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Minireview

The Study of HCV and the reproductive engineering

Takayuki Ishikawa*

Laboratory Animal Research Station, Nippon Institute for Biological Science
3331-114 Kamisasao, Kobuchizawa, Hokuto city, Yamanashi 408-0041, Japan

INTRODUCTION

It is estimated there are currently 2 million Hepatitis C virus (HCV) carriers in Japan. Every year 25,000 people die of HCV-induced hepatocellular carcinoma (HCC).

HCC has become a major social problem, with fatalities due to the disease projected to increase. HCV is infective only to humans and chimpanzees. Until the report by Wakita et al. in 2005 [1], there was no established technique for propagating HCV particles in vitro. Identification of the viral proteins has been carried out using genetic engineering to express the cloned viral genome in cultured cells. The viral genome, a single-stranded RNA of approximately 9.4 kb, codes a polyprotein of around 3,100 amino acids.

At present, ten viral proteins have been identified along the polyprotein, starting at the N-terminal (Fig.1). These viral proteins have been reported to exert a variety of biological activity. In this paper we will focus on the core protein, which has been most numerous reported in connection with HCC, providing an overview of research and introduction the reproductive engineering that is contributing to this research.

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*Correspondence: T. Ishikawa

Biological activity of the core protein

HCV has not been reported to integrate into the human genome as do other flaviviruses and pestiviruses. The viral proteins produced by HCV are likely involved directly or indirectly in the development of HCC. HCV proteins have been confirmed to show a variety of biological activities in host cells *in vitro* experimental systems.

In particular, many research reports suggest that the core protein is involved in cell transformation and signal transduction associated with cell proliferation. Ray et al. have reported that

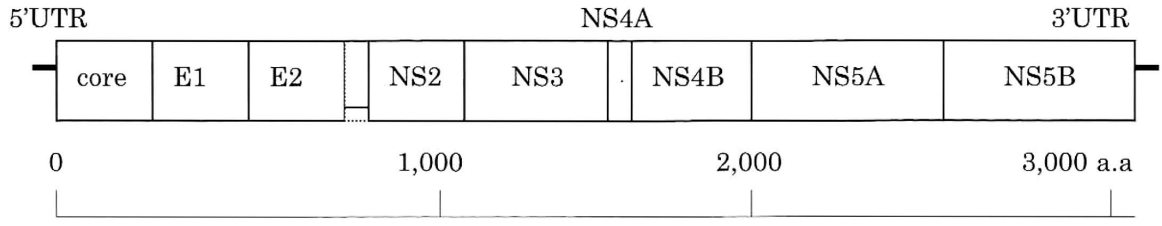

Fig. 1. Structure of HCV core polyprotein.

The viral genome, a single-stranded RNA of approximately 9.4 kb, codes a Polyprotein of around 3,100 amino acids. The core protein is located at the N-terminal of polyprotein.

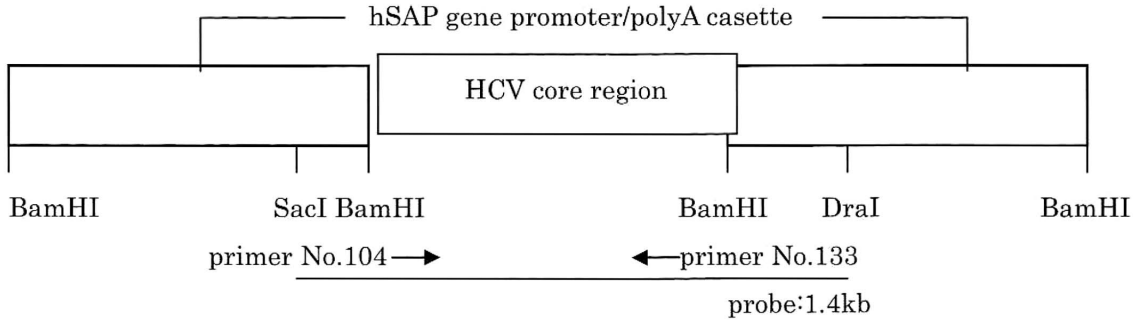

Fig. 2. Structure of hSAP/HCV core transgene.

Schematic illustration of transgene structure. The coding region of HCV core protein (genotype II/1b) has BamHI site at both 5' and 3' terminals. The hSAP gene promoter and polyA signal are derived from a genomic clone of the human SAP gene. The positions of primers are indicated by arrows.

Balbc 3T3 [2] cells and rat embryonic fibroblasts [3] transform like tumor cells when the core protein is coexpressed with the H-ras oncogene. Using the luciferase assay, Kato et al. [4] studied the effect of HCV proteins on the intracellular expression of transcription factors. They showed that the core protein has the greatest effect on intracellular signal transduction, in particular activating the NFκB pathway that is involved in apoptosis and the inflammatory response.

Yoshida et al. [5] found that the core protein increases BclX transcriptional activity through the MAP-SRE pathway, which is involved in the cell survival signal, and inhibits Fas-mediated hepatic cell apoptosis. Otsuka et al. [6] have reported that the core protein activities NFκB, SRE, AP-1, and other pathwaya that induce cell growth, and that it also activates transcription of the p53 gene, which in contrast to the former can arrest the cell cycle. This contradictory biological activity of the core protein suggests that HCC accompanying HCV infection is the result of the mutant interaction of a variety of triggers arising in the host, such as the inflammatory response against viral proteins and age-related changes in hepatic cells, rather than being induced by the activity of the core protein alone. It also shows the limits to *in vitro* HCC study.

Core protein research using transgenic mice

Gordon et al. [7] succeeded in producing transgenic (Tg) mice using microinjection of cloned genes into the male pronucleus of a fertilized oocyte. The distinguishing characteristic of this method is the ability to assess the biological activity of the introduced foreign genes in living mice.

The method is suitable for analysis of the viral proteins of a virus such as HCV for which there exist no appropriate animal model and that is characterized by long-term persistent infection. Accordingly, a number of research groups are trying to produce Tg mice that will express HCV proteins [8-11]. Moriya et al. [11] produced Tg mice that express, under the control of the HBx (Hepatitis B virus X) gene promoter, the HCV core gene, envelope gene or nonstructural (NS) protein gene, respectively. Moriya's group used these mice to compare and study the effects of the expression of each viral protein on the hepatic cells of living mice. The expression of core proteins comparable with those in chronic hepatitis C patients and an increase in free radicals in hepatic cells were found in the livers of core Tg mice. Moreover, hepatic adenomas with fatty changes grew in individuals over 16 months old, some of which developed HCC. It seems unlikely that this model faithfully recreates the pathogenesis of HCC in HCV carriers, since these Tg mice have immune tolerance to the core protein and so do not develop an inflammatory response. Nevertheless, this research is noteworthy for being the first to demonstrate the oncogenic potential of the core protein.

We produced Tg mice [12] that express the core protein under the control of the human SAP gene promoter (Fig.2). As in the Tg mice mentioned above, our mice developed fatty livers and hepatic

adenomas in individuals 57 weeks old and older (Fig.3).

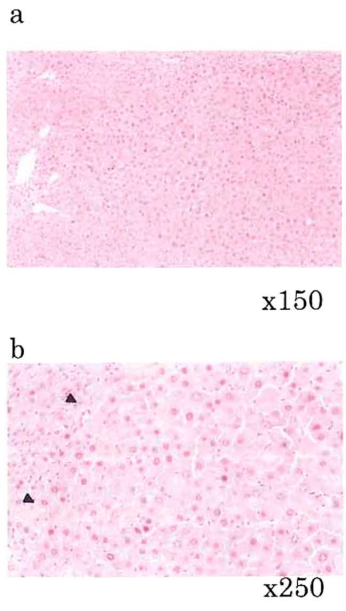

Fig. 3. Histopathology of hepatocellular adenoma in a transgenic mouse.
The neoplastic nodule compresses the adjacent parenchyma. a. x150
Hypertrophic neoplastic cells proliferate irregularly without normal hepatic structures. b. x250

Histopathological study of individuals over 80 weeks old revealed a high frequency (about 80%) of malignant B-lymphomas (follicular center cell mixed-type) in addition to hepatic lesions (Fig.4). A recent epidemiological study by Casato et al. [13] showed a high incidence of non-Hodgkin's B-lymphoma and cryoglobulinemia in chronic hepatitis C patients. Moreover, Sansanno et al.'s immunocytochemical study of non-Hodgkin's B-lymphoma occurring in chronic hepatitis C patients demonstrated that the HCV core protein is localized in lymphoma tissue [14]. Thus, these lymphoproliferative disorders have come to be recognized as extrahepatic lesions of HCV carriers. In our mice, the core gene was also expressed in the spleens, lymph nodes, and lymphoma tissue in addition to liver tissue (Fig.5). We expect that our mice will provide abundant research data for determining the oncogenic potential of the core protein on B cells and elucidating the etiologic mechanism of B-cell lymphoma in chronic hepatitis C patients.

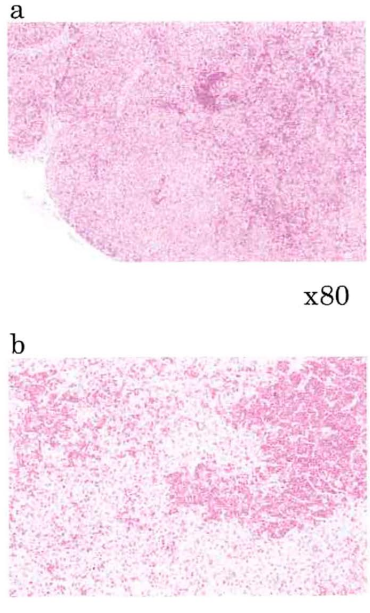

Fig. 4. Histopathology of malignant lymphoma in the mesenteric lymph node of a transgenic mouse.
Lymphoma cells replace most of the lymphoid tissues. a. x80
The neoplastic cells shows pleomorphic and mixed-cell appearances.

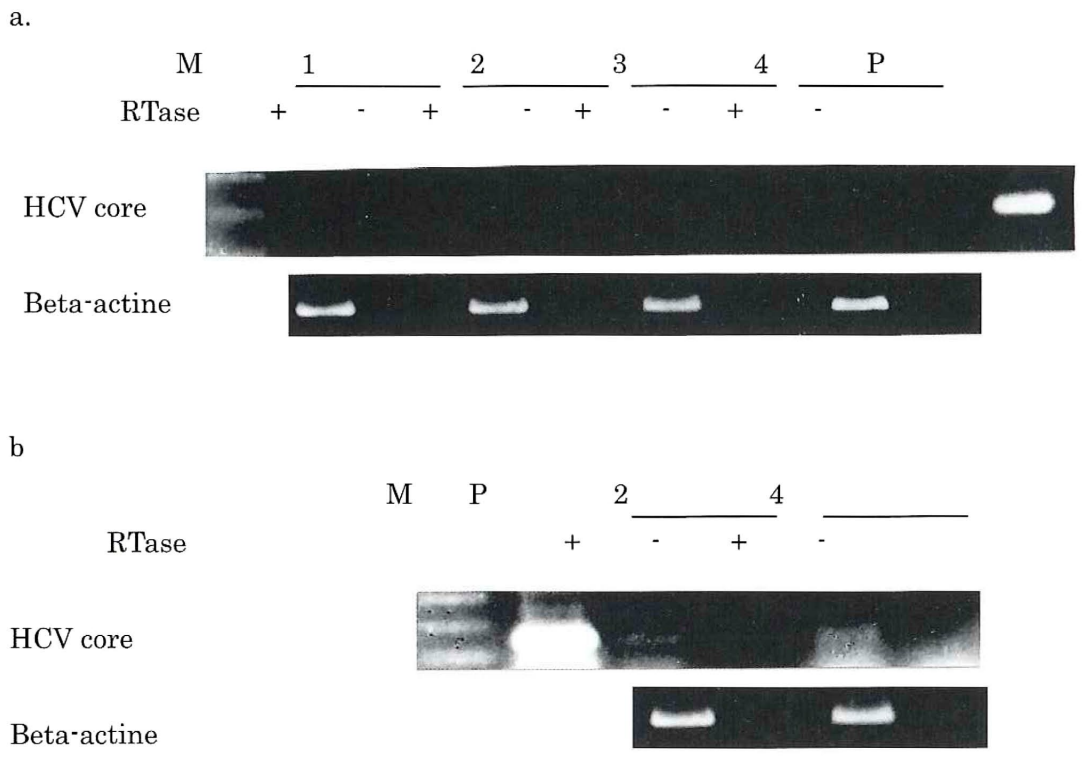

Fig. 5. Expression of the HCV core transgene in the liver and malignant lymphoma of the transgenic mice.

RT-PCR analysis in the liver of the transgenic mice. a.

RT-PCR analysis in the malignant lymphoma of the transgenic mice. b.

M:size marker, P:PCR on the genomic DNA of transgenic mouse.

CONCLUSION

HCV shows high host specificity, and there is still no appropriate infection model for it. Accordingly, Tg mice are vital to the elucidation of the relationship between HCV and HCC. Tg mice are produced using various reproductive engineering including embryo manipulation, gene microinjection, and embryo transfer. Large numbers of coeval mice are needed in order longitudinally analyze Tg mice with low reproductive efficiency (the weaning rate of our Tg mice is about 49%) for that reason in vitro fertilization and early embryo cryopreservation method are also indispensable. In writing this review, we realized again that the reproductive engineering established by scientific pioneers, have spread widely through many academic fields, providing significant benefits to numerous researchers.

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