REVIEW ARTICLE

Genetic polymorphism in IL-28 B and LMP-7 genes: Role in HCV-induced Hepatocellular Carcinoma

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Key words:
HCV; IL 28B; LMP-7; HCC

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ABSTRACT

Hepatocellular carcinoma (HCC) is a major health problem that differs epidemiologically across the world. Its pathogenesis is contributed to many factors, and chronic hepatitis viral infections which considered the main risk factor in Egypt. Host genetic factors are considered the most important determinants for the development of HCC especially Single-nucleotide polymorphisms (SNPs). Hepatitis C Virus (HCV) infection is a major health problem with 185 million people infected globally based on the estimation of the World Health Organization (WHO). Persistent infection with HCV can cause liver cirrhosis and/or hepatocellular carcinoma (HCC). HCC is considered the fifth most common type of cancer, killing about 600,000 patients every year. About 30% of HCV- infected patients clear the infection naturally, while the remaining 70% develop chronic disease of which 1 - 2% experience HCC. Determining HCV-infected patients are at risk of developing HCC is crucial whose need continuing surveillance for early detection and treatment of HCC and other liver disease. A number of factors including viral, environmental, and genetic factors, work together to determine the progression of disease in HCV infection.

INTRODUCTION

Hepatitis C Virus (HCV) infection is a major health problem with 185 million people infected globally based on the estimation of the World Health Organization (WHO). Persistent infection with HCV can cause liver cirrhosis and/or hepatocellular carcinoma (HCC). HCC is considered the fifth most common type of cancer, killing about 600,000 patients every year[1].

About 30% of HCV- infected patients clear the infection naturally, while the remaining 70% develop chronic disease of which 1 - 2% experience HCC. Determining HCV-infected patients are at risk of developing HCC is crucial whose need continuing surveillance for early detection and treatment of HCC and other liver disease. Several factors including viral, environmental, and genetic factors, work together to determine the progression of disease in HCV infection[2].

Interleukin 28B gene is present on human chromosome 19q and its antiviral effect is primarily through activating the JAK-STAT pathway. By activating IFN-stimulated genes, IL28B modifies the immune response to antiviral infection[3].

The major histocompatibility complex (MHC) class II region on chromosome 6 contains the gene for low molecular mass protein-7. It encodes a catalytic subunit of proteosome complex and contribute to antigen sampling step of MHC class I pathway. Cytotoxic CD8+T cells recognition of the antigen depend on a number of important steps in antigen processing, including LMP-7 that can alter the pool of peptides available for class I antigen presentation, increasing the CD8+T cells'response to viral antigens[4].

Interleukin 28B:

The interleukin-28B (IL-28B) gene, commonly referred to as IFN λ3, is a recently described member of the family of IFN-related cytokines that exhibits similar biologic characteristics with type I IFNs. The IFN-stimulated genes (ISG) that serve as mediators of antiviral activity, apoptosis, and immunomodulation are activated by the genes of this family of cytokines, which cluster on human chromosome 19. These genes have antiviral function[5].

Mechanism of action of IL 28B:

The IFN- λ s or type III IFNs bind to a specific receptor complex but otherwise share many functional characteristics with the type I IFNs[6]. Three members of this family are identified as IL28A (IFN λ 2), IL28B (IFN- λ 3), and IL29 (IFN- λ 1). The nomenclature used to describe the IFN- λ family reflects their structural and functional resemblance to both the interleukin family of cytokines (more particularly, IL10) and the type I IFNs. IFN- λ s have been demonstrated to be up-regulated in the presence of viruses and double-stranded DNA and to have antiviral activity like type I IFN[6].

The IFN- λ s differ from type I IFNs by their binding to a particular heterodimeric receptor complex made up of the IFN- λ specific alpha subunit (IL28RA) and the IL-10 beta receptor subunit (IL10RB)[7]. The Janus kinase (JAK) and protein tyrosine kinase 2 are activated by binding of IFN- λ to this complex, which in turn causes phosphorylation and activation of the signal transducer and activator of transcription (STAT) protein kinases[6].

When STAT proteins are phosphorylated, they dimerize (either as homodimers or STAT1/STAT2 heterodimers) and are translocated to the nucleus, this leads to downstream activation, through transcriptional activation of a host of genes with immunomodulatory functions, called IFN-stimulated genes (ISGs) as in **Figure 1**. The exact number of genes that the IFN- λ s up-regulate is unknown, it is in the hundreds.The IFN- λ s like type I IFNs have been demonstrated to exhibit antiviral properties both in vitro and in vivo, including activity against HCV replication, and recent research has revealed that the HCV inhibitory effect of IFN- λ 3 is substantially dependent on signaling through the JAK/STAT pathway[8].

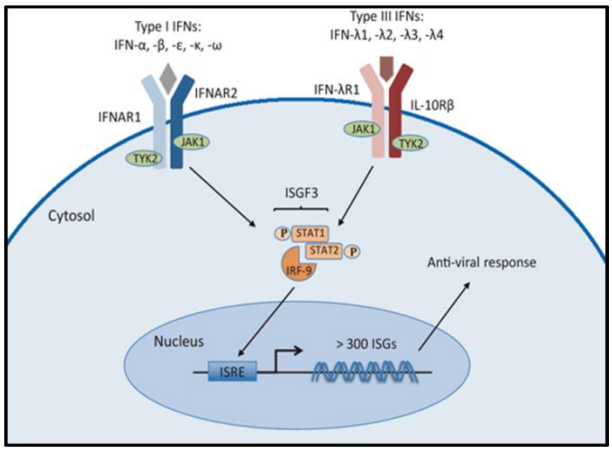

Fig. 1: Type I and type III IFNs signal through distinct heterodimeric receptors[36].

Despite the fact that IFN- λ s may be less-potent inhibitors of HCV replication than IFN-α[9], the expression of IFN- λ receptors seems to be more restricted, with particularly high expression in the liver[10] which may suggest that IFN- λ s may be particularly important for hepatotropic viruses.

IL 28B and hepatocellular carcinoma:

It was found that the rs12979860 C/T polymorphism which is located three kilo-bases upstream of the interleukin 28 B (IL-28 B) gene, encoding for the type III IFN λ 3 is strongly correlated with more than a two folds difference in response to HCV drug therapy, both in patients of European descent and in African-Americans[11].

On the basis of this TaqMan assay, at this locus there are 3 probable outcomes referred to as genotypes, and thus each sample was described as C/C, C/T, or T/T ("IL28B type")[12]. Numerous studies show a significant association between IL-28B rs12979860 C/T polymorphism and the progression of HCC.

The carriage of the T allele of this polymorphism was directly linked with the occurrence of HCC in transplanted patients (with viral and non-viral cirrhosis) in whom the occurrence of liver cancer was explicitly searched in the explanted liver and confirmed at histology. Additionally, this allele was found in 43.5% of HCC cases and in only 30% of HCC-free cases, showing highly significant differences in the genotype frequency trends between these two groups[13].

HCC patients with HCV infection carried the T allele more frequently than HCC patients without the infection. In multivariate analysis, the presence of the T allele was revealed to be an independent predictor of the presence of HCC, along with other recognized risk factors for this malignancy, such as male gender, advanced age, and body mass index[14].

Numerous recent research on IL-28B rs12979860 T/C polymorphism have concenterated on the connection with the development of hepatitis virus-related HCC[15,16].

According to research in other groups, carrying the T allele has been demonstrated to be a reliable indication of progression to an advanced stage[14,17,18]. Individuals with the genotype IL28B TT had a significantly higher risk of HCC recurrence than patients with the genotype TG/GG.

Patients with a T allele in rs12979860 had a high risk of developing liver cirrhosis and HCC according to Fabris and colleagues and Eurich and colleagues[14,17], numerous risk factors for HCC recurrence have also been identified, such as the presence of cirrhosis, elevated levels of AFP, and multiplicity of tumors[19].

Low Molecular Weight Polypeptide 7:

Major histocompatibility complex (MHC) class- I is a crucial molecule that initiates or directs the immune response by presenting foreign- or self-antigens to Cytotoxic T lymphocyte which play critical roles in the pathophysiology of liver damage caused by HCV and lowering of viral load[20].

The class II region of the MHC includes HLA-DR, -DQ and -DP in addition to genes involved in MHC class I antigen processing and presentation, such as those encoding the low molecular weight polypeptides (LMP2 and LMP7) and the transporters associated with antigen processing (TAP1 and TAP2)[21].

The antigen recognition by cytotoxic CD8 + T cells is dependent upon a number of crucial steps in antigen processing, including LMP2 and LMP7 that can change the pool of peptides available for class I antigen presentation through enhanced substrate cleavage after

basic and hydrophobic amino acid residues compared to the constitutive proteasome catalytic subunits[22].

This mechanism has the ability to alter the CD8 + T cell response to viral antigens both by increasing the variety of produced peptides and by encouraging the generation of peptides with carboxyl terminal amino acid residues that more strongly bind MHC- I molecules. Consequently, genetic variation of LMP2/LMP7 may be crucial for the immune response to HCV infection[23].

Structure of LMP-7:

LMP2 and LMP7 are interferon gamma (IFN-γ)-inducible subunits of a large protease complex known as the proteasome, which is involved in the breakdown of intracellular proteins and the production of antigenic peptides that are displayed by MHC class I molecules[24].

LMP2 and LMP7 can replace constitutively synthesized homologues (LMP2 for delta, MECL-1 for Z, and LMP7 for X) during proteasome assembly to form immunoproteasomes along with a third IFN- γ-inducible subunit MECL-1[25]. Immunoproteasomes, and LMP7 in particular, may be crucial for the production of antigenic peptides. The LMP7 propeptide is polymorphic, with Lys (K) or Gln (Q) found at position -24[26].

Mechanism of action of LMP-7:

When HCV virus infect host cells, the INFs (α, β, γ) are released which stimulate the formation of immunoproteosomes [catalytic core that is composed of low molecular polypeptide- 2 (LMP-2), LMP-7 and LMP-10] that produce immunogenic epitopes that bind to major histocompatibility complex (MHC) I molecules and when presented to CD8+ CTLs, enhances antigen presentation which in turn cause an antiviral response in the infected organism[27].

The type and growth of malignant tumors are affected by genetic and environmental factors. HLA classes I and II molecules must present immunogenic tumor peptides in order to start positive immune responses against malignancies[28]. The production and processing of the peptide are necessary for the effective presentation of peptide-HLA complexes (pMHC) on the cell surface. Being a multifunctional proteasome, LMPs are double-subunit and multicatalytic complex particles, that are dispersed throughout the cytosol and nucleus of the cell[29].

Intracellular proteins are converted into into peptides by proteasomes and immunoproteasomes. LMP2 (Proteosome 20S Subunit beta type-9) and LMP7 (Proteosome 20S Subunit beta type-8) subunits integration into newly assembling immunoproteasomes affects proteolytic activity and proteasome peptide synthesis in response to IFN- γ stimulation as in **Figure 2**[30].

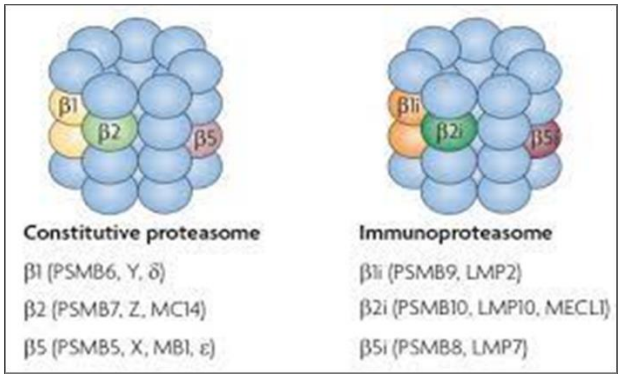

Fig. 2: Subunit composition of the active sites of the constitutive proteasome and immunoproteasome[37].

LMP-7 and hepatocellular carcinoma:

LMP2 and LMP7 products are two different subunits of a multifunctional proteasome that are essential for the selective degradation of endogenous proteins into peptides that can bind to human MHC class- I molecule[31]. Limited polymorphisms in the coding areas of human LMP2/LMP7 genes have been found in earlier studies. LMP2/LMP7 genes and HCV infection have only been explored in few number of studies.

By producing a range of peptides and favoring the generation of peptides with carboxyl-terminal amino acid residues that bind MHC-I molecules more tightly, this mechanism has the potential to change how CD8+ T cells react to viral antigens. As a result, LMP2/LMP7 genetic diversity may be crucial for the immune reaction to HCV infection[23].

Some studies[32,33] found an important correlation between LMP-7 SNP and viral clearance in response to pegylated IFN and ribavirin therapy. Others discovered a direct connection between LMP-7 polymorphism and the development of hepatic fibrosis in Egyptian individuals with HCV genotype 4.

However, it has been discovered that LMP-7 genetic variant does not significantly contribute to the development of HCC in individuals with advanced fibrosis[34,35].

CONCLUSION

Host, environmental and viral factors seem to play a major role in deciding the progression of chronic hepatitis C to hepatic cirrhosis and HCC, a process that sometimes takes many decades. HCV-infected patients, host and environmental factors tend to be more significant in assessing the progression of the liver disease to cirrhosis and HCC than viral ones.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

REFERENCES

1. Li T, Fan, J, Qin, LX, Zhou J, Sun, H.C, Qiu SJ, Ye QH, Wang, L Tang ZY. Risk factors, prognosis, and management of early and late intrahepatic recurrence after resection of primary clear cell carcinoma of the liver. Ann Surg Oncol, 2011;18(7), 1955–1963.
2. Papatheodoridis G, Hatzakis A. Public health issues of hepatitis C virus infection. Best Pract Res Clin Gastroenterol, 2012; (26),371-80.
3. Par A, Par G, Tornai I, Szalay F, Varszegi D, Frater E, Papp M, Lengyel et al. IL28B and IL10R -1087 polymorphisms are protective for chronic genotype 1 HCV infection and predictors of response to interferonbased therapy in an East-Central European cohort. BMC Res Notes, 2014; 7(12).
4. Vargas-Alarcon G, Gamboa R, Zuniga J, Fragoso JM, Hernandez-Pacheco G, Londono J, Pacheco-Tena C et al. Association study of LMP gene polymorphisms in Mexican patients with spondyloarthritis. Hum Immunol, 2004; 65(12),1437–1442.
5. de Veer MJ, Holko M, Frevel M, Walker E, Der S, Paranjape JM, Silverman RH, Williams BR. Functional classification of interferon-stimulated genes identified using microarrays J Leukoc Biol, 2001; 69(6),912–920.
6. Ank N, West H, Bartholdy C, Eriksson K, Thomsen AR, Paludan SR. Lambda interferon (IFN-λ), a type III IFN, is induced by viruses and IFNs and displays potent antiviral activity against select virus infections in vivo. Journal of virology. 2006;80(9): 4501-4509.
7. Sheppard P, Kindsvogel W, Xu W, Henderson K, Schlutsmeyer S, Whitmore TE et al. IL-28, IL-29 and their class II cytokine receptor IL-28R. Nature immunology. 2003; 4(1): 63-68.
8. Zhang X, Angkasekwinai P, Dong C, Tang H. Structure and function of interleukin-17 family cytokines. Protein & cell.2011; 2(1): 26-40.
9. Robek MD, Boyd BS, Chisari FV. Lambda interferon inhibits hepatitis B and C virus replication. Journal of virology. 2005;79(6): 3851-3854.
10. Hermant P, Michiels T. Interferon-λ in the context of viral infections: production, response and therapeutic implications. Journal of innate immunity. 2014; 6(5): 563-574.
11. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature. 2009; 461(7262): 399-401.
12. Tillmann HL, Thompson AJ, Patel K, Wiese M, Tenckhoff H, Nischalke HD, et al. A polymorphism near IL28B is associated with spontaneous clearance of acute hepatitis C virus and jaundice. Gastroenterology.2010;139(5): 1586-1592.
13. Fabris C, Falleti E, Cussigh A, Bitetto D, Fontanini E, Bignulin S, et al. IL-28B rs12979860 C/T allele distribution in patients with liver cirrhosis: role in the course of chronic viral hepatitis and the development of HCC. Journal of hepatology. 2011;54(4): 716-722.
14. El–Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology. 2007;132(7): 2557-2576.
15. Hou J, Zhang H, Liu J, Zhao Z, Wang J, Lu Z, et al. YTHDF2 reduction fuels inflammation and vascular abnormalization in hepatocellular carcinoma. Molecular cancer. 2019;18(1): 1-17.
16. Koolivand M, Allamehzadeh Z, Ahmadi A, Taheri, RA, Hassanpour K, Zeini A, Farnoosh G. The study of IFNL3 gene Rs12979860 polymorphism in the hepatitis C virus patients and healthy population in Tehran Province, Iran. Jundishapur Journal of Microbiology. 2020;13(5): 6.
17. Eurich D, Boas-Knoop S, Bahra M, Neuhaus R, Somasundaram R, Neuhaus P, et al. Role of IL28B polymorphism in the development of hepatitis C virus-induced hepatocellular carcinoma, graft fibrosis, and posttransplant antiviral therapy. Transplantation. 2012;93(6): 644-649.
18. Rivero-Juárez A, Camacho Espejo Á, Perez-Camacho I, Neukam K, Caruz A, Mira, J. A,et al. Association between the IL28B genotype and hepatitis C viral kinetics in the early days of treatment with pegylated interferon plus ribavirin in HIV/HCV co-infected patients with genotype 1 or 4. Journal of antimicrobial chemotherapy. 2012;67(1): 202-205.
19. Cabibbo G, Petta S, Barbara M, Attardo S, Bucci L, Farinati F, et al. Hepatic decompensation is the major driver of death in HCV-infected cirrhotic patients with successfully treated early

hepatocellular carcinoma. Journal of hepatology. 2017;67(1): 65-71.

20. Hiroishi K, Kita H, Kojima M, Okamoto H, Moriyama T, Kaneko T, et al. Cytotoxic T lymphocyte response and viral load in hepatitis C virus infection. Hepatology. 1997; 25(3): 705-712.
21. Van Kaer L. Accessory proteins that control the assembly of MHC molecules with peptides. Immunologic research. 2001; 23(2): 205-214.
22. Gaczynska M, Goldberg AL, Tanaka K, Hendil KB, Rock KL. Proteasome subunits X and Y alter peptidase activities in opposite ways to the interferon-γ-induced subunits LMP2 and LMP7. Journal of Biological Chemistry. 1996; 271(29): 17275-17280.
23. Cui Q, Zhang Y, Su J, Shi C, Lei N, Ding K, Wang N. The association between the genetic polymorphisms of LMP2/LMP7 and the outcomes of HCV infection among drug users. Journal of biomedical research. 2010; 24(5): 374-380.
24. Rock KL, Goldberg AL. Degradation of cell proteins and the generation of MHC class I-presented peptides. Annual review of immunology. 1999;17(1): 739-779.
25. Akiyama KY, Yokota KY, Kagawa S, Shimbara N, Tamura T, Akioka H et al. cDNA Cloning and Interferon γ Down-Regulation of Proteasomal Aubunits X and Y. Science. 1994; 265(5176): 1231-1234.
26. Prahalad SAMPATH, Kingsbury DJ, Griffin TA, Cooper BL, Glass DN, Maksymowych WP, Colbert RA. Polymorphism in the MHC-encoded LMP7 gene: association with JRA without functional significance for immunoproteasome assembly. The Journal of Rheumatology. 2001;28(10): 2320-2325.
27. Kuzushita N, Hayashi N, Kanto T, Takehara T, Tatsumi T, Katayama K, et al. Involvement of transporter associated with antigen processing 2 (TAP2) gene polymorphisms in hepatitis C virus infection. Gastroenterology. 1999; 116(5): 1149-1154.
28. Lee HJ, Ha SJ, Han H, Kim JW. Distribution of HLA-A, B alleles and polymorphisms of TAP and LMP genes in Korean patients with atopic dermatitis. Clinical & Experimental Allergy. 2001;31(12): 1867-1874.
29. Rechsteiner M, Hill C.P. Mobilizing the proteolytic machine: cell biological roles of proteasome activators and inhibitors. Trends in cell biology. 2005;15(1): 27-33.
30. Groettrup M, Khan S, Schwarz K, Schmidtke, G. Interferon-γ inducible exchanges of 20S proteasome active site subunits: why?. Biochimie. 2001; 83(3-4): 367-372.
31. Driscoll J, Brown MG, Finley D, Monaco JJ. MHC-linked LMP gene products specifically alter peptidase activities of the proteasome. Nature. 1993; 365(6443): 262-264.
32. Omran MH, Fotouh BE, Youssef SS, Ibrahim NE, Nabil W, Mahdy ESM, et al. Association between low molecular polypeptide 7 single nucleotide polymorphism and response to therapy in hepatitis C virus infection. World Journal of Hepatology. 2013; 5(3): 97.
33. Ibrahim A, Abd El-Azim S, Abd El-Azim M. Association of MnSOD Ala16Val genotype and activity with hepatocellular carcinoma risk in HCV-infected Egyptian patients. Arab Journal of Gastroenterology. 2010;11(1): 19-23.
34. El Awady MK, Omran MH, Ibrahim MK, Moustafa AM, Dawood RM, Bader El Din N G, et al.Low Molecular Mass Polypeptide 7 single nucleotide polymorphism is associated with the progression of liver fibrosis in patients infected with hepatitis C virus genotype 4. Clin Lab. 2016; 62: 381-387.
35. Wu B, Cui J, Yang XM, Liu ZY, Song F, Li, L, et al. Cytoplasmic fragment of CD147 generated by regulated intramembrane proteolysis contributes to HCC by promoting autophagy. Cell death & disease. 2017; 8(7): 2925-2925.
36. Galani IE, Koltsida O, Andreakos E. Type III interferons (IFNs): emerging master regulators of immunity. Crossroads between innate and adaptive immunity V. 2015;1-15.
37. Groettrup M, Kirk CJ, Basler M. Proteasomes in immune cells: more than peptide producers?. Nature Reviews Immunology. 2010;10(1): 73-78.