“P53 Codon 72 Single Base Substitution in Viral Hepatitis C and Hepatocarcinoma Incidences”

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Abstract Viral infection with hepatitis C virus (HCV) has a high propensity in becoming chronic and it is the major cause of hepatocellular carcinoma (HCC) worldwide. This review was basically established to illustrate the putative role of the P53 gene Arg72Pro polymorphism on various cancer models and viral infections, focusing on HCV and HCC incidences. Authors studied the 72 G/C single base substitution of P53 gene at codon 72 using various polymorphic techniques. Intriguingly, authors investigated that the P53 codon 72 plays a crucial role as risk factor in several cancer models. Others found that there is no association between codon 72 genotypes and HCV disease severity or liver cancer. Moreover, the lack of a significant relationship between this polymorphism and risk of HCC shows that it does not predispose towards hepatocarcinogenesis and the frequent loss of the proline allele in HCV-associated carcinogenesis of the liver plays some critical role in hepatocarcinogenesis. Amazingly, there is a significant correlation between male homozygotes for

P53 72Pro with HCV type 1b infection. However, there was no significant difference between the P53 polymorphism and HCV genotypes 2a and 2b. It was concluded that the P53 gene polymorphism at codon 72 has been investigated as potential risk factor in several cancer models and HCV infections.

Keywords P53 codon 72 · Hepatitis C · Hepatocarcinoma

Introduction

Viral infection with hepatitis C virus (HCV) is one of the major causes of hepatocellular carcinoma (HCC) worldwide. The HCV infection has a high propensity in becoming chronic and the majority of HCV carriers have histological evidence of liver inflammation and chronic damage, although there is a very wide spectrum of severity and progression rate [1]. Hepatocellular carcinoma, the most important primary hepatic cancer worldwide [2], ranks fifth as cause of cancer mortality worldwide [3]. Furthermore, hepatocellular carcinoma arises through a multistep process of genetic derangement in which recurrent gene mutations affecting cell cycle and apoptosis are involved [4]. Unfortunately, the mechanisms leading to liver cell injury, inflammation, and fibrosis in chronic HCV are not fully understood. However, there is evidence to suggest that apoptosis of liver cells may play a significant role in the pathogenesis of the viral infection with HCV [5, 6].

Oncogenicity and P53 Function

Lack of tumor suppressor function, which increases the oncogenic activity rate, results from genetic alterations

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such as mutation, insertion, or deletion within tumor cells, which ultimately causes abnormal gene expression [7]. The P53 gene is a well-known tumor suppressor gene, and the most frequently mutated gene in various human cancer. Moreover, P53 protein serves as a DNA motif binding-specific transcription factor in the regulation of genes that promote growth arrest or apoptotic response to cellular stress [8].

The P53 gene plays a crucial role to acts as the ‘guardian of the genome’. It is able to successfully regulate the expression of cell-cycle control gene (TP21) in response to DNA damage. Amazingly, half of all human malignancies including; colon, bone, lung, breast, lymphoma, cervical, skin, gastric, ovary, brain and urological cancers exhibit P53 mutations and it is frequently accompanied by wild-type P53 inactivation. Regarding to mutational variations, P53 mutations most frequently occur within a highly conserved region comprising exons 4–8 [9]. Many studies investigated whether P53 exon 4–8 mutations are associated with various cancers in tumor grade, stage, metastasis, tumor progression, tumor prognosis and response to different anticancer drugs [10–13]. Unfortunately, the results have not been consistent.

Over 20 years ago, a new P53 mutation structure was discovered based on mobility differences when performing P53 protein electrophoresis, resulting in a new concept of single nucleotide polymorphism (SNP) and carcinogenesis. This difference in mobility was owing to a polymorphism located at P53 codon 72. Normal genetic variation at codon 72 is associated with a proline (Pro) to arginine (Arg) substitution without additional association with carcinogenesis. However, this concept was not fully approved, after more advanced evidence of the association of the codon 72 polymorphism with cancer development and association with viruses such as the cases of HCC associated with HCV [14].

The P53 tumor suppressor gene, located at chromosome 17p13.1, plays an important role in the carcinogenesis of different organs and tissues being involved in gene transcription, DNA synthesis and repair, and programmed cell death. Additionally, the P53 protein is a transcriptional factor that can naturally undergo function-dependent conformational changes. Structural mutations result in altered proteins with aberrant conformation affecting its normal physiological functions [15].

Viral Infections and Functional Inactivation of P53 Codon 72 Polymorphism

The functional inactivation of P53 can be induced by some viral proteins for example the case of the hepatitis C virus (HBV) X protein, which binds P53 and cause

conformational changes, and the E6 proteins of human papilloma virus (HPV) or the large T antigen of Simian virus 40 (SV40), which accelerate P53 degradation [16]. The P53 gene displays common polymorphisms in the general population, one of which, characterized by an Arg to Pro substitution at codon 72 in the transactivation domain of exon 4, has been investigated as risk modifier in several cancer models [17, 18]. The variant Pro allele has been shown to be less efficient to suppress cell transformation and to induce apoptosis and to be less sensitive to degradation by viral oncogenic proteins [19]. The two alleles also show different ability to bind the P53-related protein P73 and the Pro variant has been reported to be non-randomly inactivated by loss of heterozygosity (LOH) in some tumor types [20].

The functional inactivation of the P53 gene is one of the most common abnormalities in HCC and many other tumors [21]. It encodes a 393 amino acid nuclear phosphoprotein that binds specific DNA sequences in the human genome, and it is of critical importance for the regulation of cell cycle and maintenance of genomic integrity [22–24]. Authors hypothesized that alterations in the genome or function of P53 may be important events in hepatocarcinogenesis [25]. Frequent loss of one allele and mutations in the remaining allele have been reported to occur in diverse human cancer types including HCC [26, 27].

Intriguingly, Yu et al. [17] found that P53 gene polymorphism involved in hepatocarcinogenesis may determine individual susceptibility to HCC. The P53 wild-type is polymorphic at residue 72 in exon 4, where a single-base change causes a substitution of Pro for Arg (CCC/CGC) in the transactivation domain [14]. Many authors have tried to find an association between the presence of the Arg allele and the susceptibility of cancer of the cervix and laryngeal cancer but the results are controversial [28–30]. The frequency of the Pro variant has been studied as a potential risk factor for cancer of the lung, breast, large bowel and HCC, with inconsistent results [31–35]. Scientists suggest that the frequency of the Arg allele varies between ethnic groups [36]. We suggest that the P53 Arg72Pro polymorphism arose spontaneously in Asians/Japanese after the human ancestor population separated into Caucasians and Asians in the early history of Man.

Functional Activities of P53 Codon 72 Polymorphism

P53 single nucleotide polymorphism at codon +72 in exon 4 results in expression of either Arg or Pro at codon 72. This polymorphism is located in a proline-rich domain, which is essential for its DNA-binding ability to induce apoptosis. Additionally, these two polymorphisms have

different functional activities. Marin et al. found that some tumor-derived P53 mutants can bind to P73 and inactivate its bio-function. Furthermore, this binding activity of P53 mutants can be interrupted by whether the P53 variants are encoded by codon 72. Generally, Arg72 might retain a less prohibitory effect to avoiding mutant P53 binding to P73. The P73 gene, a P53 homologue, can activate P53 responsive promoters and induce apoptosis in P53 defected cells. The ability of mutant P53 to bind P73, neutralize P73-induced apoptosis and transform cells in cooperation with EJ-Ras was enhanced when codon 72 encoded Arg [20].

The Arg72 variant also appeared to more effectively interact with HPV-E6 *in vitro*, and to more easily be degraded through the ubiquitin proteasome pathway, resulting in inactivation of P53 gene and the induction of HPV-related tumor development [16]. Thereafter, Thomas et al. [18] revealed that Arg72 more likely acts dominantly in transcriptional regulation of P53 downstream targets that induce apoptosis or repress the transformation of primary cells. In 1999, it was shown that the two genetic variants of P53 codon 72 were not functionally equivalent and the Arg72 variant induces apoptosis and suppresses tumor growth more efficiently than the Pro72 variant. Another study provided further evidence that the Arg72 variant is at least 5 times more efficient in apoptosis induction, by comparing it with the Pro72 variant [37]. Furthermore, the Pro72 variant appears to induce higher levels of G1 arrest [38].

Amazingly, authors reported that fibroblasts and lymphocytes isolated from Arg72 homozygote centenarians and sexagenarians (Arg+) underwent oxidative-stress-induced apoptosis with higher proportions than those of proline allele carriers (Pro+). These data suggest that the TP53 codon 72 polymorphism might contribute to apoptotic variability among the elderly, which is potentially relevant to age-related pathologic conditions, such as myocardial ischemia [39].

P53 Codon 72 Polymorphism and HCV-Associated HCC

Table 1 illustrates the previous studies examined the P53 Arg72Pro single nucleotide polymorphisms and their roles in HCC incidences and HCV infection cases. The P53 gene displays a common genetic Arg/Pro polymorphism at codon 72 with functional significance and it has been investigated as risk factor in several cancer models. There are various studies examined the P53 single gene polymorphism at codon 72 and HCC and HCC-associated HCV infection. Leverì et al. [40] investigated P53 codon 72 polymorphisms in 340 HCV-infected subjects at different

stages of disease including, 84 HCC patients. They concluded that there is no association between codon 72 genotypes and disease severity or liver cancer was observed. Anzola et al. [21] examined P53 codon 72 genotypes in 97 biopsy samples withdrawn from 67 Basque HCC patients histologically diagnosed with HCC and 111 blood samples withdrawn from Basque residents as a control group. The polymorphism was examined by both single strand conformation polymorphism analysis and allele specific polymerase chain reaction. Moreover, Fisher's exact test was performed to evaluate the data. Authors showed that there were no significant differences in the frequency of codon 72 polymorphism genotypes between patients with HCC and healthy controls. They added that, there is a frequent loss of proline allele in HCV-positive carriers. Finally, they concluded that the lack of a significant relationship between this polymorphism and risk of HCC shows that it does not predispose towards hepatocarcinogenesis in this population and the frequent loss of the proline allele in HCV-associated carcinogenesis of the liver plays some critical role in hepatocarcinogenesis.

Intriguingly, Minouchi et al. [41] believed that there is a significant correlation between male homozygotes for P53 72Pro with HCV type 1b infection. They arose this amazing conclusion after comparing the P53 polymorphisms on exon 4 (CGC vs. CCC, P53Arg vs. P53Pro, A2 allele vs. A1 allele at codon 72, respectively) among 75 cases with HCV infection and 232 non-infected control subjects. P53 gene polymorphism was analyzed by the polymerase chain reaction–single strand conformation polymorphism method using DNA from peripheral blood leukocytes. They resulted that there was no significant correlation between HCV infection and the P53 polymorphism. However, there were significantly higher P53Pro homozygotes when comparing male volunteers infected with HCV type 1b with controls matched by sex and age. They added that there was no significant statistical difference between the P53 polymorphism and HCV genotypes 2a and 2b.

Okada et al. [35] used 98 tissue specimens of regenerative nodules obtained from 15 cirrhotic volunteers for analysis and divided them to 20 cases of chronic hepatitis C and 2 cases of fatty liver were used as controls. After that, DNA was extracted from each of regenerative nodules, and direct nucleotide sequencing was performed on P53 gene exon 5 in 7 of 98 DNA samples (7.1 %) from regenerative nodules in 6 cases of cirrhosis. Moreover, Subcloning analysis revealed that mutation sites differed in each subclone and the incidences of the mutation varied from 7.7 to 58.8 % depending on individual nodules. They concluded that P53 gene mutations were frequently found in cirrhotic livers compared with patient livers with chronic hepatitis, suggesting that P53 mutations at the stage of cirrhosis may be a causative factor that may potentially lead to HCC.

Table 1 P53 Codon 72 Polymorphism in HCV-Associated HCC

Ref.	Ethnicity (n)	Patient sub-groups	HCV-HCC patients group, n (%)			Controls group, n (%)			P value	Conclusion
			72 G/G	72 G/C	72 C/C	72 G/G	72 G/C	72 C/C		
[40]	Italian (340)	HCV (+)	43 (50.6)	36 (42.4)	6 (7.0)	One control group (88) (Carriers, no cirrhosis, no HCC) 48 (54.5) 33 (37.5) 7 (8.0)			0.05	No association between genotypes and disease severity or liver cancer.
		Cirrhosis (+)	31 (38.3)	44 (54.3)	6 (7.4)	48 (54.5)	33 (37.5)	7 (8.0)	0.01	
		HCC (+)	46 (53.5)	33 (38.4)	7 (8.1)	48 (54.5)	33 (37.5)	7 (8.0)	0.001	
[21]	Spain (164)	HCV/HBV (+)	13 (32.50)	25 (62.50)	2 (5.00)	Three control group (111) HCV/HBV (–) 33 (57.90) 22 (38.60) 2 (3.50)			0.034	Frequent loss of the Pro allele in HCV-HCC plays role in HCC.
		Cirrhosis (+)	29 (45.31)	31 (48.44)	4 (6.25)	No cirrhosis 17 (51.52) 16 (48.48) 0 (0.00)			<0.05	
		Vascular invasion (+)	14 (53.85)	11 (42.31)	1 (3.84)	Vascular invasion (–) 34 (47.89) 34 (47.89) 3 (4.22)			<0.05	
		Alcohol (+)	3 (27.27)	7 (63.64)	1 (9.10)	Alcohol (–) 43 (50.00) 40 (46.51) 3 (3.49)			<0.05	
[41]	Japan (120)	Cirrhosis (+)	91 (92.9)	7 (7.1) ^a		Not Mentioned – – –			>0.01	Significant correlation between male homozygotes for 72Pro with HCV type 1b infection.
		HCV (+)	20 (100)	0.00 (0.00) ^a		–	–	–	0.000	
		Fatty liver (+)	2 (100)	0.00 (0.00) ^a		–	–	–	0.000	
[36]	Japan (75)	M. HCV type-1 (+)	10 (42)	8 (33)	8 (33)	One Control Group HCV (–) (232) 25 (56) 18 (40) 2 (4)			0.039	1-Mutations were found in cirrhotic livers compared with chronic hepatitis livers. 2-It may be a causative factor that may lead to HCC.
		F. HCV type-1 (+)	5 (46)	5 (46)	1 (9)	18 (44)	15 (37)	8 (20)	0.726	

n represents size of samples, % represents percentage of samples, + represents present or positive, – represents absent or negative, 72 G/G represents Arg72Arg or wild-type, 72 G/C represents Arg72Pro, 72 C/C represents Pro72Pro

^a (72 G/C + 72 C/C) or mutant-type

Conclusion

The current review article crystallizes the powerful role of P53 gene in HCV infected cells' death "Apoptosis" and how this phenomenon is associated directly with the hepatitis disease and its progression, since hepatitis C is the major cause of hepatocellular carcinoma. We concluded from the present review the putative role of the P53 gene Arg72Pro polymorphism on various cancer models and viral infections, focusing on HCV and HCC incidences. Furthermore, it was concluded that the P53 gene polymorphism at codon 72 has been investigated as potential risk factor in several cancer models and HCV infections. Intriguingly, male HCV patients not females, with genotype-1 not genotypes-2, have a significant correlation when comparing homozygotes for P53 72Pro with heterozygotes.

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