International Immunopharmacology 28 (2015) 901–905

Contents lists available at ScienceDirect

International Immunopharmacology

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

LncRNA HULC affects the differentiation of Treg in HBV-related liver cirrhosis

Jie Zhao [2], Ye Fan [2], Kunpeng Wang, Xuhao Ni, Jian Gu, Hao Lu, Yunjie Lu, Ling Lu, Xinzheng Dai *[,1], Xuehao Wang **[,1]

Liver Transplantation Center of the First Affiliated Hospital and Key Laboratory on Living Donor Liver Transplantation, Ministry of Health, Nanjing Medical University, Nanjing, Jiangsu Province, PR China

ARTICLE INFO

Article history:
Received 1 March 2015
Accepted 14 April 2015
Available online 4 May 2015

Keywords:
HBV-related cirrhosis
Tregs
Long non-coding RNAs
HULC
p18

ABSTRACT

Background and aims: Recently, a couple of the long noncoding RNAs (lncRNAs) have been proved to participate in hepatocellular carcinoma development and progression. However, their associations with liver cirrhosis have not been reported. In this study, we aimed to identify the affection of HULC on regulatory T cells (Tregs) differentiation in HBV-related liver cirrhosis.

Methods: Seven lncRNAs were chosen as candidate lncRNAs based on the association with liver disease. The candidate lncRNAs were validated by RT-qPCR. Additional flow cytometry of Tregs was performed in 34 HBV-related liver cirrhosis patients and 34 healthy volunteers. To investigate the function of HULC, HULC expression was modified by gene overexpression via lentivirus vector. RIP assay was performed further to validate the association between HULC and p18.

Results: Circulation Tregs and HULC were significantly up-regulated in plasma samples of HBV-related cirrhosis patients. In addition, overexpression of HULC by lentivirus vector elevated Treg frequency in vitro. Furthermore, RIP assay showed that HULC down-regulated the level of p18 directly.

Conclusions: We confirmed the effects of HULC on Tregs differentiation in HBV-related liver cirrhosis. In addition, it was proved that HULC regulates the function of Tregs through down-regulated the level of p18 directly.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Liver fibrosis and the end-stage cirrhosis represent the final pathways of almost all chronic liver diseases [1]. Chronic liver injury caused by hepatitis B virus (HBV) infection usually arouse inappropriate and persistent wound healing responses in liver followed by liver fibrosis, and loss of liver function [2]. During HBV infection, immune responses are significant for clearance of virus. Formerly many studies have underscored the importance of adaptive immunity in HBV infection [3]; Recent studies was focusing on the role of immunoregulatory cells. Increasing evidence from mouse and human studies have emphasized the crucial role of $CD4^+$ T cells in liver inflammation and fibrosis [4,5]. $CD4^+$ T helper cells was subdivided into four main subsets, based on their expression profile of surface markers and secreted cytokines: T helper (Th) cell type 1, Th2, Th17 and regulatory T cells (Tregs) [6].

The long noncoding RNA (lncRNA) is commonly defined as a non-protein-coding RNA molecule longer than 200 nucleotides [7,8], which is capable to regulate gene expression via modulation of chromatin remodeling, transcription, or post-transcriptional mRNA processing, participation in protein function or localization, and intercellular signaling [9]. Based on the high throughput screening, Guttman et al. found more than a thousand highly conserved lncRNAs in mammals [10].

Recently, numbers of studies have demonstrated that a couple of lncRNAs, hepatocellular carcinoma up-regulated long non-coding RNA (HULC) [11], HOX transcript antisense RNA (HOTAIR), Linc00152, maternally expressed 3 (MEG3), Linc-p21, Ula1p (ULA1) and colon cancer associated transcript 1 (CCAT1) [12] participated in hepatocellular carcinoma (HCC) development and progression. However, their associations with liver cirrhosis have not been reported. Thus, we aim to identify the effects of HULC on Tregs differentiation in HBV-related liver cirrhosis

In this study, we hypothesized that HULC may contribute to Treg differentiation via down-regulation of p18 in HBV-related liver cirrhosis.

* Correspondence to: Xinzheng Dai, The Liver Transplantation Center of the First Affiliated Hospital, Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, Jiangsu Province, PR China. Tel.: +86 25 83718836 6476; fax: +86 25 83672106.
** Correspondence to: X. Wang, The Liver Transplantation Center of the First Affiliated Hospital, Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, Jiangsu Province, PR China.
E-mail address: wangxuehao1941@hotmail.com (X. Wang).
[1] These authors contributed equally to this work.
[2] Co-first authors.

http://dx.doi.org/10.1016/j.intimp.2015.04.028
1567-5769/© 2015 Elsevier B.V. All rights reserved.

2. Materials and methods

2.1. Clinical samples

Thirty-four patients with HBV-related liver cirrhosis during February 2013 to December 2014 were recruited from the First Affiliated Hospital of Nanjing Medical University (Nanjing, Jiangsu, China). All patients had negative histories of exposure to either chemotherapy or radiotherapy before, and there was no co-occurrence of diagnosed cancers. Control plasma samples were obtained from healthy volunteers without any health problems during their health check-ups at the First Affiliated Hospital of Nanjing Medical University. The research protocol was approved by the Institutional Ethics Committee of the First Affiliated Hospital of Nanjing Medical University. Written informed consent was obtained from every participant to participate in this study. Peripheral blood samples were collected in EDTA anti-coagulated evacuated tubes.

2.2. Fluorescence activated cell sorting of CD4 + and CD4 + CD25 + Foxp3 + cells

Ficoll density gradient (Sigma Aldrich, St. Louis, MO, USA) was used in isolating peripheral blood mononuclear cells (PBMCs) from whole blood. Purification of human $CD4^+$ T cells and $CD4^+CD25^+Foxp3^+$ cells when sufficient PBMCs could be obtained from donors, they were chosen for further purification. $CD4^+$ T cells and $CD4^+CD25^+Foxp3^+$ cells were purified using a fluorescence activated cell sorting (FACS) Aria cell sorter (Becton Dickinson, Palo Alto, CA, USA) after the staining with anti-CD4-fluorescein isothiocyanate (FITC) and/or anti-CD25-phycoerythrin (PE) antibodies. The purity of isolated cells was 90%.

Cells were cultured in RPMI 1640 medium (Gibco, CA, USA) containing 10% fetal calf serum (Gibco, CA, USA). IL-2 (300 U/mL, PeproTech, USA) was added to enable survival of the lymphocytes in vitro.

2.3. Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)

Total RNA from $CD4^+$ T cells and $CD4^+CD25^+Foxp3^+$ cells was extracted by TIANGEN (TIANGEN, Beijing, China) according to the manufacturer's protocol. cDNAs from all sample were synthesized from 1 mg of total RNA by PrimeScript RT Master Mix kit (Takara, Dalian, China). The expression of HULC, MEG3, lincRNA-p21, LincRNA00152, UCA1, GAPDH and CCAT1 was analyzed by qRT-PCR using Quantifast SYBR Green PCR Kit (Qiagen, Duesseldorf, Germany) at 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. Primer sequences were listed in Table 1. Expression levels were calculated relative to GAPDH. Data analyses were performed using the $2^{-\triangle\triangle Ct}$ method.

2.4. Protein extraction and Western blotting

Total protein was extracted using cell lysis buffer for Western and RNA Immunoprecipitation in accordance with the manufacturer's instruction. Typically, 20 mg of the protein was loaded per lane. Protein samples were resolved using SDS-PAGE and transferred onto a polyvinylidene fluoride membrane (Roche, CA, USA). The membranes were blocked with 5% non-fat dry milk for 1 h at room temperature and incubated at 4 °C overnight with anti-p18 antibody (Abcam, London, UK). Sequentially, the secondary antibodies were conjugated to horseradish peroxidase, and the proteins were visualized via chemiluminescence (Beyotime, Shanghai, China). GAPDH (Abcam, London, UK) was used to normalize the quantity of the protein.

Table 1
Primer sequences used in qRT-PCR.

Target gene	Forward primer	Reverse primer
UCA1	CTCTCCATTGGGTTCACCATTC	GCGGCAGGTCTTAAGAGATGAG
CCAT1	CATTGGGAAAGGTGCCGAGA	ACGCTTAGCCATACAGAGCC
MEG3	GCCAAGCTTCTTGAAA	GGCCTTCCACGGAGTAGAGCGAGTC
Linc00152	CTCCAGCACCTCTACCTGTTG	GGACAAGGGATTAAGACACACA
HULC	ATCTGCAAGCCAGGAAGAGTC	CTTGCTTGATGCTTTGGTCTGT
HOTAIR	GCAGTGGGGAACTCTGACTC	TTGAGAGCACCTCCGGGATA
LincRNA-p21	GGGTGGCTCACTCTTCTGGC	TGGCCTTGCCCGGGCTTGTC
GAPDH	AATGGGCAGCCGTTAGGAAA	GCGCCCAATACGACCAAATC

2.5. Lentivirus production and transduction

To further investigate the function of HULC, HULC expression was modified by gene overexpression via lentivirus vector. We modified the commercial LV-HULC vector lentiviral constructs (Genscript, Nanjing, China) to overexpress HULC in T cells. All lentiviral vectors expressed GFP and the efficiency of infection was measured under a fluorescent microscope based on GFP expression.

2.6. Ribonucleoprotein immunoprecipitation (RIP)

RIP experiments were performed using a Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, MA, USA), according to the manufacturer's instructions. Antibody for RIP assays of p-18 (CST, MA, USA) was used. Coprecipitated RNAs were detected by RT-PCR. Gene-specific primers used for detecting HULC are presented above.

2.7. Statistical methods

The results of qRT-PCR and other variables were expressed as the mean (S.E.M.). Student's *t*-test and Mann–Whitney unpaired test analysis of variance were used to evaluate statistical differences in patients and controls. Statistical analysis was performed using STATA 9.2 and presented with the GraphPad prism software. In all cases, $P < 0.05$ was considered significant.

3. Results

3.1. $CD4^+CD25^+FoxP3^+$ Treg in blood of HBV-related cirrhosis patients was more frequent than in healthy controls

We characterized $CD4^+CD25^+FoxP3^+$ T cells and $CD4^+$ T cells respectively as Treg and $CD4^+$ T cells, and determined the ratio of Tregs in total $CD4^+$ T cells as the frequencies. $CD4^+CD25^+FoxP3^+$ Tregs are abundantly present in blood of HBV-related cirrhosis patients but nearly absent in healthy volunteers (Fig. 1A).

3.2. Expression of HULC and HOTAIR was up-regulated and the expression of MEG3 was down-regulated in Tregs of HBV-related cirrhosis patients

To confirm the involvement of HULC, MEG3, lincRNA-p21, LincRNA00152, UCA1, HOTAIR and CCAT1, we examined the expression levels of RNAs in peripheral Tregs from 34 patients and 34 healthy volunteer by qRT-PCR. We found that the expression levels of HULC and HOTAIR were remarkably increased and the expression level of MEG3 was decreased in HBV-related cirrhosis (Fig. 1B).

3.3. Upregulated HULC promoted Tregs differentiation in vitro

To explore the effect of HULC on Tregs, we initially determined to increase the level of HULC in T cells. Subsequently, T cells from the healthy donors were treated with LV-HULC or LV-NC. Fig. 2A showed the efficacy of lentivirus production and transduction. As shown in Fig. 2B, HULC overexpression resulted in an increase in the ratio of Tregs/T cells. These data suggest that up-regulated HULC contributes to Tregs differentiation.

Download English Version:

https://daneshyari.com/en/article/2540381

Download Persian Version:

https://daneshyari.com/article/2540381

Daneshyari.com