

Expression of microRNA-155 precursor in peripheral blood mononuclear cells from hepatitis C patients after antiviral treatment

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Summary. – Chronic hepatitis caused by Hepatitis C virus (HCV) is the main source of liver cirrhosis, hepatocellular carcinoma, and extra-hepatic diseases. After treatment-induced resolution of hepatitis C, the persistence of HCV RNA in serum and peripheral blood mononuclear cells (PBMCs) is often observed. An expression of the precursor of microRNA-155 (miR-155) called BIC can be the factor responsible for a course of HCV infection. Therefore, we assessed the relationship between BIC expression and HCV RNA status in sera and PBMCs samples of 64 hepatitis C patients treated with interferon α (IFN- α) + ribavirin. High expression of BIC in PBMCs was determined in 100% of patients that harbored HCV RNA in serum and PBMCs. Further, we found that 83% of PBMCs samples were BIC-positive in a group of patients that eliminated HCV RNA only from serum. The lowest expression of BIC was found in patients that eliminated HCV RNA from both serum and PBMCs.

Keywords: Hepatitis C virus; microRNA-155; peripheral blood mononuclear cells; BIC; antiviral treatment

Introduction

An estimated 170–200 million people worldwide are infected with HCV that belongs to the genus *Hepacivirus*, the family *Flaviviridae*. HCV infection is a major cause of chronic hepatitis that frequently progresses to the cirrhosis and hepatocellular carcinoma (Sy and Jamal, 2006). Currently available anti-HCV therapies (IFN + ribavirin) are effective in about 50% of the patients (McHutchison *et al.*, 1998). Thus, the quest for a new effective antiviral strategy is the current problem.

Although the liver is the main location of HCV replication, the ample data indicate that HCV can infect extra-hepatic tissues including PBMCs (Zignego *et al.*, 2007).

Several studies described a detection of HCV RNA in PBMCs collected from patients with chronic hepatitis C (Bare *et al.*, 2005; Blackard *et al.*, 2006). HCV tropism for PBMCs is also supported by a broad spectrum of lymphoproliferative disorders including type II mixed cryoglobulinemia and non-Hodgkin lymphoma (Craxi *et al.*, 2008). Moreover, it was documented that HCV infection can persist at low level in PBMCs many years after spontaneous or antiviral treatment-induced serum resolution (Pham *et al.*, 2004; Gallegos-Orozco *et al.*, 2008). The mechanism responsible for HCV RNA persistence in PBMCs after completion of the antiviral therapy remains unclear.

MicroRNAs belong to the class of endogenous small RNA molecules, 20–25 nts in length that take part in the regulation of metabolic processes in humans (Bartel, 2004). One of the regulatory sequences miR-155 has emerged as the gene expression regulator crucially involved in human innate immunity (Haasch *et al.*, 2002). Mature miR-155 is the product originated from pri-miR-155 sequence, shortly called BIC (Eis *et al.*, 2005). Inducible expression of mature miR-155 and its precursor BIC was observed after stimulation of macrophages

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Abbreviations: BIC = precursor of microRNA-155; DLBCL = diffuse large B-cell lymphomas; HCV = Hepatitis C virus; IFN- α/β = interferon α ; miR-155, 122 = microRNA-155, 122; PBMCs = peripheral blood mononuclear cells; pri-miR = primary microRNA

by polyriboinosinic:polyribocytidylic acid [poly(I:C)] and after exposure of the cells to pro-inflammatory IFN- α/β (O'Connell *et al.*, 2007). However, the relationship between HCV infection and expression of BIC has not been established yet.

To address this issue, we investigated an association between BIC expression in PBMCs and the presence of HCV RNA in patients' sera and PBMCs after anti-HCV treatment. Our findings revealed a strong association between BIC expression in PBMCs and HCV RNA persistence after completion of antiviral treatment.

Materials and Methods

Blood samples. Blood samples were collected from 64 patients with chronic hepatitis C infection aged 9 to 21 years after antiviral treatment (IFN- α 2b + ribavirin). PBMCs and serum were isolated from 5 ml of blood by density gradient centrifugation on Histo-paque 1,077 (Sigma).

Total RNA. Total RNA was extracted from serum and PBMCs samples by modified guanidinium thiocyanate/phenol/chloroform technique (Chomczynski and Sacchi, 1987).

RT-PCR. Total RNAs (6 μ g) from sera and PBMCs were reverse-transcribed and amplified by MasterAMP™ Tth DNA Polymerase (Epicentre® Biotechnologies) with external HCV-specific primers (forward: CCACCATGAATCACTCCCCTGT, reverse: GCTCATGGTGCACGGTCTACGAGACCT) in reaction steps – 20 mins at 70°C and 3 mins at 94°C followed by 35 cycles of 94°C for 20 secs, 50°C for 20 secs, 72°C for 20 secs, and 72°C for 7 mins. The product of the first reaction was amplified with internal primers (forward: GTCTTCACGCAGAAAGCGTCTAGCC, reverse: CACTCGCAAGCACCTATCAGGCAG) in the second-round of PCR step (2 mins in 94°C, 30 cycles of 94°C for 40 secs, 55°C for 40 secs, 72°C for 40 secs, and 72°C for 10 mins). HCV-specific PCR product of 278 bp was detected by PAGE (Dulak and Funk, 1996).

BIC expression was evaluated also by RT-PCR. Random cDNAs were synthesized according to the manufacturer's instruction by using RT kit Improm II (Promega) and then amplified with BIC-specific primers (forward: TAATCCAAGAACTAGTCACCC, reverse: GGTTCACGTGAATAACCTGGAC) as follows: 5 mins at 94°C, 94°C for 30 secs, 60°C for 44 secs, 72°C for 40 secs, and 72°C for 7 mins. The appearance of BIC-specific PCR product of 209 bp was determined after 30 PCR cycles by PAGE (van den Berg *et al.*, 2003). Control of gene expression was performed in the same cDNA samples using the same RT-PCR profile as described above and β -actin-specific primers of following sequences: forward: CAAAGACCTGTACGCCAACACA and reverse: AACCGACTGCTGTACCTTCAC (Delpuech *et al.*, 2002).

Results and Discussion

HCV RNA presence in serum and PBMCs was determined in 64 patients that underwent antiviral treatment. According to the obtained results, these patients could be divided into 3 groups: group 0/0 (n = 37), where HCV RNA was detected

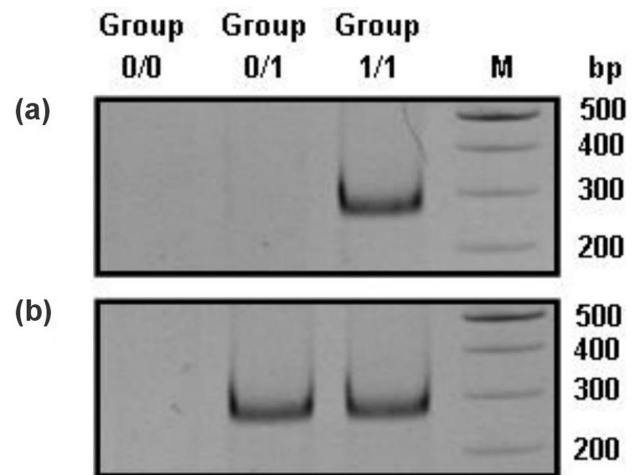


Fig. 1

Detection of HCV RNA in sera (a) and PBMCs (b) by RT-PCR and PAGE

DNA size marker (lane M).

neither in sera nor in PBMCs, group 0/1 (n = 12), where HCV RNA was detected only in PBMCs and group 1/1 (n = 15), where HCV RNA was found in both sera and PBMCs (Fig. 1a,b). Accumulated evidence indicated that cells from lymphoid system including PBMCs are capable to support HCV infection *in vivo* and *in vitro* (Zignego *et al.*, 2007). Our study confirmed HCV RNA presence in PBMCs in 27 out of 64 patients after anti-HCV treatment. This finding remained in line with the results of others, who demonstrated that HCV RNA may persist in lymphoid cells after spontaneous or therapy-induced resolution of hepatitis (Pham *et al.*, 2004; Gallegos-Orozco *et al.*, 2008). Despite serious implications of HCV reactivation after antiviral therapy, the mechanism of HCV persistence in PBMCs remains elusive. Recently, microRNAs turned out to be potent regulators of viral infections including HCV infection (Pedersen *et al.*, 2007). MicroRNA-122 expression was shown as an important stimulator of HCV replication *in vitro* (Jopling *et al.*, 2005; Henke *et al.*, 2008). Another representative of microRNA family miR-155 was found to be a common target of the mammalian inflammatory response to the infection. In our study, we decided to evaluate the involvement of miR-155 precursor (BIC) in observed HCV RNA persistence. The correlation between BIC expression and its functional product miR-155 has been recently studied. Eis *et al.* (2005) found that pre-miR-155 may be released from primary BIC gene transcripts or from spliced and polyadenylated BIC RNA. Moreover, they reported limited correlation between BIC transcript and mature miR-155 in diffuse large B-cell lymphomas (DLBCL). It was found that human embryonic kidney cells 293 (HEK-293T)

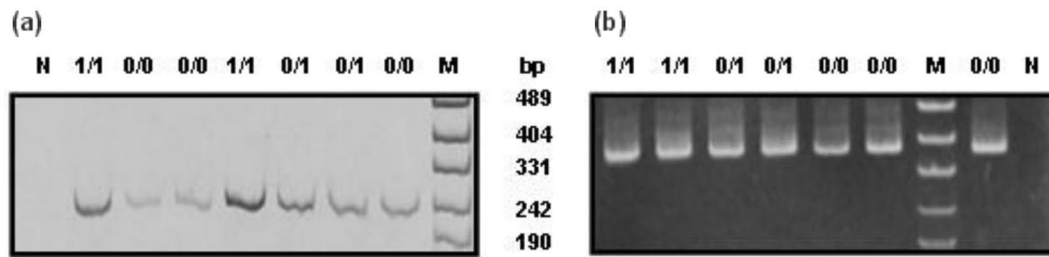


Fig. 2

Detection of BIC expression in PBMCs by RT-PCR and PAGE

BIC (a) and β -actin (b) mRNA in PBMCs from patient's group 0/0, 0/1, 1/1. Negative control (lane N), DNA size marker (lane M).

transfected with plasmid pcDNA3.BIC produced detectable amounts of mature miR-155 supporting the idea that non-spliced BIC RNA could be a template for miR-155 (Eis *et al.*, 2005). Other reports indicated a precise correlation between BIC transcripts and mature miR-155 in various human lymphoma cell lines Akata(+), Akata(-), Ramos(-), and Ramos/AW (Rahadiani *et al.*, 2008), DLBCL (Rai *et al.*, 2008) and mouse macrophages (O'Connell *et al.*, 2007). Rai *et al.* (2008) found that although the level of expression of spliced form of BIC was often higher than that of non-spliced transcripts, the amount of spliced cytosolic transcripts closely reflected the amount of non-spliced BIC. These results may approve the quantification of BIC as a surrogate measure for the functional, mature miR-155 (Rai *et al.*, 2008). Indeed, many attempts to define the expression of miR-155 in DLBCL have interchangeably used BIC or mature miR-155 (van den Berg *et al.*, 2003; Kluiver *et al.*, 2005; Eis *et al.*, 2005; Lawrie *et al.*, 2007). In our study expression of BIC and β -actin were evaluated by RT-PCR in all PBMCs samples (Fig. 2 a,b) and then matched with HCV RNA status. BIC expression was confirmed in PBMCs in 67% of group 0/0 patients, e.g. in 25 out of 37 patients (Fig. 3). In PBMCs samples from patients that eliminated HCV RNA from serum only (group 0/1), BIC expression was demonstrated in 83% cases. In group of patients that harbored HCV RNA in both sera and PBMCs after anti-viral treatment (group 1/1), the BIC was expressed in 100% of PBMCs samples. Moreover, the level of BIC expression in groups 0/0 and 0/1 was relatively lower compared with the BIC expression level found in PBMCs samples from group 1/1 (Fig. 2a). At the same time, the expression level of β -actin remained unchanged as determined in the same samples independently of HCV RNA status or BIC expression (Fig. 2b). We show here that HCV RNA presence in sera and PBMCs in patients after anti-viral treatment is associated with the most frequent and elevated expression of BIC in PBMCs. We can speculate that the modulation of PBMCs function triggered by BIC or/and miR-155 may lead to HCV RNA persistence. A link between BIC expression and the expression of putative target genes taking part in antiviral immune

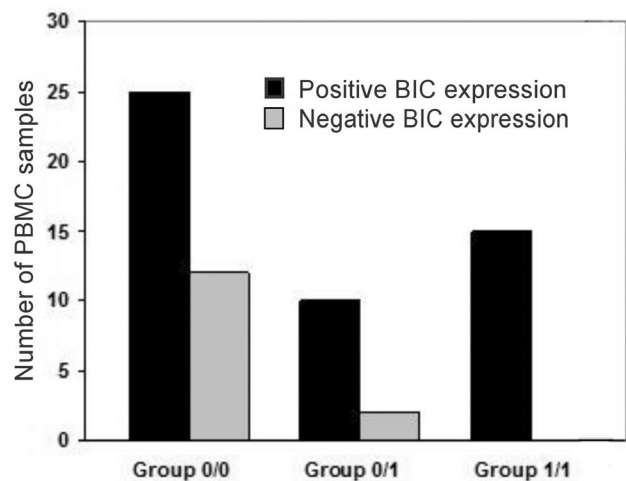


Fig. 3

Proportions of BIC-positive and BIC-negative PBMCs in indicated groups of HCV patients

response has not been fully established. It is supposed that miR-155 may activate immune system to the invading pathogens and/or may prevent enormous immune response (Rai *et al.*, 2008). It was shown that the activation-induced cytidine deaminase is an important target for regulation by miR-155 (Teng *et al.*, 2008). Our observation that HCV RNA persistence in sera and PBMCs was accompanied by the high and ubiquitous expression of BIC in PBMCs suggested that BIC expression could influence the efficiency of IFN- α therapy. We can hypothesize that the relative lack of viral response to the interferon therapy of HCV infection may be associated with BIC-dependent blunted interferon cell signaling. Although the better understanding of the BIC role in viral response to the interferon therapy requires further studies, yet our results demonstrated for the first time that HCV RNA persistence after interferon treatment was accompanied by the increased expression of BIC in PBMCs.

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