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Short communication

Mdm2 Snp309 G allele displays high frequency and inverse correlation with somatic P53 mutations in hepatocellular carcinoma

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ABSTRACT

Loss of function of the p53 protein, which may occur through a range of molecular events, is critical in hepatocellular carcinoma (HCC) evolution. MDM2, an oncogene, acts as a major regulator of the p53 protein. A polymorphism in the MDM2 promoter, SNP309 (T/G), has been shown to alter protein expression and may thus play a role in carcinogenesis. MDM2 SNP309 is also associated with HCC. However, the role of SNP309 in hepatocarcinogenesis with respect to TP53 mutations is unknown. In this study, we investigated the distribution of the MDM2 SNP309 genotype and somatic TP53 (the p53 tumor suppressor gene) mutations in 99 human HCC samples from Africa, Europe, China and Japan. Samples exhibited striking geographical differences in their distribution of SNP309 genotypes. The frequency and spectrum of p53 mutations also varied geographically; TP53 mutations were frequent in Africa, where the SNP309 T/T genotype predominated but were rare in Europe and Japan, where the SNP309 G allele was present more frequently.

TP53 mutations were detected in 18% (4/22) of SNP309 T/G and G/G and 82% (18/22) of SNP309 T/T genotype holders; this difference was statistically highly significant (P-value = 0.0006).

Our results indicated that the presence of the SNP309 G allele is inversely associated with the presence of somatic TP53 mutations because they only coincided in 4% of HCC cases. This finding suggests that the SNP309 G allele may functionally replace p53 mutations, and in addition to known etiological factors, may be partly responsible for differential HCC prevalence.

1. Introduction

Hepatocellular carcinoma (HCC), the most common liver malignancy, is among the five leading causes of cancer death in the world. The incidence of HCC varies greatly worldwide, depending on the distribution of well-known environmental risk factors such as hepatitis B virus (HBV) and hepatitis C virus (HCV) infections and dietary exposure to aflatoxins [1]. HCC is thought to be mainly an environmental disease; however, not all individuals with exposure to risk factors develop cancer even over the long term. On the other hand, familial clustering and early onset of HCCs in some populations suggest an inherited genetic predisposition to liver cancer [2]. Germline polymorphisms of several genes have been studied as potential risk factors for HCCs [3–5]. However, the pathogenesis of human HCC is a multistage process with the involvement of a series of genes, including oncogenes and tumor suppressor genes; germline polymorphisms of these genes may also determine individual susceptibility to HCC.

The p53 tumor suppressor gene (TP53) is of critical importance for regulating cell cycles and maintaining genomic integrity. TP53 also is a common target for inactivation during liver carcinogenesis. Although this inactivation may be largely due to mutations in the p53 gene, recent evidence suggests that other mechanisms may be involved in p53 inactivation. For instance, the hepatitis B virus-encoded X antigen (HBxAg) binds to and inactivates wild-type p53 [6,7]. Interaction of p53 with a cellular oncoprotein, MDM2, also inactivates p53, via increasing its degradation and/or blocking p53 transcriptional activation [8–10].

In a recent study, a functional single nucleotide polymorphism at nucleotide 309 (T > G) in the promoter region of MDM2 has been reported. Interestingly, cells with the 309 G/G genotype have an enhanced affinity to bind stimulatory protein Sp1 and also show heightened MDM2 expression and a significant attenuation of the p53 pathway compared with those carrying the 309 T/T genotype [11]. Furthermore, SNP309 has been shown to be associated with earlier age of onset of certain hereditary and sporadic cancers in humans [11,12].

In this study, we investigated the distribution of the SNP309 genotype in 99 human HCCs that were previously characterized for TP53 alterations from HCC endemic and rare geographical areas.

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Table 1
Distribution of P53 mutations and SNP309 genotypes in HCC samples from different geographical regions and Hardy–Weinberg equilibrium states of MDM2 genotypes.

Samples	P53 Mut.	SNP309 genotypes			Hardy–Weinberg Equilibrium			
		T/T	T/G	G/G	p	q	Chi-square	*P*-value
Africa (n = 33)	11 (33%)	31	2	0	0.97	0.03	0.03	1
Japan (n = 13)	1 (8%)	0	10	3	0.38	0.62	5.08	0.08
China (n = 21)	5 (23%)	7	3	11	0.40	0.60	10.39	0.001
Europe (n = 32)	5 (16%)	11	16	5	0.59	0.41	0.04	1
Total (n = 99)	22 (22%)	49	31	19	0.65	0.35	9.54	0.0015

Mut, mutations; p and q refer to allele frequencies of T and G, respectively.

Table 2
Inverse relationship between p53 mutation and SNP309 G genotype of MDM2 gene in all HCC samples and without African samples.

		Genotype Frequency		OR (95%CI)	*P*-value
		T/T	T/G + G/G		
All HCC samples	p53 Wt	31	46	0.15 (0.03–0.52)	0.0006
	p53 Mut	18	4		
African samples excluded	p53 Wt	11	44	0.15 (0.03–0.7)	0.0065
	p53 Mut	7	4		

Wt, wild-type; Mut, mutant; OR, odds-ratio; CI, confidence interval.

Our findings revealing the differential occurrence of the SNP309 genotype in mutant and wild-type p53 carriers enhance the understanding of HCC aetiology in a multi-regional context.

2. Materials and methods

We analyzed a total of 99 DNA samples (isolated from histologically confirmed tumor and nontumor liver tissue of the patients) from HCC patients living in different geographical regions, including Mozambique (n = 16), South Africa (excluding Mozambique: n = 17), China (n = 21), Japan (n = 13), Europe (Germany, France, Spain, Turkey, Israel and USA: n = 32). Characteristics of these tumors and methods for DNA isolation have been described previously [13,14].

SNP309 (T/G) of MDM2 were genotyped using PCR amplification of the first intron of the MDM2, followed by MspA1I (Promega) digestion as described elsewhere [15].

All statistical analyses were conducted using R functions in 'genetics' and 'stats' packages (http://www.r-project.org) [16,17]. Pearson's Chi-squared test with simulated *P*-value (based on 10,000 replicates) was applied to test whether the populations were in Hardy–Weinberg equilibrium with respect to MDM2 SNP309 polymorphism. The association between the P53 mutation status and the SNP309 genotypes was assessed using Fishers' exact tests.

3. Results

Ninety-nine samples with known p53 status (n = 99) were genotyped for SNP309. The observed genotypic frequency of SNP309 in HCC patients was distributed as 49% T/T genotype carriers (n = 49), 31% T/G genotype carriers (n = 31), and 19% G/G genotype carriers (n = 19).

Remarkable differences in the allele frequencies for each SNP309 genotype between patients from different geographical regions were observed. The G allele was the most common in the 13 Japanese HCC patients (100%); three of them were homozygous (23%) and 10 of them (77%) were heterozygous (Table 1). Interestingly, there was no wild-type SNP309 genotype carrier (T/T) among Japanese HCC patients (n = 13), although the Japanese population was in HW equilibrium (*P*-value = 0.08). Contrastingly, 31 out of 33 South African patients were wild-type for SNP309 (94%), but only two were heterozygous (6%), while there was no patient with the G/G genotype. Genotypic distributions of African and European populations did not exhibit HW disequilibrium (Table 1). The allele frequencies were highly divergent between African and other populations in which G allele was frequent (Table 1).

Distribution of T/T and T/G – G/G genotypes together was similar between patients from two geographically distant regions: China and Europe [wild-type genotype frequency 33% (7/21) vs. 34% (11/32); mutant genotype frequency 67% (14/21) vs. 66% (21/32), respectively]. However, heterozygote genotype frequency varied drastically between Chinese and European HCC patients [15% (3/21) vs. 50% (16/32)].

We then analyzed whether a significant correlation between the TP53 mutation and SNP309 genotypes existed. Interestingly, 18 of 22 (82%) TP53 mutations were concentrated in 49 (37%) HCC cases displaying the T/T genotype (Table 2). Among the 19 cases homozygous for G/G, three of them had TP53 mutations (16%) and only one of 31 cases of heterozygous T/G displayed somatic TP53 mutations (3%) (Table 2). Considering both T/G and G/G genotypes together (dominant model), only 4% of the 99 HCCs were positive for both p53 gene mutation and the G genotype (Tables 1 and 2).

We next examined whether there was a statistical interaction between the G genotype and TP53 mutations using Fisher's exact test. There was a highly significant inverse relationship between the presence of TP53 mutation and the G allele (*P*-value, two-sided = 0.0006; odds-ratio = 0.153; 95% CI = 0.03–0.52).

We next excluded African patients from the statistical analysis to prevent the potential bias from the high percentage of p53 mutations and SNP309 wild-type genotype carriers in Africa. The inverse relationship between the presence of TP53 mutation and the G genotype was sustained (*P*-value, two-sided = 0.0065; odds-ratio = 0.148; 95% CI = 0.03–0.7).

4. Discussion

Given the importance of the p53 pathway in HCC development, it is of interest to investigate the potential impact of the SNP309 genotype on its own and in combination with p53 mutation status in hepatocellular carcinomas. Here, we analyzed two genetic alterations, one of which is somatic and another of which is germline: TP53 mutations and SNP309 polymorphism. We evaluated dominant and additive models (G/G and G/T genotypes together) because Bond et al. showed a twofold increase in the MDM2 protein for cell lines with the heterozygous (G/T) genotype and a fourfold increase for cell lines with the homozygous variant (SNP309 G/G) genotype [11].

Our study provides evidence for an inverse association between the presence of the SNP309 mutant genotype and p53 mutation in HCC patients. However 4% of our HCC population displayed TP53 mutations despite having SNP309 G allele. The presence of the TP53

mutations in these samples could at least partly be explained by direct affect of known and unknown environmental factors in the etiology of these four HCC samples. Indeed, one of these patients displays G > T mutation in codon 249 of TP53, which is strongly associated with diatery aflatoxin B1 intake [6,7].

Given the functional role of SNP309 in the inhibition of the p53 pathway, the mutant genotype of this SNP may be functionally equivalent to the inactivating p53 mutations in hepatocarcinogenesis. In fact, numerous studies have shown that overexpression of MDM2 is an important event in carcinogenesis; in addition, MDM2 amplification occurs mostly in the absence of p53 mutation, supporting the concept that MDM2 amplification and p53 mutation are alternative mechanisms of p53 dysfunction [8,18,19]. In agreement with our hypothesis, a recent study shows that invasive bladder cancer patients with wild-type SNP309 (T/T) were prone to displaying p53 mutations [20].

On the other hand, our study also provides evidence that the p53 pathway was disrupted either by p53 mutation or the SNP309 G allele in 68/99 HCCs (68%). Thus, the p53 pathway may be more frequently altered in HCCs than previously thought.

Previous studies proposed that high levels of MDM2 resulting from the SNP309 G allele and just one wild-type p53 allele in Li-Fraumeni patients produce a severely weakened p53 tumor suppressor pathway, resulting in a higher mutation rate, poorer DNA repair processes and reduced apoptosis, which lead to faster and more frequent tumor formation [11,21]. Because the G genotype might substitute the need for p53 gene mutation or weaken the p53 tumor suppressor pathway, we suggest that this genotype may contribute to hepatocellular carcinoma risk. Because of lack of healthy control samples in corresponding ethnic groups or countries, we were not able to test this hypothesis with a case–control study. However, in a recent study, Dharel et al. reported that SNP309 is associated with the presence of hepatocellular carcinoma in Japanese patients with chronic hepatitis C [22]. In concordance with the study by Dharel et al., two recent studies indicated an association between the G genotype and risk for hepatocellular carcinoma in Moroccan and Korean patients with chronic hepatitis B infections [23,24].

Our study, together with the studies by Dharel et al., Yoon et al. and Ezzikouri et al., infers that the variations in HCC development not only depend on somatic mutations occurring in the tumor itself but also host genetic factors. Although additional work is necessary to confirm, these findings may raise the possibility that the high prevalence of HCC in some geographical regions, in addition to environmental factors, could be partly due to the high frequency of SNP309 G alleles in the people of these regions.

Conflict of interest

There is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mrfmmm.2009.11.008.

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