logistic regression models. >T2: multiple tumors of >5 cm in diameter or tumor(s) involving a major branch of the portal vein or hepatic veins.

Discussion

FUT2 encodes alpha (1,2) fucosyltransferase, which catalyzes the addition of terminal alpha (1,2) fucose residues on glycans such as Globo H and Lewis Y. Increasing evidence indicates that Globo H and Lewis Y are highly overexpressed in various types of malignant tumors including breast, liver, prostate, and pancreatic cancer [3, 20-23]. Although it is generally accepted that *FUT2* has an important role in cancer, associations between *FUT2* polymorphisms and clinical characteristics of HCC have not been analyzed. Thus, we assessed whether *FUT2* SNPs are associated with HCC risk or with the clinical features of HCC.

FUT2 is associated with several chronic diseases such as Crohn's disease and several autoimmune or immune-mediated chronic diseases [24, 25]. For example, *FUT1/FUT2* is predominantly expressed in M1 inflammatory macrophages, and the expression of these genes is highly correlated with the level of *TNF* (which encodes tumor necrosis factor) in patients with rheumatoid arthritis (RA) [26]. In addition, the non-secretor allele of *FUT2* SNP rs601338 confers susceptibility to type 1 diabetes and resistance to infections [25]. About 90% of HCC cases arise in individuals with chronic liver inflammation and fibrosis caused by damage to the liver by, for example, alcohol consumption [27, 28]. Indeed, in our current data, we observed that alcohol consumption was associated with HCC (Table 1) and that the non-secretor allele of *FUT2* SNP rs1047781 displayed significant association with the AST/ALT ratio, which is an indicator of liver damage (Table 5). Based on a test for alkaline phosphatase (ALP) in the blood, it has recently been reported that preoperative ALP levels could be used to monitor and predict recurrence in high-risk HCC patients [29]. The *FUT1* SNP rs2071699 and the *FUT2* SNP rs1047781 are also associated with serum ALP levels among the Japanese population [30].

FUT2 includes some ethnic group–specific polymorphisms [31], but the non-secretor phenotypes are present within most populations [32]. In the present study, we included the synonymous SNP rs281377 and non-synonymous SNPs rs1047781, rs601338, and rs602662 as candidate SNPs to investigate the association with HCC development. The wild-type rs1047781 (A) encodes the "secretor" allele of *FUT2*, whereas rs1047781 (T) encodes the non-secretor allele. The presence of at least one polymorphic T allele of rs1047781 resulted in a high association with a clinical stage of III or IV and with larger tumor size for individuals with HCC (Table 4). In addition, *FUT2* rs1047781 may be the susceptible locus for recurrence of colorectal cancer in a population from southern China [14]. Overexpression of Lewis Y, which is the glycan product of FUT1 and FUT2, promotes human epididymis protein 4–mediated invasion and metastasis of ovarian cancer [33]. HCC is a highly vascularized tumor with frequent intrahepatic metastasis [34-36]. Cheng *et al.* noted that Globo H, which is another glycan product of FUT2, might shed from cancer cells through microvesicles, resulting in enhanced angiogenic activity [37].

We found no significant association between FUT2 polymorphisms and AFP levels, which is similar to findings from a previous study of individuals with HCC [16]. Recently, the use of AFP levels in HCC diagnosis has been doubted because of the significant rates of false-positive and

false-negative findings [38]. By using a glycan array, we recently identified several CACAs that have a better predictive sensitivity than AFP [4]. In addition, alpha-fetoprotein fraction L3 (AFP-L3), which is synthesized by malignant cells and incorporates a fucosylated oligosaccharide, has been shown to be a better early diagnostic and prognostic marker for HCC [39, 40].

In conclusion, our findings suggest that gene–clinical characteristic interactions might alter the susceptibility for HCC development. This study provides new information on the association of *FUT2* polymorphisms with the clinical pathology of HCC in the Taiwanese population.

Table 4. Odds ratio (OR) and 95% confidence interval (CI) of clinical status and *FUT2* rs1047781 genotypic frequencies in 339 HCC patients.

Variable	Genotypic frequencies			
	AA (N = 95)	AT + TT (N = 244)	OR (95% CI)	*p*-value
HCC stage				
I or II	70 (73.7%)	152 (62.3%)	1.00	0.048*
III or IV	25 (26.3%)	92 (37.7%)	1.695 (1.003–2.865)	
Tumor size				
≤T2	72 (75.8%)	153 (62.7%)	1.00	0.022*
>T2	23 (24.2%)	91 (37.3%)	1.862 (1.089–3.183)	
Lymph node metastasis				
No	91 (95.8%)	237 (97.1%)	1.00	0.531
Yes	4 (4.2%)	7 (2.9%)	0.672 (0.192–2.350)	
Distant metastasis				
No	89 (93.7%)	232 (95.1%)	1.00	0.606
Yes	6 (6.3%)	12 (4.9%)	0.767 (0.279–2.107)	
Vascular invasion				
No	82 (86.3%)	196 (80.3%)	1.00	0.197
Yes	13 (13.7%)	48 (19.7%)	1.545 (0.795–3.003)	
Child-Pugh grade				
A	75 (78.9%)	183 (75.0%)	1.00	0.444
B or C	20 (21.1%)	61 (25.0%)	1.250 (0.705–2.215)	
HBsAg				
Negative	58 (61.1%)	140 (57.4%)	1.00	0.537
Positive	37 (38.9%)	104 (42.6%)	1.164 (0.718–1.890)	
Anti-HCV				
Negative	41 (43.2%)	136 (55.7%)	1.00	0.037*
Positive	54 (56.8%)	108 (44.3%)	0.603 (0.374–0.973)	
Liver cirrhosis				
Negative	20 (21.1%)	47 (19.3%)	1.00	0.710
Positive	75 (78.9%)	197 (80.7%)	1.118 (0.621–2.010)	

The ORs and their 95% CIs were estimated by logistic regression models. >T2: multiple tumors of >5 cm in diameter or tumor(s) involving a major branch of the portal vein or hepatic veins. *$p < 0.05$.

Table 5. Association of *FUT2* genotypic frequencies with HCC-related laboratory status.

Characteristic	α-Fetoprotein[a] (ng/mL)	AST[a] (IU/L)	ALT[a] (IU/L)	AST/ALT ratio[a]
rs281377				
TT	3381.7 ± 966.6	129.3 ± 16.6	108.0 ± 12.7	1.53 ± 0.10
TC + CC	3979.4 ± 2150.3	154.4 ± 39.3	133.6 ± 31.6	1.35 ± 0.09
p value	0.777	0.498	0.376	0.331
rs1047781				
AA	3927.2 ± 1899.4	108.5 ± 21.5	108.0 ± 25.0	1.22 ± 0.07
AT + TT	3365.3 ± 1001.2	145.6 ± 20.1	116.3 ± 14.0	1.59 ± 0.11
p value	0.778	0.288	0.761	0.037*

Mann-Whitney U-test was used between two groups.
[a]Mean ± S.E.

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Competing Interests

The authors have no conflict of interest.

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