Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Epithelial cell adhesion molecule expression in hepatic stem/progenitor cells is controlled by the molecular clock system

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ARTICLE INFO

Article history:
Received 8 June 2018
Accepted 20 June 2018
Available online xxx

Keywords:
Chronic hepatitis
Potential hepatic stem-like cells
EpCAM
Clock
Circadian rhythm
LEF

ABSTRACT

The circadian rhythm, which regulates various body functions, is transcriptionally controlled by a series of clock gene clusters. The clock genes are related to the pathology of various kinds of diseases. Although there is evidence of serious sleep disorders in patients with chronic hepatitis, the liver regeneration mechanism under chronic hepatitis conditions and its association with the clock genes are not clear. In this study, the influence of the circadian locomotor output cycles kaput (CLOCK), which is one of the clock genes, on a 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-induced hepatitis animal model was investigated. The appearance of potential hepatic stem-like cells (epithelial cell adhesion molecule [EpCAM]-positive cells) is an initial critical step in liver regeneration during chronic inflammation. The results showed a considerable number of hepatic EpCAM-positive cells in the wild-type (WT) mice 1 week after the DDC feeding. However, the number of EpCAM-positive cells in the *Clock*-mutant (*Clk/Clk*) mice decreased, and their hepatitis was worse compared with the WT mice. In addition, the expression of *Epcam* mRNA, which is a functional marker of potential hepatic stem-like cells, was controlled by LEF1, which was regulated by CLOCK. The results of this study will facilitate the elucidation of the liver regeneration mechanisms, including those at the molecular level, and may assist in the development of new treatment modalities in the future.

1. Introduction

Chronic hepatitis is a worldwide health problem [1]. The accumulation of the virus after infection, along with fat, or by unidentified causes, can lead to an inflammatory state, and chronic hepatitis induces cirrhosis and, ultimately, hepatocellular carcinoma [2,3]. Patients with chronic hepatitis are required to receive antiviral drugs and interferon therapy for viral hepatitis to prevent progression of the disease to cirrhosis [4]. However, there are currently challenges to achieving a cure, including problems such as the high adaptability of the virus, a narrow therapeutic index, a high cost of the drugs, and severe side effects [5]. In addition, the prevalence of hepatitis with various non-viral etiologies such as onalcoholic steatohepatitis has rapidly been increasing in Asian regions [6]. Therefore, the development of innovative therapies for the treatment of hepatitis is necessary.

The functional recovery of a damaged organ is achieved by supplementing the lost cells, which is possible by the various cells that can differentiate [7]. Somatic stem cells, which are undifferentiated cells in an organ, can enable the restoration of the organ by differentiating into the only cells constituting the organ during the repair of the damaged organ [8–10]. In a previous study, a cell,

Abbreviations: Clock, circadian locomotor output cycles kaput; Cry, cryptochrome; Bmal1, brain and muscle arnt-like 1; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; EpCAM, epithelial cell adhesion molecule; Lef1, lymphoid enhancer-binding factor-1; Tcf4, transcription factor 4.

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https://doi.org/10.1016/j.bbrc.2018.06.117
0006-291X/

which was undifferentiated, was observed in chronic hepatitis, and it clearly differentiated and multiplied to contribute to the regeneration of the liver function and reduction of inflammation severity [11]. These cells are called potential hepatic stem-like cells. The presence of potential hepatic stem-like cells has been confirmed in various other liver diseases in humans. Although these cells may be applied in regenerative medicine for the treatment of liver diseases, their characteristics remain unknown.

The circadian rhythm is controlled by a biological clock, consisting of a feedback mechanism involving a transcription factor group called clock genes [12,13]. The molecular clock machinery play an important role for the adaptation of physiological and behavioral activities to environmental cues. On the other hand, the association between the onset of various diseases, including mental conditions and the biological clock, has been attracting attention clinically and in the development of pharmaceutical products for targeted therapy [14–16]. However, chronic hepatitis is a disease in which an association with the molecular mechanisms of the biological clock has not yet been clarified. Although the changes in the expression of one of the clock genes of a somatic stem cell existing in the skin of the *Bmal1* knockout mouse were reported [17], the association between the potential hepatic stem-like cells and the molecular clock machinery in chronic hepatitis is unclear.

In this study, we analyzed the relationship between potential hepatic stem-like cells and the molecular clock machinery in chronic hepatitis.

2. Materials and methods

2.1. Animals

Clock mutant (*Clk/Clk*) mice with an ICR background and wild-type (WT) mice of the same strain were housed in a light-controlled room (12-h light/dark cycle) at 24 ± 1 °C and humidity of 60 ± 10% with food (CE-2; CLEA, Osaka, Japan) and water ad libitum. Mice were fed with CE-2 containing 0.1 mg/kg 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC; Tokyo Chemical Industry, Tokyo, Japan) for a maximum of 8 weeks to prepare mouse models of chronic hepatitis. Mice were cared for in accordance with the guidelines established by the Animal Care and Use Committee of Kyushu University.

2.2. Measurement of serum aminotransferase levels

Mouse blood sample was collected in tubes at 13:00 (0, 1, 2, 4, 6, and 8 weeks after the initiation of the DDC-containing diet) and was centrifuged at 3000 rpm for 15 min. The supernatant was transferred to another tube to obtain the blood serum. Serum alanine aminotransferase (ALT) levels were measured using a Transaminase CII Test Kit (Wako Pure Chemical Industries, Osaka, Japan).

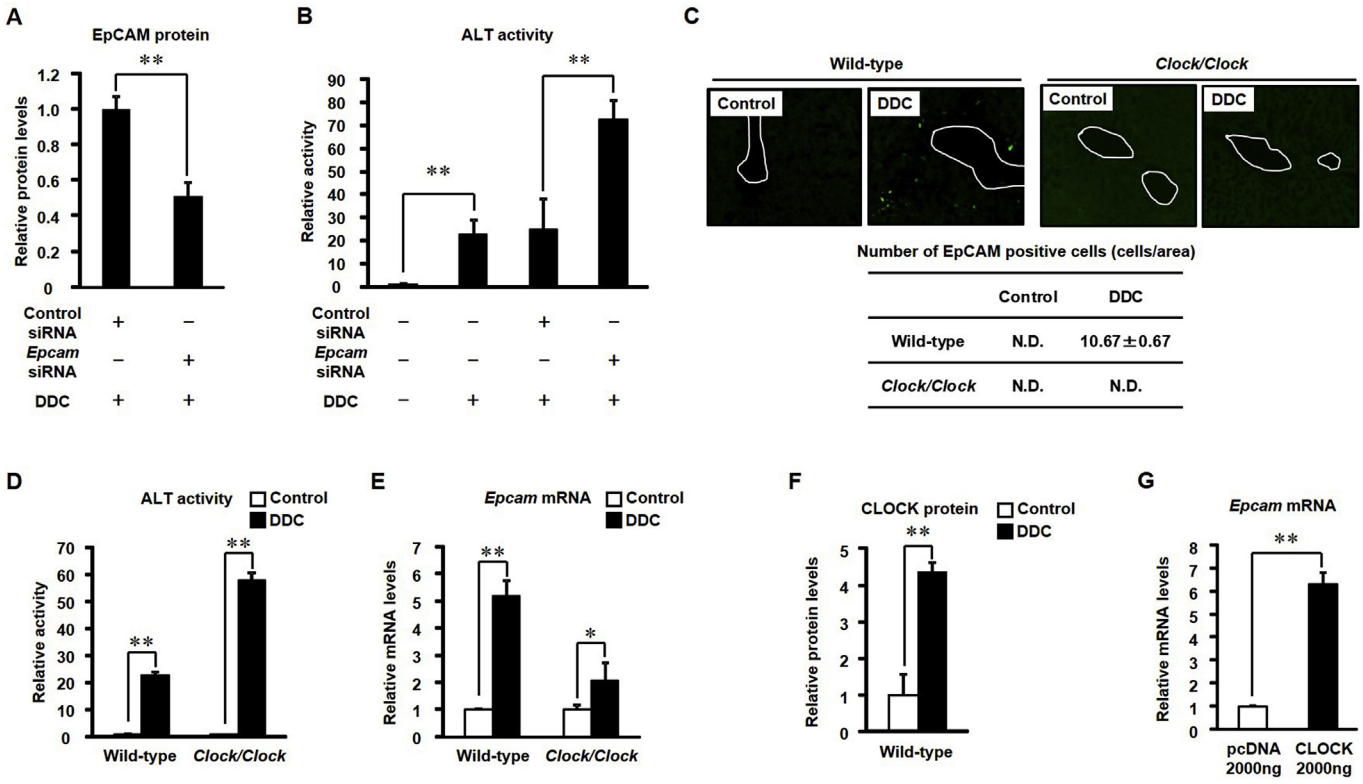

Fig. 1. Influence of the *Clock* mutation on the DDC-induced expression of EpCAM in the liver of mice. (A) EpCAM protein levels at day 7 in the liver of mice treated with *Epcam* siRNA after the initiating feeding with DDC-containing diet. Each value represents the mean ± S.E. (n = 3–5). **; P < 0.01 compared between the two groups. (B) Downregulation of EpCAM by siRNA suppresses the DDC-induced elevation of plasma ALT activity at day 7 after initiating feeding with DDC-containing diet. (C) Upper panels show histological features of hepatic cells of wild-type and *Clock* mutant (*Clock*/*Clock*) mice fed with the control (CE-2) or DDC (0.1%)-containing diet for a week. Green color indicates EpCAM positive cells. Lower panels show the quantification of the number of EpCAM positive cells in the liver of wild-type and *Clock*/*Clock* mice fed with a control or DDC-containing diet for a week. (D) The activity of ALT in the plasma of wild-type and *Clock* mutant mice fed with a DDC containing diet for a week. (E) The mRNA levels of *Epcam* in the liver of wild-type and *Clock*/*Clock* mice fed with a control or DDC-containing diet for a week. The relative mRNA level of the control diet feeding group was set at 1.0. (F) The protein levels of CLOCK in the liver of wild-type and *Clock*/*Clock* mice fed with a control or DDC-containing diet for a week. The relative protein level in mice fed with the control diet was set at 1.0. (G) The mRNA levels of *Epcam* in cultured Hepa1-6 cells transfected with CLOCK expressing vectors. At 48 h after transfection, mRNA levels of *Epcam* were assessed by qRT-PCR. For panels B–G, Each value represents the mean ± S.E. (n = 3). **P < 0.01 compared between the two groups.

Please cite this article in press as: H. Kimura, et al., Epithelial cell adhesion molecule expression in hepatic stem/progenitor cells is controlled by the molecular clock system, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.06.117