Hepatitis C Virus (HCV) and Hepatocellular carcinoma (HCC): Association and molecular causes of HCV transformation to HCC

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Abstract
Hepatitis C virus infection has become a major health problem affecting approximately 170 million people every year. Also around 350,000 people die every year due to this virus. In India, around 6-12 million people get infected with Hepatitis C virus. Different studies have reported the role of Hepatitis C viral proteins mainly Core, NS3, NS5B in progressing to Hepato-Cellular Carcinoma but the exact cause and effect relationships are not known. A meta-analysis was first conducted using web tools and data published in last 25 years. Secondly in silico work was also conducted and correlated with the existing study.

Interaction of HCV proteins with TP53 (Tumor Suppressing protein p53) in developing HCC was also carried out. Role of different HCV proteins has been observed in host signaling pathways which alter mechanism of tumor expression competence of host system. This study showed that HCV infection is not just limited in causing liver damage but can also lead to extra-hepatic-manifestations which may make way for severity in liver. This may further lead to challenges in development of an effective treatment against HCV, in spite of the number of Direct Acting Antivirals (DAAs) and therapies in use. More studies on molecular diagnosis, further research on DAAs and therapies can contribute in developing better therapeutics.

Keywords: Hepatitis C virus, Hepato-cellular-carcinoma, miRNA-122, Hepatitis C proteins.

Introduction

Approximately 170 million people in the world are chronically infected from Hepatitis C virus (HCV)[10]. Of the various countries on the rise of HCV cases, Central and North Africa and East Asia have the higher similarities of mortalities with about 35,000 deaths each year. On the basis of studies reported from different regions in India, it is estimated that around 6-12 million people are infected by Hepatitis C virus [7]. The World Health Organization aims to eradicate HCV virus, the public health problem by the end of 2030.

Long term retention and infection of HCV lead to multiple stages of liver diseases. The initial stage begins with the fat deposition in liver i.e. steatosis followed by fibrosis and finally cirrhosis. It has been found that cirrhosis develops in approximately 10-15% patients of chronic HCV infection. Chronic alcohol consumption, aflatoxin exposure, non-alcoholic liver disease and hemochromatosis are some of the risk factors that can exaggerate the infection[26]. It has been reported that tissue studies of HCC liver show presence of envelope and core proteins of HCV, nevertheless to establish the cause and relationship between HCV and HCC, molecular mechanism of HCV-HCC association and the translational consequences of possible interventional viral RNA with host DNA during the process of tumor suppressor proteins.

The Hepatitis C virus belongs to the family Flaviviridae and is a single stranded, positive sense RNA virus. Being an RNA virus, it can intervene with any of the protein synthesis of host cells specifically those involved in growth and repair. Through this present study, we have aimed to study the gene level and protein level involvement of the HCV proteins with that of the host cell. This will help in prediction of early indicators and points of therapeutic interventions in order to prevent transformation of HCV into HCC. Present study focuses on possible molecular causes responsible for converting HCV infection into chronic liver disease and/or HCC.

Material and Methods

Meta-analysis study conducted: A meta-analysis was performed first using Web of Knowledge, PubMed, Scopus, Science direct, Google scholar for data published in the last 25 years. The journals consulted included Journal of Hepatology, Journal of Virology, Cancer and Metastasis Reviews, Cancer Discovery, Journal of Hepatology and Gastroenterology, Journal of Oncology, The New England Journal of Medicine, Journal of Clinical Oncology etc. The selected papers were grouped into three different categories i.e. distribution of genotypes, genomic and proteomic level interaction of virus and host factors. Different proteins of HCV were also focused and their roles in different host metabolic pathways were studied. We also conducted a broad literature search on TP53 (tumor suppressing protein p53) and its role in HCV-infected patients developing HCC. Role of three HCV proteins viz. core protein, NS3 and NS5A was studied in depth.

***In silico* studies on interaction of HCV proteins with p53 protein of the host:** We conducted an *in silico* analysis of the interaction between human tumor suppressing protein p53 and HCV non-structural proteins viz. Core (PDB ID: IXF5, Chains A and B) NS3 (PDB ID: 2OC8, chains A and

C) and NS5B (PDB ID:1C2P, chains A and B) performing docking by using ClusPro online docking server. Moreover, the structure of these two proteins was visualised by using PyMol.

Results

Observations on Meta-analysis study conducted: Hepatitis C virus which belongs to the genus *Hepacivirus* and family *Flaviviridae*, is a human hepatotropic virus and is genetically heterogenous with different 8 genotypes and is further divided into various subtypes[33]. The virus has been grouped into 8 clades (Figure 1) which shows 30%-35% difference among each other and at least 105 subtypes, called as genotypes which show nucleotide sequence difference by less than 15% [34]. These subtypes are designated as 1a, 1b, 2a, 2b, 3a etc. and are most prevalent and commonly identified.

The different genotypes of HCV virus show fundamental differences from each other and show diversification in genomic sequences from each other. Earlier HCV (parenterally transmitted) and HEV (enterically transmitted) were termed as non-A and non-B hepatitis[7]. Genotyping based on 5'NCR (non-coding region) showed that genotype 1 with the prevalence of 38.25% and genotype 3 with the prevalence of 53.69% are the most dominant genotypes found in India. The percentage nucleotide identity between the HCV genotype 3 was 95.26% ±0.12% and for genotype 1, it was 94.67% ±0.11%[21].

The nucleotide similarity reported among the four (1, 2, 3 and 4) different isolates was 94.55% ± 0.96%. Other subtypes of HCV genotype 3 were reported and were identified as 3i, 3j and 6I[21]. The most predominant genotypes that have been reported so far, are genotype 1 and genotype 3. Genotype 3a including 1a infects the drug users in Europe and genotype 4a is commonly found in Middle East countries. HCV genotype subtypes 1b, 2b and 2a are however identified in Asia whereas patients of older age groups have been reported throughout Europe especially among patients with the past history of blood transfusion.

There are number of studies on the HCV infection from different parts of India[14]. Different genotypes circulating have also been studied[4,25,28]. The study carried out by Chaudhri and his coworkers[4] had selected only patients that were diagnosed with chronic liver symptoms. They had studied a total of 2640 blood serum from patients with liver disease. Among these, 375 (25.3%) were chronic liver cases and were positive for HCV antibodies. Out of this, 19% patient had blood transfusion history. RT-PCR study showed that 97 cases had acute liver disease, of which 12.4% were genotype 1b, 2.1% genotype 2a and 9.3% genotype 2b. The dominant genotypes which were reported in acute liver cases, were 3a accounting approximately 28.9 % and 42.3% for 3a genotype.

The patients who had chronic liver disease with active HCV viral infection were 323 in number. Out of this, 31(9.6%) were genotype 1b, 2(0.6%) genotype 2b and 3(0.9%) genotype 2a. The dominant genotypes which were reported in chronic liver cases, were genotypes 3b (47.7%), genotype 3a (34.7%) and 1.2% reported as mixed infection of 3a and 3b genotypes[4]. Genotype 3 is the most dominant genotype reported from India followed by genotype 1, 4, 2 5, 6 and mixed genotypes (Figure 2).

The study reported by Malhotra and his coworkers[24] in which total 1500 samples were studied for Ab-HCV positive, 65% were male and 35% were females. Between 25-53 years of age, sample were almost 38% in number, one fourth were 26.025% between age 35-45 years, 3.32% were between 11-20 years and 1.03% were 60 years of age. The Kaithal place alone reports around more than one third (37.37%) of HCV positive infection. Additionally, Fatehabad and Kaithal together reported up to 60% HCV positive infections[24]. The studies carried out by Mansell et al[25] have reported that dominant HCV genotypes from Australia and New Zealand were 2a (10%), 3a and 1(45%) (Table 1). In Japan, 80% have reported HCV genotype 1b positive, 15% infected with 2a genotype and 5% infected with 2b genotype

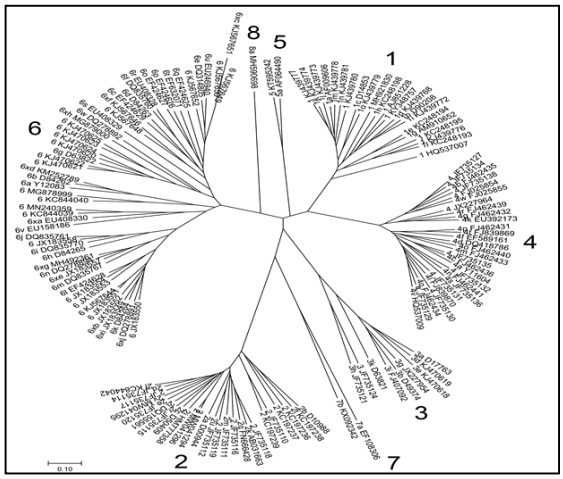

Fig. 1: Different genotypes of HCV virus and their subtypes (ICTV classification of Viruses)

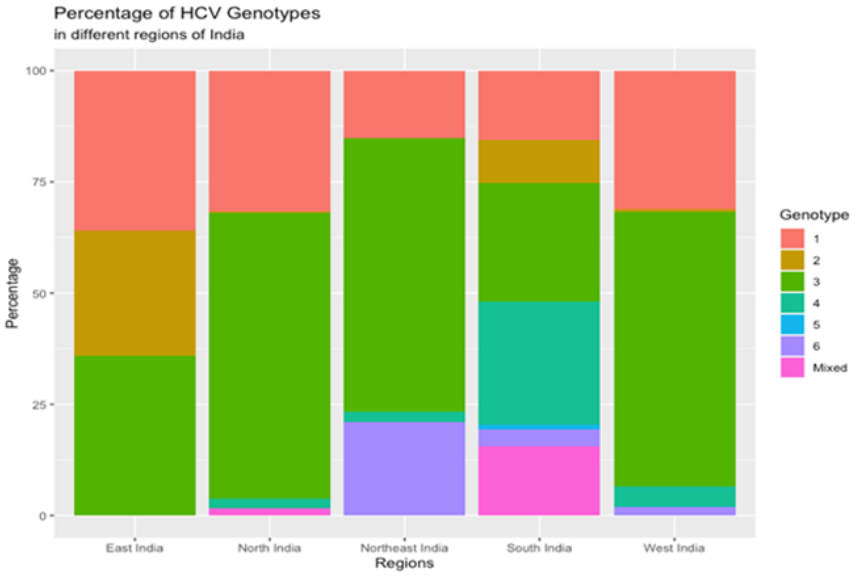

Fig. 2: Percentage of HCV genotypes from different parts of India

Table 1
Showing dominant HCV genotypes from difference parts of the world

Country	**Dominant HCV Genotypes**
Australia and New Zealand	3a &1(45%); 2a (10%)
Japan	1b (80%), 2a (15%),2b (5%)
Northern China	2b (30%), 2a (30%)
Southern China	1b (over 90%)
Middle East	4
Europe and United States	1a

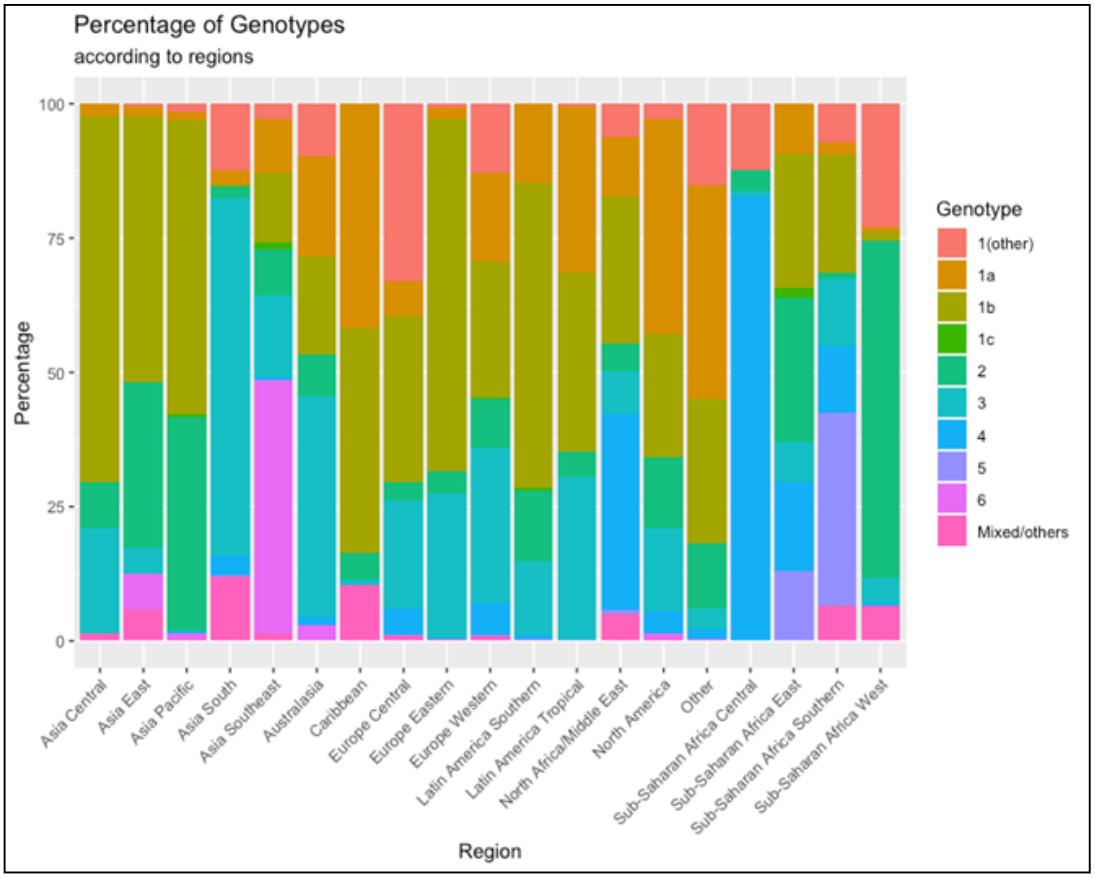

Fig. 3: Percentage of HCV genotypes from different parts of the world

The globally genotype 1 was dominant among all the genotypes of Hepatitis C virus which accounts for around 46% of all infections followed by genotype 3 (22%), genotype 2 and genotype 4 (13% each) (Figure 3). The subtype 1b was found to be dominant, globally accounting around 22% of all HCV genotype infection.

The progression from Hepatitis C virus infection to liver carcinoma involves an intricate and complex interplay between host and viral factors resulting in a CLD (chronic liver disease), cirrhosis and finally to the development of cancer[8]. According to genomic studies, several molecular mechanisms drive this transformation. The Hepatitis C virus sets liver towards chronic inflammation, leading to persistent oxidative stress. This inflammation generates reactive oxygen species (ROS) which causes DNA damage and mutations in main liver parenchymal cells i.e. hepatocytes[6]. Gradually, these accumulations of genetic alterations/aberrations can lead to malignancy.

Specific HCV proteins, more predominantly core protein, NS3, NS5A and NS5B have been reported with host cellular pathways[3]. The HCV core protein affects cellular signaling pathways including the p53 tumor suppressor pathway which results in genomic instability. Studies have reported that HCV NS5A and NS5B interact with host DNA repair mechanisms, potentially causing errors in DNA replication and interfering in repair mechanisms. These interactions result in chromosomal aberrations and mutations that make hepatocytes more susceptible to malignancy.

HCV infection is also associated with significant epigenetic changes in the host genome such as DNA methylation, alterations in micro RNA expression and histone modifications[13,46]. Persistent infection of HCV leads to telomere shortening and dysfunction. Chromosome ends are protected by telomeres and when they fail to function, Hepatitis C virus leads to chromosomal instability, a hallmark feature of cancer[12]. In Hepatocytes, telomere shortening can result in aneuploidy and end-to-end chromosome fusions which exacerbate genomic instability in liver carcinoma. Furthermore, HCV has evolved defense mechanisms against the host immune system which leads to chronic liver damage and prolonged infection[39].

Infected cells are able to thrive and gradually acquire genetic changes because of this immune evasion[35]. Cirrhosis, the end-stage liver disease caused by a persistent HCV infection, is characterized by widespread fibrosis and regenerating nodules. The risk of genetic mistakes during DNA replication is increased by the ongoing cycle of liver cell death and regeneration and thus contributing to liver carcinoma[2,37].

Future studies on the signaling pathways and genetic changes associated to this transformation could offer novel perspectives on the prevention and management of liver carcinoma in people with HCV infection[32]. Proteomic research has shown that the core protein intrudes with p53's tumor-suppressing functions, promoting it towards genomic instability while NS5A protein activates the PI3K/AKT/mTOR signaling pathways, enhancing cellular processes[29,31]. Proteomic analysis has identified alterations in the expression of ECM proteins such as fibronectin collagen and laminin in HCV-infected livers/liver cells[18,27,41].

The virus replicates in the cytoplasm of the host cell and the polyprotein is cleaved into four different structural and seven non-structural proteins by host and viral signal peptidases[9,23]. It has been reported that three main proteins of this virus (Core, NS3 and NS5B) are involved in causing hepato-cellular carcinoma as proved from different cell culture experiments[1,15,16,20,30,43].

The DNA related alterations like hypermethylation, hypomethylation are closely related with different human diseases like cancers. The DNA hypermethylation has been reported at some promoter regions of tumor suppressor genes in case of HCV infected patients which may contribute to HCC in such patients and these genes are mainly inactivated by the hypermethylation at promoter region of CpG island[19].

The NS5B protein interacts with Rb protein and leads to the degradation of this protein in case of HCV patients and promotes cancer like conditions in these patients. To find out whether NS5B protein expression triggers the expression of responsive genes of E2F, luciferase reporter constructs were employed for the MAD2 (Mitotic-Check- point protein) and p107 (Rb, family member protein) promoters[19,45]. The expression of both these promoters was increased to 3-5-fold when the NS5B was ectopically added to the Huh cell lines. However, when mutant Rb protein was added, it did not bind with the E2F, therefore the NS5B did not activate the promoter expression by 3-5-fold[11,40]. The above data shows that the Rb protein-mediated repression of E2F dependent transcription is reversed by NS5B protein.

The core protein of HCV viruses contains such ORF regions called as core+1 or ARFP. The two isoforms of core+1 have been so far reported which are as core+1/S and core+1/L[38]. It has been reported that core+1 interacts with the host machinery which accelerates the cell cycle leading towards liver carcinogenesis. Additionally, the severity of liver disease in chronic HCV patients has been associated to immune responses of core+1/ARFP[38]. Previous studies have also reported that NS3 protein of HCV virus interacts with the PPM1A protein[36,42].

The NS3 protein colocalizes the PPM1A from nucleus to the cytoplasm in cultured hepatoma cells infected with HCV[22]. It was found that NS5A protein colocalizes the p53 protein in the perinuclear region and also inhibits its transcriptional activity[17].

Observations on *in silico* studies to determine role of HCV proteins in causation of HCC: The docking scores for the different proteins (Core, NS3 and NS5B) were -1092.4, -1077.9 and -1152.7 respectively which reflect the high affinity between these proteins. The amino acid interactions between these proteins were visualized by using PyMOL (Figure 4, 5a, 5b, 6, 7, 8 and 9a, 9b). The precise amino acids of the viral proteins and its sites of interaction with the p53 amino acids are shown in tables 2, 3 and 4.

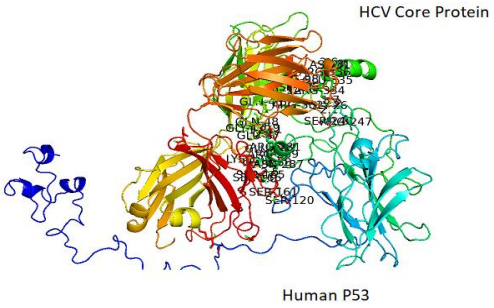

Fig. 4: Core protein of HCV complexed with p53 protein

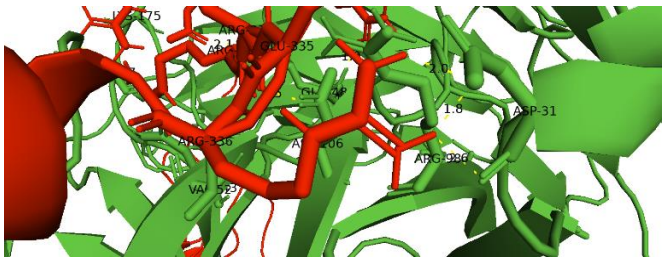

Fig. 5 (a): Closer view of p53(red) and HCV core protein (green) interaction

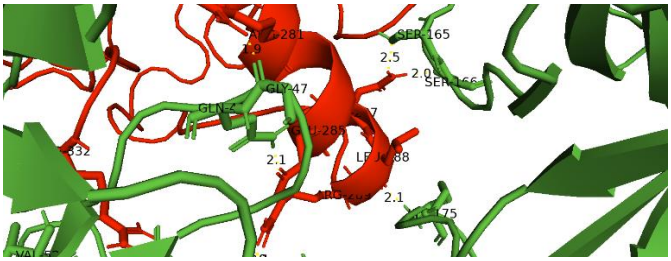

Fig. 5 (b): Closer view of p53(red) and HCV core protein (green) interaction

Table 2
Amino acids Interaction of HCV Core with p53 at various positions

HCV Core (amino acids)	p53 (amino acids)
Ser165	Asn287
Glu87	Arg287
Gln48	Glu285
Lys17	Leu288
Ser166	Asn287
Gly47	Arg281
Gly26	Ser240
Asp31	Arg336
Asp106	Arg334
Ser161	Ser120

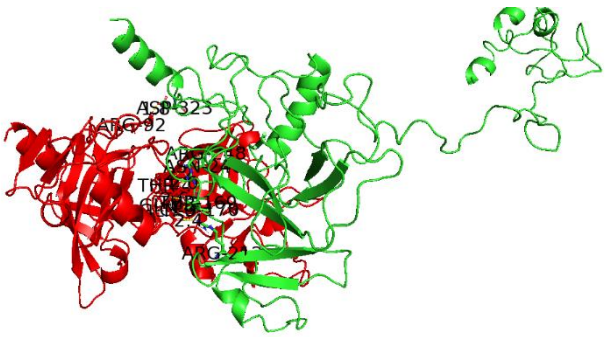

Fig. 6: NS3 protein of HCV complexed with p53 protein

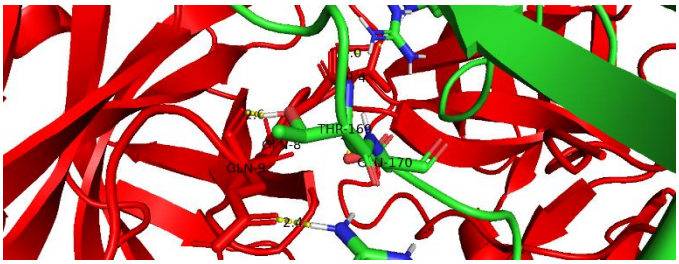

Fig. 7: Closer view of p53(red) and HCV NS3 protein (green) interaction

Table 3
Amino acids Interaction of HCV NS3 with p53 at various positions

HCV NS3 (amino acids)	p53 (amino acids)
Thr4	Asn246
Arg92	Asp323
Gln8	Arg248
Gln8	Glu170
Gln9	Thr169
Gln9	Arg212

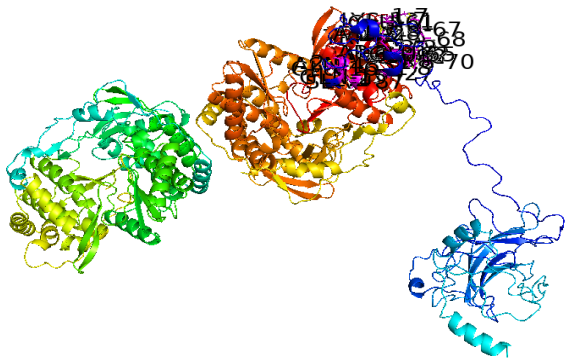

Fig. 8: NS5B protein of HCV complexed with p53 protein

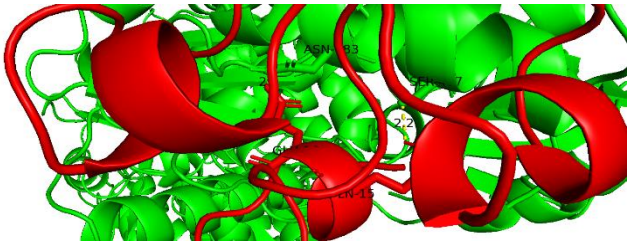

Fig. 9(a): Closer view of p53(red) and HCV NS5B protein (green) interaction

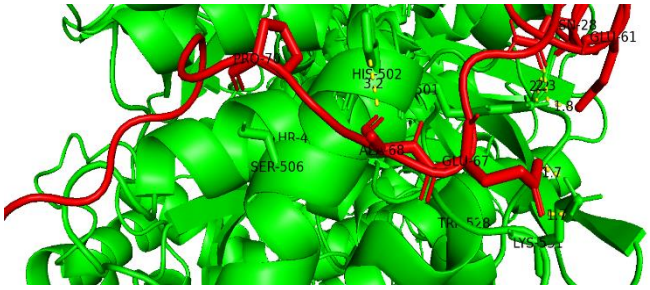

Fig. 9(b): Closer view of p53(red) and HCV NS5B protein (green) interaction

Table 4
Amino acids Interaction of HCV NS5B with p53 at various positions

HCV NS5B (amino acids)	p53 (amino acids)
Arg501	Asn28
Arg501	Glu61
Lys531	Glu67
Asn483	Gln16
Ser487	Gln15
Arg489	Glu55
Ser506	Pro70
His502	Ala68

Discussion

HCV is closely related to HCC, however, the exact mechanism by which this virus causes HCC, is not yet completely understood. The advances in *in silico* research and evidences have proved that HCV regulates the host metabolic pathways leading to hepato-cellular-carcinoma. Our study aimed to understand the molecular mechanism and effect of this virus on different tumor suppressing genes. In this study, out of 10 different proteins of the virus, we have mainly focused on three proteins Core, NS3 and NS5B because these proteins have gained more importance as these viral proteins interact with different host signaling pathways and have ability of cell-transformation leading to different cancerous conditions.

In HCV infected patients, the Rb protein is downregulated and its downregulation creates the favorable environment for HCV RNA replication. Upon HCV infection, the NS3 colocalizes Rb protein to the cytoplasm miRNA-122 which is approximately 22-25 nucleotides long present on the chromosome. Around 70% of this miRNA are present in the mammalian liver.

Conclusion

The present study observes that HCV infection hinders the host metabolic processes at both genomic and proteomic levels by interfering with multiple host signalling pathways that are important for cell proliferation, survival and apoptosis. The sequencing of 5'NCR could differentiate the HCV genotypes, however, the classification of subtypes could be possible by analyzing the nucleotide sequence of core region. Additionally, evidence suggests that HCV viral proteins cause HCC by suppressing number of host tumor suppressing proteins but these results are still controversial.

The deep molecular study is required to assess the role of p53 in case of virus infected-hepato-carcinogenesis. Also, the study of other viral proteins needs to be studied in detail and if any involvement of these proteins appears in HCC, the reliable molecular predictors of HCC can be developed.

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References

1. Abdallah C., Lejamtel C., Benzoubir N., Battaglia S., Sidahmed-Adrar N., Desterke C., Lemasson M., Rosenberg A.R., Samuel D., Bréchot C., Pflieger D., Le Naour F. and Bourgeade M.F., Hepatitis C virus core protein targets 4E-BP1 expression and phosphorylation and potentiates Myc-induced liver carcinogenesis in transgenic mice, *Oncotarget*, **8(34)**, 56228–56242 **(2017)**

2. Aborehab N.M., Kandeil M.A., Sabry D., Rabie R. and Ibrahim I.T., Circular SERPINA3 and its target microRNA-944 as potential molecular biomarkers in hepatitis C virus-induced hepatocellular carcinoma in Egyptian population, *Non-coding RNA Resear.*, **8(3)**, 401–412 **(2023)**

3. Chaturvedi V.K., Singh A., Dubey S.K., Hetta H.F., John J. and Singh M., Molecular mechanistic insight of hepatitis B virus mediated hepatocellular carcinoma, *Microb. Pathogene.*, **128**, 184–194 **(2019)**

4. Chaudhuri S., Das S., Chowdhury A., Santra A., Bhattacharya S.K. and Naik T.N., Molecular epidemiology of HCV infection among acute and chronic liver disease patients in Kolkata, India, *J. Clin. Viro.*, **32(1)**, 38-46 **(2005)**

5. Choo Q.L., Kuo G., Weiner A.J., Overby L.R., Bradley D.W. and Houghton M., Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome, *Sc.*, **244(4902)**, 359-362 **(1989)**

6. D'souza S., Lau K.C., Coffin C.S. and Patel T.R., Molecular mechanisms of viral hepatitis induced hepatocellular carcinoma, *World J. Gastroentero.*, **26(38)**, 5759–5783 **(2020)**

7. Fowsiya J. and Madhumitha G., Pharmacognostical standardization and antibacterial activity of the dried fruit of Carissa edulis vahl, *Res. J. Chem. Environ.*, **28(2)**, 18-24 **(2024)**

8. Goossens N. and Hoshida Y., Hepatitis C virus-induced hepatocellular carcinoma, *Clinical and Molecular Hepatology*, **21(2)**, 105–114 **(2015)**

9. Hayashi S., Nagaoka K. and Tanaka Y., Blood-based biomarkers in hepatitis B virus-related hepatocellular carcinoma, including the viral genome and glycosylated proteins, *Internat. J. Mol. Sc.*, **22(20)**, 11051 **(2021)**

10. Hepatitis C Virus Fact Sheet, WHO Report 164 **(2013)**

11. Hernando E., Nahlé Z., Juan G., Diaz-Rodriguez E., Alaminos M., Hemann M., Michel L., Mittal V., Gerald W., Benezra R. and

Lowe S.W., Rb inactivation promotes genomic instability by uncoupling cell cycle progression from mitotic control, *Nature*, **430(7001)**, 797-802 **(2004)**

12. Huang C.F., Awad M.H., Gal Tanamy M. and Yu M.L., Unmet needs in the post-DAA era: the risk and molecular mechanisms of hepatocellular carcinoma after HCV eradication, *Clin. Mol. Hepato.*, **30 (3),** 326-344 **(2024)**

13. Irshad M., Gupta P. and Irshad K., Molecular basis of hepatocellular carcinoma induced by hepatitis C virus infection, *World J. Hepato.*, **9(36)**, 1305–1314 **(2017)**

14. Jain P., Prakash S., Gupta S., Singh K.P., Shrivastava S., Singh D.D., Singh J. and Jain A., Prevalence of hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus and hepatitis E virus as causes of acute viral hepatitis in North India: A hospital-based study, *Ind. J. Med. Microbio.*, **31(3)**, 261-265 **(2013)**

15. Jiang Y.F., He B., Li N.P., Ma J., Gong G.Z. and Zhang M., The oncogenic role of NS5A of hepatitis C virus is mediated by up-regulation of survivin gene expression in the hepatocellular cell through p53 and NF-κB pathways, *Cell Bio. Internat*, **35(12)**, 1225–1232 **(2011)**

16. Kasprzak A. and Adamek A., Role of hepatitis C virus proteins (C, NS3, NS5A) in hepatic oncogenesis, *Hepatology Tesearch: The official Journal of the Japan Soc. Hepato.*, **38(1)**, 1–26 **(2008)**

17. Lan K.H., Sheu M.L., Hwang S.J., Yen S.H., Chen S.Y., Wu J.C., Wang Y.J., Kato N., Omata M., Chang F.Y. and Lee S.D., HCV NS5A interacts with p53 and inhibits p53-mediated apoptosis, *Oncogene*, **21(31)**, 4801-4811 **(2002)**

18. Li Z., Xu J., Cui H., Song J., Chen J. and Wei J., Bioinformatics analysis of key biomarkers and potential molecular mechanisms in hepatocellular carcinoma induced by hepatitis B virus, *Med.*, **99(20)**, e20302 **(2020)**

19. Lim J.S., Park S.H. and Jang K.L., Hepatitis C virus Core protein overcomes stress-induced premature senescence by down-regulating p16 expression via DNA methylation, *Cancer Letters*, **321(2)**, 154–161 **(2012)**

20. Liu J., Ding X., Tang J., Cao Y., Hu P., Zhou F., Shan X., Cai X., Chen Q., Ling N., Zhang B., Bi Y., Chen K., Ren H., Huang A., He T.C. and Tang N., Enhancement of canonical Wnt/β-catenin signaling activity by HCV core protein promotes cell growth of hepatocellular carcinoma cells, *PloS One*, **6(11)**, e27496 **(2011)**

21. Lole K.S., Jha J.A., Shrotri S.P., Tandon B.N., Prasad V.M. and Arankalle V.A., Comparison of hepatitis C virus genotyping by 5′ noncoding region-and core-based reverse transcriptase PCR assay with sequencing and use of the assay for determining subtype distribution in India, *J. Clin. Microbio.*, **41(11)**, 5240-5244 **(2003)**

22. Long J., Wang Y., Li M., Tong W.M., Jia J.D. and Huang J., Correlation of TP53 mutations with HCV positivity in hepatocarcinogenesis: Identification of a novel TP53 microindel in hepatocellular carcinoma with HCV infection, *Oncology Reports*, **30(1)**, 119-124 **(2013)**

23. Mahmoudvand S., Shokri S., Taherkhani R. and Farshadpour F., Hepatitis C virus core protein modulates several signaling pathways involved in hepatocellular carcinoma, *World J. Gastroentero.*, **25(1)**, 42-58 **(2011)**

24. Malhotra P., Malhotra V., Malhotra N., Singh I., Chugh A. and Chaturvedi A., Epidemiological profile of hepatitis C patients at India's new Hub–Haryana, *Adv. Res. Gastroentero. Hepato.*, **1(1)**, 001-006 **(2015)**

25. Mansell C.J. and Locarnini S.A., Epidemiology of hepatitis C in the East, In Seminars in liver dis., by Thieme Medical Publishers, Inc., **15(1)**, 15-32 **(1995)**

26. Mizuguchi Y., Takizawa T., Yoshida H. and Uchida E., Dysregulated miRNA in progression of hepatocellular carcinoma: A systematic review, *Hepato. Res.*, **46(5)**, 391-406 **(2016)**

27. Moldogazieva N.T., Mokhosoev I.M., Zavadskiy S.P. and Terentiev A.A., Proteomic profiling and artificial intelligence for hepatocellular carcinoma translational medicine, *Biomed.*, **9(2)**, 159 **(2021)**

28. Narahari S., Juwle A., Basak S. and Saranath D., Prevalence and geographic distribution of Hepatitis C Virus genotypes in Indian patient cohort, *Infect. Gene. Evol.*, **9(4)**, 643-645 **(2009)**

29. Ng C.K., Dazert E., Boldanova T., Coto-Llerena M., Nuciforo S., Ercan C., Suslov A., Meier M.A., Bock T. and Schmidt A., Integrative proteogenomic characterization of hepatocellular carcinoma across etiologies and stages, *Nature Communicat.*, **13(1)**, 2436 **(2022)**

30. Qadri I., Iwahashi M., Capasso J.M., Hopken M.W., Flores S., Schaack J. and Simon F.R., Induced oxidative stress and activated expression of manganese superoxide dismutase during hepatitis C virus replication: role of JNK, p38 MAPK and AP-1, *The Biochem. J.*, **378(3)**, 919–928 **(2004)**

31. Reungoat E., Grigorov B., Zoulim F. and Pécheur E.I., Molecular crosstalk between the hepatitis C virus and the extracellular matrix in liver fibrogenesis and early carcinogenesis, *Cancers*, **13**, 2270 **(2021)**

32. Shen C., Jiang X., Li M. and Luo Y., Hepatitis virus and hepatocellular carcinoma: recent advances, *Cancers*, **15**, 533 **(2023)**

33. Simmonds P., Genetic diversity and evolution of hepatitis C virus–15 years on, *J Gen. Viro.*, **85(11)**, 3173-3188 **(2004)**

34. Smith D.B., Pathirana S., Davidson F., Lawlor E., Power J., Yap P.L. and Simmonds P., The origin of hepatitis C virus genotypes, *The J. Gen. Viro.*, **78(2)**, 321–328 **(1997)**

35. Tang K., Lin W., Wang D., Hu X., Chen Z., Chen J., Liang B., Zhang L., Qin P. and Wu D., Potential Role of MAP3K14 in Hepatocellular Carcinoma: A Study Based on Comprehensive Bioinformatical Analysis and Validation, *J. Cancer*, **15(9)**, 2731–2745 **(2024)**

36. Verga-Gérard A., Porcherot M., Meyniel-Schicklin L., André P., Lotteau V. and Perrin-Cocon L., Hepatitis C virus/human interactome identifies SMURF2 and the viral protease as critical elements for the control of TGF-β signalling, *FASEB Journal: Official Publication of the Feder. Am. Soc. Exper. Bio.*, **27(10)**, 4027–4040 **(2013)**

37. Vianello C., Monti E., Leoni I., Galvani G., Giovannini C., Piscaglia F., Stefanelli C., Gramantieri L. and Fornari F., Noncoding RNAs in Hepatocellular Carcinoma: Potential Applications in Combined Therapeutic Strategies and Promising Candidates of Treatment Response, *Cancers*, **16(4)**, 766 **(2024)**

38. Walewski J.L., Keller T.R., Stump D.D. and Branch A.D., Evidence for a new hepatitis C virus antigen encoded in an overlapping reading frame, *RNA (New York, N.Y.)*, **7(5)**, 710–721 **(2001)**

39. Wang M.D., Diao Y.K., Yao L.Q., Fan Z.Q., Wang K.C., Wu H., Gu L.H., Xu J.H., Li C. and Lv G.Y., Emerging role of molecular diagnosis and personalized therapy for hepatocellular carcinoma, *iLIVER*, **3(1)**, 100083 **(2024)**

40. Whitaker L.L., Su H., Baskaran R., Knudsen E.S. and Wang J.Y., Growth suppression by an E2F-binding-defective retinoblastoma protein (RB): contribution from the RB C pocket, *Mol. Cell Bio.*, **18(7)**, 4032–4042 **(1998)**

41. Xu M., Xu K., Yin S., Chang C., Sun W., Wang G., Zhang K., Mu J., Wu M. and Xing B., In-Depth Serum Proteomics Reveals the Trajectory of Hallmarks of Cancer in Hepatitis B Virus–Related Liver Diseases, *Mol. Cell Proteo.*, **22(7)**, 100574 **(2023)**

42. Xu Z., Choi J., Yen T.S., Lu W., Strohecker A., Govindarajan S., Chien D., Selby M.J. and Ou J., Synthesis of a novel hepatitis C virus protein by ribosomal frameshift, *The EMBO J*, **20(14)**, 3840–3848 **(2001)**

43. Zhao P., Malik S. and Xing S., Epigenetic mechanisms involved in HCV-induced hepatocellular carcinoma (HCC), *Front. Onco.*, **11**, 677926 **(2021)**

44. Zhou Y., Zhao Y., Gao Y., Hu W., Qu Y., Lou N., Zhu Y., Zhang X. and Yang H., Hepatitis C virus NS3 protein enhances hepatocellular carcinoma cell invasion by promoting PPM1A ubiquitination and degradation, *J. Exper. Clin. Cancer Res*., **36(1)**, 1-13 **(2017)**

45. Zhu L., Zhu L., Xie E. and Chang L.S., Differential roles of two tandem E2F sites in repression of the human p107 promoter by retinoblastoma and p107 proteins, *Mol. Cell Bio.*, **15(7)**, 3552-3562 **(1995)**

46. Zhu Q., Xie J., Mei W. and Zeng C., Methylated circulating tumor DNA in hepatocellular carcinoma: A comprehensive analysis of biomarker potential and clinical implications, *Cancer Treat. Rev.*, **128**, 102763 **(2024)**.

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