RESEARCH ARTICLE

RECK Gene Promoter rs10814325 Polymorphism in Egyptian Patients with Hepatocellular Carcinoma on Top of Chronic Hepatitis C Viral Infection

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Abstract

Background: The reversion-inducing-cysteine-rich protein with Kazal motifs (RECK) gene is a novel transformation suppressor gene linked to several malignancies. Objective: To analyze any association between RECK gene rs10814325 single-nucleotide polymorphism (SNP) and HCC susceptibility with various clinico-pathological and laboratory data. Materials and Methods: RECK gene rs10814325 SNP was estimated, using real-time PCR, in 30 HCC patients on top of HCV infection, 30 HCV related cirrhotic patients and 30 healthy controls. Results: No special pattern of association could be detected on comparing the RECK gene rs10814325 genotypes(P=0.5), or alleles(P=0.49) among the studied groups. HCC patients with TT genotype had younger age (mean of 54.1±6.0 years vs 60.6±10.2 years for TC/CC genotypes, P=0.035). Abdominal distension was significantly greater in TT genotype patients (75% vs 30% for TC/CC genotypes, P=0.045). The TT genotype was present in 75% of patients with lymph node metastasis. Serum GGT levels were higher in TT genotype patients [80 (48.5-134.8) IU/L vs 40 (33-87.5) IU/L for TC/CCgenotypes], and lower limb edema was observed in 60% for TT vs 20% for TC/CCgenotypes, but both just failed to reach significance (p=0.05 and p=0.06 respectively). Conclusions: RECK gene rs10814325 T>C could not be considered a risk factor for HCC development on top of HCV, but may be related to the disease progression and metastasis.

Keywords: Hepatocellular carcinoma - HCV related-cirrhosis - RECK gene - gene polymorphism - real time PCR

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Introduction

Hepatocellular carcinoma (HCC) is the fifth malignant tumor in incidence worldwide and despite current progress with the treatment of cancers, existing therapies are limited in their abilities to cure HCC and fatality remains high, making it the third most common cause of death from cancer worldwide (Shiraha et al., 2013; Mlynarsky et al., 2015).

Among HCC predisposing factors, chronic hepatitis C and B with associated liver cirrhosis represent the two major risk factors for its development, being implicated in more than 70% of HCC cases worldwide (Chung et al., 2012; El-Serag and Kanwal, 2014).

Egypt has the highest hepatitis C virus (HCV) prevalence in the world (Lavanchy, 2011), probably due to extensive iatrogenic transmission during the era of parenteral antischistosomal therapy. A significant number of HCV infected patients do not respond to the currently available interferon and ribavirin combination treatment, leading to an estimate of more than 350,000 deaths every year from HCV-related liver cirrhosis and HCC (Mohd Hanafiah et al., 2013).

In addition, individual genetic predisposition may have a role in HCC development risk. Cumulative studies have suggested an association between particular single nucleotide polymorphisms (SNPs) in selected candidate genes and the increasing risk of HCC development (Weng et al., 2010; Chen et al., 2011).

The reversion-inducing-cysteine-rich protein with Kazal motifs (RECK) gene is a tumor suppressor gene that has been identified on human chromosome 9p13-p12, with a length of 87kb. The glycoprotein product is anchored to the plasma membrane via a COOH-terminal glycosylphosphatidyl inositol (GPI)-modification, and has a significant effect on tumorigenesis through negative regulation of matrix metalloproteinases (MMPs). It was also found to inhibit tumor angiogenesis and invasion through the extracellular matrix. RECK was found to be down-regulated in many human cancers and was clinically associated with lymph node metastasis (Clark et al., 2007; Chung et al., 2012).

Thirteen SNPs have been identified on RECK gene, four of them are in the coding region of exons 1, 9, 13

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and 15, and the remaining 9 are in introns 5, 8, 10, 12, 15 and 17 (Meng et al., 2008). RECK rs10814325 SNP is located at promoter sites and was predicted to be able to change transcription factor-binding sites by TRANSFAC® (Eisenberg et al., 2002).

The aim of this study was to analyze the distribution pattern of rs10814325 SNP of the RECK gene in Egyptian patients with HCC on top of HCV infection, in HCV-related cirrhosis group, and in a group of healthy individuals, in an attempt to find an association that might exist between this polymorphism and the risk of developing HCC. Association with various clinical and laboratory data of the patients was also investigated.

Materials and Methods

This cross-sectional study was carried out between March 2013 and August 2014 at Kasr Al-Aini Hospital,

Table 1. Demographic, Clinical, Laboratory and Radiological Data of the Three Studied Groups.

	HCC (n=30)	Cirrhosis (n=30)	Control (n=30)	P
Age (Years)*	56.2 ± 8.1	56.8 ± 8.1	57.2 ± 8.8	0.91
Male/Female†	23/7	23/7	23/7	0.999
Smoking‡	18 (60%)	17 (56.7%)	17 (56.7%)	0.96
Alcohol intake‡	1 (3.3%)	1 (3.3%)	0 (0.0%)	0.6
Family history‡	5 (16.7%)	0 (0.0%)	--	**0.05**
Bilharziasis‡	22 (73.3%)	20 (66.7%)	--	0.57
Cachexia‡	6 (20%)	10 (33.3%)	--	0.24
Jaundice‡	17 (56.7%)	17 (56.7%)	--	0.999
Abdominal distension‡	18 (60%)	28 (93.3%)	--	**0.002**
Lower limb edema‡	14 (46.7%)	27 (90%)	--	**<0.001**
Hematemesis‡	10 (33.3%)	13 (43.3%)	--	0.43
Melena‡	10 (33.3%)	15 (50%)	--	0.19
Encephalopathy‡	6 (20%)	12 (40%)	--	0.09
Child Pugh stage‡				
A	8 (26.7%)	3 (10%)		
B	13 (43.3%)	13 (43.3%)		0.19
C	9 (30%)	14 (46.7%)		
TLC (x10³ cell/μl)§	5.65 (3.97-8.17)	4.25 (3.22-8.07)	6.5 (5.35-7.67)	**0.08**
Hb (g/dl)*	11.1 ± 2.2a	10.2 ± 1.88[a]	14± 0.9[b]	**<0.001**
Plt (x10³/mm³)§	135 (96.8-163.3) [a]	110 (67.8-156.3)[a]	285.5 (197.8-340.5)[b]	**<0.001**
PT (seconds)§	15 (13.3-16.8) [a]	16 (15-17.9)[a]	12 (12-12.4)[b]	**<0.001**
PC (%)§	72.5 (58-79.5) [a]	63.3(48.8-75)[a]	98 (94-100)[b]	**<0.001**
INR§	1.3 (1.16-1.52) [a]	1.38 (1.2-1.6)[a]	1 (1-1.1)[b]	**<0.001**
BUN (mg/dL)§	30 (21-41) [a]	26 (20-37.8)[a]	20.5 (17.8-25.8)[b]	**<0.001**
Creatinine (mg/dL)§	1 (0.8-1.5) [a]	1 (0.7-1.32)[a]	0.7 (0.5-0.9)[b]	**<0.001**
Total protein (g/dL)*	6.67 ± 1.2 [a]	6.6 ± 0.86[a]	7.37 ± 0.55[b]	**0.003**
Albumin (g/dL)*	2.85 ± 0.74 [a]	2.6 ± 0.48[a]	4.1 ± 0.34[b]	**<0.001**
AST (IU/L)§	68 (46-96.3) [a]	62 (45.3-91.8)[a]	18.5 (15-26.5)[b]	**<0.001**
ALT (IU/L)§	35.5 (20-51) [a]	34.5 (27-51.3)[a]	12.5 (10-17)[b]	**<0.001**
GGT (IU/L)§	53.5 (40-118.3) [a]	76.5 (29.5-96.8)[a]	28.5 (23.8-32.3)[b]	**<0.001**
ALP (IU/L)§	112 (75-174.3)[a]	100 (66-200)[a]	79 (57.3-90)[b]	**0.002**
TBIL (mg/dL)§	1.45 (0.97-3.27)[a]	1.7 (1.1-5.27)[a]	0.75 (0.5-0.9)[b]	**<0.001**
DBIL (mg/dL)§	0.45 (0.28-1.42)[a]	1 (0.4-2)[a]	0.1 (0-0.1)[b]	**<0.001**
HCV RNA (IU/ml)§	101,500 (67,500-172,500)	93,000 (74,500-149,000)	--	0.7
AFP (ng/mL)§	59.5 (7.47-388)	8.1 (4.4-15.3)	--	**0.003**
Liver size‡				
Shrunken	8 (26.7%)	11 (36.7%)		0.07
Average	10 (33.3%)	15 (50%)	--	
Enlarged	12 (40%)	4 (13.3%)		
Spleen status‡				
Average	3 (10%)	0 (0%)		0.16
Enlarged	25 (83.3%)	29 (96.7%)	--	
Removed	2 (6.7%)	1 (3.3%)		
Enlarged Lymph Node‡	4 (13.3%)	0 (0%)	--	0.11
Ascites‡				
Absent/mild	19 (63.4%)	7 (23.3%)	--	**0.004**
Moderate/severe	11 (36.6%)	23 (76.6%)		

*Data are presented as mean ± SD; † Data are presented as number; ‡ Data are presented as number (Percent); § Data are presented as median (25th-75th); Groups bearing the same initials are not statistically different at P < 0.05; HCC, hepatocellular carcinoma; TLC, total leukocyte count; Hb, hemoglobin; Plt, platelets; PT, prothrombin time; PC, prothrombin concentration; INR, international normalized ratio; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, Gamma glutamyl transferase; ALP, alkaline phosphatase; TBIL, total bilirubin; DBIL, direct bilirubin; HCV RNA, hepatitis C virus ribonucleic acid; AFP, alpha-fetoprotein.

Table 2. Frequency Distribution of RECK Gene Genotypes and Alleles

	HCC (n=30)	Cirrhosis (n=30)	Control (n= 30)	P
Genotypes				
TT	20 (66.7%)	23 (76.7%)	19 (63.3%)	
TC	9 (30%)	6 (20%)	9 (30%)	0.8
CC	1 (3.3%)	1 (3.3%)	2 (6.7%)	
TC/CC*	10 (33.3%)	7 (23.3%)	11 (36.7%)	0.5
Alleles				
T allele	49 (81.7%)	52 (86.7%)	47 (78.3%)	0.49
C allele	11 (18.3%)	8 (13.3%)	13 (21.7%)	

Data are presented as number (percent); *versus TT.

Table 3. Clinical, Laboratory and Radiological Data of Different Genotypes of the HCC Group

	TT (n=20)	TC/CC (n=10)	P
Age	54.1±6.0	60.6±10.2	**0.035**
Family history*	4 (20%)	1 (10%)	0.64
Bilharziasis*	14 (70%)	8 (80%)	0.68
Cachexia*	4 (20%)	2 (20%)	0.99
Jaundice*	12 (60%)	5 (50%)	0.7
Abdominal distention*	15 (75%)	3 (30%)	0.045
Lower limb edema*	12 (60%)	2 (20%)	0.06
Hematemesis*	7 (35%)	3 (30%)	0.99
Melena*	7 (35%)	3 (30%)	0.99
Encephalopathy*	4 (20%)	2 (20%)	0.99
Child Pugh stage*			
A	4 (20%)	4 (40%)	0.45
B	10 (50%)	3 (30%)	
C	6 (30%)	3 (30%)	
TLC (x10³ cell/μL)†	5.9 (3.9-8.9)	5 (4-6.83)	0.6
Hb (g/dL)‡	11. ± 2.2	11.4 ± 2.3	0.68
Plt (x10³/mm³)†	131.5 (99-167.3)	138.5 (84.8-176.0)	0.97
PT (sec)†	15.2 (13.5-17.7)	14.8 (12.1-17.1)	0.47
PC (%)†	69.5 (56.8-79)	77 (55.8-81.5)	0.55
INR†	1.3 (1.2-1.6)	1.28 (1-1.55)	0.37
BUN (mg/dL)†	30 (20-44.5)	31 (25.8-39.5)	0.4
Creatinine (mg/dL)†	0.99 (0.8-1.5)	1.07 (0.8-1.4)	0.88
Total protein (g/dL)‡	6.7 ± 1.3	6.55 ± 1.1	0.7
Albumin (g/dL)‡	2.75 ± 0.66	3 ± 0.89	0.35
AST (IU/L)†	71 (49.0-96.8)	59 (39.3-94.3)	0.6
ALT (IU/L)†	40 (18.5-50.8)	31.5 (20-52)	0.98
GGT (IU/L)†	80 (48.5-134.8)	40 (33-87.5)	**0.05**
ALP (IU/L)†	120.5 (79.5-170)	92.5 (72-208.8)	0.66
TBIL (mg/dL)†	1.35 (0.93-2.58)	2.18 (0.97-4.3)	0.39
DBIL (mg/dL)†	0.45 (0.23-1.15)	0.57 (0.28-2.45)	0.69
HCV RNA (IU/ml)†	101,000 (71,750 - 183,623.3)	122,000 (64,000 - 168,500)	0.64
AFP (ng/ml)†	86.7 (5.8-350)	23.9 (8.3-774.5)	0.73
HFL size (cm)‡	3.63 ± 1.64	4.46 ± 3.1	0.34
Liver size*			
Shrunken	6 (30%)	2 (20%)	
Average	7 (35%)	3 (30%)	0.7
Enlarged	7 (35%)	5 (50%)	
Spleen status*			
Average	1 (3%)	2 (20%)	
Enlarged	17(85%)	8 (80%)	0.28
Removed	2 (10%)	0 (0.0%)	
Enlarged lymph nodes*	3 (15%)	1 (10%)	0.99
Ascites*			
Absent/mild	12 (60%)	7 (70%)	0.28
Moderate/severe	8 (40%)	3 (30%)	
Number of hepatic foci*			
One/two foci	18 (90%)	7 (70%)	0.2
Three/four foci	2 (10%)	3 (30%)	
Focal site*			
Right lobe	17 (85%)	8 (80%)	
Left lobe	3 (15%)	1 (10%)	0.34
Both lobes	0 (0.0%)	1 (10%)	
Focal lesion echogenicity*			
Hypoechoic	20 (100%)	8 (80%)	0.1
Hyperechoic	0 (0%)	2 (20%)	

*Data are presented as number (percent); † Data are presented as median (25^{th}-75^{th}); ‡Data are presented as mean ± SD.

Cairo University. It was conducted on a total number of 90 subjects divided into 3 groups: Group I: including 30 patients with HCC on top of HCV infection and cirrhosis, Group II: including 30 patients with liver cirrhosis on top of HCV taken as a pathological control group, and Group III: including 30 healthy subjects taken as a normal control group.

Patients older than 18 years and those with HCV RNA positive testing were included while those with HBsAg positive testing and secondary metastatic liver tumors were excluded. The study was conducted according to the ethical guidelines of the declaration of Helsinki with informed consent taken from all participants beforehand.

All participants were subjected to full history taking and full clinical examination. In addition, group I and II patients were examined radiologically with ultrasonography and triphasic C.T. Participants underwent the following tests: CBC, PT, PC, INR, total & direct bilirubin, AST, ALT, GGT, ALP, total protein and albumin, urea and creatinine, alpha fetoprotien (AFP) level assayed by electrochemiluminescence on cobas e411, (Roche Diagnostics, Germany), HBsAg assayed by 3rd generation ELISA using kit supplied by Murex Biotech Limited, UK, HCV RNA and identification of RECK gene polymorphism (T/C) (rs10814325) using Applied Biosystems Step oneTM Real Time PCR system, Biosystems Life Technologies Inc, Canada.

Identification of the RECK gene polymorphism (T/C) (rs10814325)

DNA was extracted from EDTA vacutainer using

QIAamp DNA blood Mini kit (Qiagen GmbH -Germany) and stored at -20°C till time of amplification. Real-time PCR allelic discrimination assay was designed using TaqMan SNP Genotyping Assay for RECK gene polymorphism (T/C) (rs10814325), ID: (C_27084758_10) (Applied Biosystems).

The SNP Genotyping Assay (40x) was diluted to a 20x working stock using nuclease free water, vortexed, centrifuged and aliquots were stored at -20°C. Each reaction was performed in a total volume of 25 µl/well that contained 12.5 µl of 2x TaqMan Universal PCR Master Mix (UMM) (0.05 units/µl Taq DNA polymerase in reaction buffer, 4 mM MgCl2, 0.4 mM of each of dNTPs), 1.25 µl (20x) working stock of SNP Genotyping Assay, 4 µl of DNA, and 7.25 µl nuclease free water. The real-time PCR reaction was conducted with an initial denaturation step at 95°C for 10 min, followed by 50 cycles, each consisting of 92°C for 15 sec and 60°C for 1.30 min.

TaqMan minor groove binder (MGB) probes from the SNP Genotyping Assay provided a fluorescence signal for the amplification of each allele, where a substantial increase in VIC dye fluorescence only indicated homozygosity for Allele 1 (mutant C allele), a substantial increase in FAM dye fluorescence only indicated homozygosity for Allele 2 (wild T allele), while a substantial increase in both VIC and FAM dyes fluorescence indicated Allele 1-Allele 2 heterozygosity.

Following PCR amplification, an endpoint plate read was performed. Sequence Detection System (SDS) Software used the fluorescence measurements resulting during the plate read to plot fluorescence (Rn) values based on the signals from each well. The plotted fluorescence signals indicated the types of alleles in each sample.

Statistical analysis

Quantitative data were summarized as mean ± SD when normally distributed and as median (25^{th}-75^{th}) when abnormally distributed. Differences between groups were detected using ANOVA with post hoc test, Kruskal Wallis, Student's t and Mann Whitney tests as appropriate. Qualitative data were summarized as number and percentages and compared by Chi square (X^2) test. All tests were two tailed and considered statistically significant at a P value <0.05. Statistical analysis was run on SPSS for mac, release 20.0 (SPSS Inc., Chicago, Ill, USA).

Results

Demographic, clinical and laboratory data of the three studied groups are summarized in Table 1. No significant association could be detected as regarding frequency distribution of RECK gene genotypes and the three studied groups (P=0.796), Table 2.Three out of four HCC patients with lymph node metastasis (75%) had TT genotype. Laboratory and radiological data for the genotypes among HCC patients are presented in Table 3.

Discussion

Hepatocarcinogenesis is a slow multistep process that involves complex pathological and biological events, during which genomic changes progressively alter the hepatic cells phenotype to produce cellular intermediates that evolve into HCC (Bakiri and Wagner. 2013). RECK gene modulates the function of membrane type-1 matrix metalloproteinase (MT1-MMP) by direct inhibition of its proteolytic activity and modulation of the endocytic pathways. This modulation can give RECK gene a key role in the regulation of angiogenesis and maintenance of vascular integrity, and hence, in tumor suppression (Zhang et al., 2012).

An ethnicity-related variation in the frequency of RECK gene rs10814325 polymorphisms has been documented. This could serve as an explanation for the contradictory findings of RECK polymorphisms in different populations (Chung et al., 2012).

This study aimed to investigate the distribution pattern of rs10814325 SNP of the RECK gene in three groups of Egyptian patients; HCC on top of HCV infection group, HCV-related cirrhosis group, and a group of healthy individuals, looking for a possible association that might exist with the risk of developing HCC.

An expected pattern of changes in laboratory data was encountered; whereby, haemoglobin, platelet count, PC, total protein and albumin showed a significant reduction in both the HCC and cirrhosis groups compared to the normal controls. On the other hand, there was a significant increase in PT, INR, AST, ALT, ALP, GGT, total and direct bilirubin, BUN and creatinine in the first two groups.

Alpha-fetoprotein was significantly higher in HCC patients than in cirrhotic patients (P=0.003). It was previously stated that, even slightly elevated AFP levels (6-20 ng/ml), could serve as a risk factor for HCC development in patients infected with HCV, in contrast, AFP levels <6 ng/ml indicate a low risk of HCC development in those patients regardless of the fibrosis stage (Tateyama et al., 2011).

Failure to detect an association between alcohol intake and HCC development is probably related to the rarity of drinking habits in our society not to an insignificance of alcohol intake for HCC development, which was repeatedly proven (Morgan et al., 2004; Liu et al., 2014).

Genetic predisposition for HCC development in patients with HCV has been previously reinforced by many studies (Wang et al., 2004; Kato et al., 2005). In our study, a trend towards positive family history of HCC was detected in HCC group compared to the cirrhosis group, where all patients with positive family history of HCC developed HCC. However; this did not reach a significant value; P=0.052. Almost 17% of HCC patients had positive history compared to 0.0% of cirrhotic patients, this might suggest a possible genetic predisposition background.

The genetic predisposition for HCC development can be also supported based on the clinico-pathological findings of our patients. Marked deterioration in the cirrhosis group patients in comparison to the HCC group was observed, despite the fact that they did not develop HCC. Lower limb edema was significantly higher in cirrhosis group (90%) compared to HCC group (46.7%), P<0.001. Abdominal distension was present in 93.3% of cirrhosis versus 60% of HCC group (P=0.002), and moderate to marked ascites were also significantly higher

in cirrhotic patients (76.6% vs 36.6%, P=0.004).

Although other clinical findings failed to reach significant levels, they were more frequently encountered in cirrhotic patients; 33.3% had cachexia compared to HCC patients (20%), haematemesis (43.3% vs 33.3%), encephalopathy (40% vs 20%), Child Pugh class C (46.7% vs 30%), and melena (50% vs 33.3%).

No special pattern of association could be detected on comparing the RECK gene rs10814325 SNP genotypes among the three studied groups (P=0.796). Insight of the small number of members in the CC genotype, collapse of genotypes TC and CC seemed appropriate to address this issue and to look for a more meaningful association, however; this did not yield a significant difference (P=0.51). Allelic distribution failed as well to detect a significant association.

Up to our knowledge, only one Taiwanese study, investigated the potential association of RECK promoter rs10814325 SNP with HCC susceptibility. In contrary to our finding, they were able to demonstrate a 1.85-fold [95% CI, 1.03-3.36] higher risk for HCC in patients inheriting at least one C allele (TC+CC) compared to TT wild type carriers, after adjusting for other variables. The allele with the highest distribution frequency in both HCC patients and healthy controls was heterozygous TC, showing a 2.1 -fold (95% CI: 1.108-4.028), while CC showing a 2.2-fold (95% CI: 1.211-3.939) higher risk for HCC (Chung et al., 2012).

On the other hand, RECK promoter rs10814325 SNP was investigated in other malignancies, yet yielding contradictory results. A recent Chinese study, testing the possible association between three RECK genetic variants and the susceptibility to advanced non-small cell lung cancer (NSCLC) as well as the chemotherapy response status, found that only the genotype distributions and allele frequencies of rs10814325 T>C were significantly different between NSCLC and controls (P<0.001). By multivariate analysis, markedly higher risk for NSCLC was observed in rs10814325 CC genotype (adjusted OR=2.302, 95% CI, 1.514-3.501, P=0.012) with TT as reference, after adjustment for other variables (Chen et al., 2014).

In accordance to our finding, a large case-control study conducted in Sweden, failed to detect an association between RECK rs10814325 polymorphism and breast cancer susceptibility, however; in the survival analysis study, only the TC heterozygotes showed a better survival compared to the TT homozygotes (P=0.02 in all cases and P=0.03 in lymph node negative cases) (Lei et al., 2007).

Another side of the argument is that RECK was basically proposed as a prognostic marker rather than a diagnostic one. A study on pancreatic cancer showed that RECK protein has negative effects on cancer invasiveness by inhibiting MMP-2 activation, supporting the hypothesis that RECK has a potential value as a prognostic molecular marker for cancer (Masui et al., 2003). This goes along with a more recent study highlighting RECK because of its correlation with metastasis and invasiveness, but not as a diagnostic marker. In their study, Alexius-Lindgren and coworkers found that RECK was down-regulated in tumors which were invasive and metastatic (Alexius-Lindgren et al., 2014). Being a cross-sectional study, our study could not detect the progression of the disease in HCC patients. However, it is to be noted that three out of four patients with lymph node metastasis had TT genotype.

Gene-environment interactions between the RECK polymorphism and environmental factors in oral cancer development were also investigated. RECK rs10814325 polymorphism was not found to be associated with oral cancer susceptibility, on the other hand, RECK TC or CC carriers with betel quid chewing had a 7.62-fold [95% CI, 2.96-19.64] risk to have oral cancer compared with TT wild-type carrier without betel quid chewing. Among betel quid chewers, TC or CC smoking carriers had a 6.68-fold (95% CI, 1.21-36.93) risk to have oral cancer compared with TT non-smoking carriers. In betel quid chewing oral cancer patients, TC or CC carriers had a 2.26-fold (95% CI, 1.19-4.29) risk of having neck lymph node metastasis compared with TT carriers. Authors concluded that gene-environment interactions between the RECK polymorphisms, smoking and betel quid may alter oral cancer susceptibility and metastasis (Chung et al., 2011). In our study, it was not possible to draw a relationship between RECK polymorphism and lymph node metastasis as only 4 HCC patients had lymph node metastasis, though this could have answered the question about the role of RECK polymorphism as a prognostic rather than a diagnostic factor.

In continuity, it was suggested that hypermethylation may lead to promoter silencing of RECK mRNA, associated with poor survival in HCC. Zhang and his colleagues found that RECK mRNA were lower in HCC tissues than in non-HCC tissues. Expression of RECK was mainly decreased in HCC patients with hypermethylation than those with demethylation, with significant correlation between RECK mRNA and poor survival after surgery (Zhang et al., 2012).

A similar study previously done in another GIT tumor, gastric carcinoma, suggested that RECK gene aberrant methylation may provide useful information for the early diagnosis and treatment of peritoneal metastasis of gastric cancer (Du et al., 2010).

The study of frequency distribution of multiple clinicopathological findings as regards the two genotype groups of RECK gene within HCC patients did not add much to the results as no overt significant association could be detected except for abdominal distension, where 75% of TT genotype had distension versus 30% of TC/CC genotypes (P=0.045). In accordance, no observed association was also detected in the work done on RECK promoter rs10814325 SNP (Chung et al., 2012), and on RECK mRNA (Zhang et al., 2012).

Different ethnic backgrounds of the patients may account for these contradictory findings. Additional studies with larger sample sizes are needed to validate the different effects of the RECK gene polymorphisms on HCC in different ethnic groups.

In conclusion, the RECK gene rs10814325 T>C could not be considered as a risk factor for the development of HCC in the presence of HCV infection as no special pattern of association was detected.

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