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Editorial

Genetic changes in the interferon sensitivity-determining region of hepatitis C virus (HCV) during the natural course of infection: an implication for the gene function in the role of chronic infection

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Nonstructural 5A gene variability of hepatitis C virus (HCV) during a 10-year follow up
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The interferon sensitivity-determining region (ISDR; aa2209–2248 of HCV-J) in the nonstructural 5A region (NS5A) of hepatitis C virus (HCV) was originally identified as the genomic functional element wherein missense mutations were closely related to the clinical efficacy of interferon treatment, as well as to serum viral loads, in genotype-1b HCV infection.[1,2] After the first reports of the ISDR, controversy arose as to its predictive value for the outcome of interferon therapy, because clinical studies in Europe and North America did not always support the relevance of ISDR,[3] although most studies in Japan, Spain, and Italy supported it.[4,5] However, recent meta-analyses have clearly supported the universal correlation between ISDR sequence and interferon resistance.[6] It is speculated that the initial discrepancy of the results might have been caused by differences in interferon regimens and patient sources.

After identification of ISDR as the key genomic element for interferon efficacy and viral replication, the molecular function of NS5A protein and its relevance to ISDR structures has been vigorously and intensively studied using NS5A protein expression in vitro or in transgenic mice. A variety of putative NS5A functions were postulated, such as binding to cellular protein kinase R (PKR),[7] TRADD,[8] Grb-2,[9] p21,[10] hVAP-33,[11] or other proteins that may influence the pathogenesis of hepatitis C by antiviral effects, apoptosis, signal transduction, cell cycle regulation, or formation of viral replication complex. Much attention has been paid to PKR, because NS5A protein was found to block the antiviral effect of PKR in an ISDR sequence-dependent manner by directly binding to PKR through the so-called PKR-binding domain, which includes the ISDR plus an additional 26 aa stretch located at the C-terminal portion (aa2209–2274). The recently developed HCV subgenomic replicon system also disclosed the importance of NS5A proteins in intracellular viral replication, because specific mutations called "cell culture-adaptive mutations" needed in its genome for efficient replication in cultured cells clustered in the central region of NS5A, particularly in the serine cluster region immediately upstream to the ISDR or the ISDR itself.[12,13] Because these mutations possibly affect phosphorylation of NS5A proteins, the role of phosphorylated NS5A protein in viral replication and interferon sensitivity has become the recent target of molecular research.

How does the HCV–ISDR structure change in a host during the natural course of disease? The answer to this question should give us important clinical information about when to start interferon therapy, whether earlier or later in HCV infection. In the current issue of the *Journal of Gastroenterology*, Fan et al.[14] report the genetic evolution of the *NS5A* gene during a 10-year follow-up of natural HCV infection in 7 patients, focusing on ISDR, PKR-binding domain, serine cluster region, and other functional domains in *NS5A* gene. To investigate changes of the genetic variability during the natural course, they performed subcloning analysis. As a result, serine residues at positions 2194, 2197, 2201, and 2204 in the serine cluster region, suggested to be important for hyperphosphorylation of NS5A protein, were highly conserved in all patients and within the quasipecies of each patient, suggesting that phosphorylation plays the crucial role in NS5A protein function. Meanwhile, subcloning analysis of the ISDR quasispecies disclosed that the wild-type ISDR (no aa substitution relative to HCV-J), or the intermediate-type ISDR (one to three substitutions) was dominant and stable throughout the observation periods in all patients. However, the ISDR quasispecies decreased over time, and the quasispecies had a tendency gradually to converge to the wild-type ISDR.

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Before the report by Fan et al., several studies already had been conducted for analyzing the natural genetic change of the ISDR.[15–19] Although these previous studies also demonstrated that the wild-type ISDR or the intermediate-type ISDR was generally stable, the observation periods were rather short, and the quasispecies complexity was not investigated in most of the studies. Importantly, Fan et al. demonstrate that the ISDR quasispecies finally tended to converge to the wild-type ISDR with a decrease of the quasispecies complexity, indicating that sequence motif of the wild-type ISDR had a crucial role functionally in establishing chronic HCV infection. Although the mutant-type ISDR (four or more aa substitutions) was not included in the study, the mutant-type ISDR was reported to be rather unstable, because nonsynonymous mutations (63%) were higher than synonymous mutations (37%), indicating that strong selective pressure of the host was exerted on the mutant-type ISDR.[16] This finding coincides with the results by Fan et al. that the HCV of wild-type ISDR ultimately survives in the course of chronic infection.

If the quasispecies complexity of the ISDR subtypes finally converges to the wild-type ISDR, it might be better to start interferon therapy early for chronic HCV infection. It is not clear why the mutant-type ISDR is unstable in a host. Part of the mechanism, however, might be explained by different interaction with cellular proteins, such as PKR induced by endogenous interferon. On the other hand, if the mutant-type ISDR was unstable and easily defeated by the wild-type ISDR in chronic infection, this weak subtype might have disappeared in the world of HCV infection. However, although the distribution is rather small, the mutant-type ISDR is still frequently found in clinical samples. Is it on the way to disappearing? Or does it have an advantage over the other types in a certain phase of infection other than the chronic phase? Recent advances in understanding of the innate immune system have disclosed that mammalian cells have two distinct innate immune pathways protecting cells from the virus: the interferon regulatory factor (IRF) system, and the interferon (IFN)—signal transduction and activator of transcription (STAT) system. In the phase of chronic infection, the IFN–STAT system might have a dominant role in viral suppression, and the wild-type ISDR is supposed to inhibit this pathway, giving a survival advantage to HCV with the wild-type ISDR. In contrast, the IRF system might be dominant in the phase of acute infection. Does the mutant-type ISDR inhibit this IRF pathway, and give a survival advantage to HCV with the mutant-type ISDR? The answer to this question requires further study, but these analyses might go far toward helping understand the mysterious function of the ISDR.

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