Similarity of the secondary structure with identical seven bases in the pregenomic/genomic regions required for the replication of hepatitis B virus and hepatitis C virus

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Abstract: We described the existence of the similarity of the secondary structure in hepatitis B virus (HBV) packaging signal ϵ and in hepatitis C virus (HCV) 3' X tail, both of which are considered to be important for the viral replication cycle. Seven nucleotide (nt) sequence (5'-GGUGGCU-3') existed both in HBV ϵ nt 3169-3175 and HCV 3' X tail nt 1'-7'. Furthermore, the 7-nt sequence paired with the 6-nt sequence (5'-AGCCUC-3') of HBV ϵ (nt 3149-3154) and (5'-AGCCUC-3') of HCV 3' X tail (nt 15'-20'), to form the very similar secondary structure with one unpaired U. We consider the possibility that the common biological characters shared by these viruses are derived from the recognition of this conserved secondary structure by the viral or cellular proteins.

Key words: HBV ϵ ; HCV 3' X tail; secondary structure; replication; hepatotrophism.

Introduction. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are closely associated with the development of hepatocellular carcinoma (HCC). Both hepatotrophic viruses are not directly cytopathic, and there are many asymptomatic healthy viral carriers. No oncogene is defined in the genomes of these viruses, but in many patients both viruses cause the chronic infection which can be a predisposition for the development of HCC. Despite of these common biological characters, these viruses have the different genome structures. As the genome, HCV has the single, positive strand RNA, and HBV has the partially double stranded circular DNA although its replication is mediated by the pregenomic RNA.¹⁾ In the replication of HBV, the priming of the negative strand DNA synthesis occurs on a bulged region of the packaging signal, ϵ .²⁾⁻⁴⁾ Although the priming site is specified onto the bulged region [nucleotide (nt) 3147-3145 (3'-CUU-5') (Fig. 1, left)], the stem and loop structure of ϵ is considered to be important for replication, particularly for the binding of viral polymerase protein.⁴⁾ In the 3' untranslated region of HCV genome, there is a 98 nucleotide (nt) sequence

designated 3' X tail.^{5),6)} This sequence is highly conserved among several subtypes of HCV, and reported to form the stem and loop structures.^{5),6)} The replication cycle of HCV is not known exactly, but 3' end of the viral genome containing this sequence is considered to take important roles for the replication and packaging.⁵⁾⁻⁷⁾ In this paper, we compared the sequence and the secondary structure of the pregenomic/genomic region of two viruses required for their replications, i.e., ϵ of HBV and 3' X tail of HCV, and report the existence of the similar secondary structures in them.

Methods. Based on the computer assisted analysis of the secondary structure and the published information of HBV packaging region²⁾ and HCV 3' X tail,^{5),6)} we compared the sequence and the secondary structure of these regions in two viruses.

Results and discussion. As shown in Fig. 1, nt 3169-3175 (5'-GGUGGCU-3') in HBV ϵ and nt 1'-7' (5'-GGUGGCU-3') in HCV 3' X tail was identical in the sequence level. This 7-nt sequence formed very similar secondary structures both in HBV ϵ and HCV 3' X tail. HBV ϵ nt 3169-3175 (5'-GGUGGCU-3') paired with nt 3149-3154 (5'-AGCCUC-3') to form a stem with one unpaired U (Fig. 1, left). This structure is regarded to

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Fig. 1. Secondary structures of HBV ϵ (left) and HCV 3' X tail (right). Enclosed are stem structures with one unpaired U, conserved both in HBV ϵ and HCV 3' X tail. 3' X tail shown here is type II/1b, and the enclosed structure also exists in type III/2a.⁵⁾ Nucleotide numbers are based on ref. 4) for HBV ϵ and ref. 5) for HCV 3' X tail.

compose one part of polymerase protein binding site.⁴⁾ As for HCV 3' X tail, nt 1'-7' (5'-GGUGGCU-3') paired with nt 15'-20' (5'-AGCCCU-3') to form a stem structure with one unpaired U (Fig. 1, right), which is very similar to that in HBV ϵ . Thus almost identical secondary structures exist in the regions considered to be indispensable for the replication of two different hepatotropic viruses. By the simple assumption, the identical 7-nt sequence (5'-GGUGGCU-3') may appear once in 4⁷ (four to the seventh) bp (16 kb) in average. The 7-nt sequence in HBV also existed in 98-nt 3' X tail of HCV. Furthermore, this 7-nt sequence paired with the 6-nt sequence (5'-AGCCPyPy-3') to form the secondary structure, which are very similar in two viruses. This similarity seems not to be the result of simple coincidence by chance but to have some unknown biological significance.

The whole genome structures and replication cycles of HBV and HCV are quite different, although these two viruses have similar features; both viruses are hepatotropic and cause chronic infection of the liver, leading to liver cirrhosis and HCC. One reason for the characteristic similarity of these virus infections may be the existence of virus specific receptors on the surface of hepatocytes. It is also possible that the viral, or cellular protein(s) binds to this conserved structure, and determine the hepatotropic characters of two viruses. The

replication machinery of these viruses may have unknown commonality. Our findings also implicated the possibility that the similar structures shared by two viruses may have evolved from the same ancestral origin. We are currently on the way to find the cellular protein which binds to this structure.

We previously reported that HBV genomic region designated 15AB, which overlaps the packaging signal ϵ , can mediate the DNA recombination and possibly accelerate the genomic instability leading to hepatocarcinogenesis.⁸⁾⁻¹¹⁾ We also consider the similar secondary structure shared in 15AB, HBV ϵ , and HCV 3' X tail may have some implication for the development of HCC.

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