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Hepatocyte growth factor upregulates interferon signaling in human hepatocytes: Possible implications for interferon therapy after liver transplantation

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Abstract: Background/Aim: Although a recurrent hepatitis C virus (HCV) infection is the leading cause of graft loss in liver transplant recipients, the optimal timing to begin interferon (IFN) therapy after LTx is still unknown. The purpose of this study is to analyze the relationships, between signaling by PEGylated IFN in human hepatocytes, with regard to hepatocyte proliferation, and immunosuppressive drugs in vitro. Methods: Experiment 1 – Normal human hepatocytes (NhHeps) were cultured with/without recombinant human hepatocyte growth factor (r-hHGF) for 48 h, and then treated with 100 IU/mL IFN at the indicated time. The expressions of double-stranded RNA-dependent protein kinase (PKR) and IFN-α-induced antiviral protein were analyzed using Western blotting for the extracted lysates from these cells. Experiment 2 – The NhHeps were cultured in 10% medium containing varying concentrations of tacrolimus (Tac), cyclosporine A (CyA), and methylprednisolone (PLS), and the cells were treated with 100 IU/mL IFN at the indicated time. Subsequently, the density of PKR was examined. Results: The expression of PKR was enhanced by HGF. PKR induction by IFN was suppressed by Tac > CyA > PLS. Conclusion: Hepatocyte proliferation induced by HGF did not interfere with the signaling by IFN. The presence of immunosuppressive drugs was therefore found to negatively affect IFN signaling.

Keywords: HCV infection, antiviral therapy, immunosuppressive drugs, liver regeneration

Introduction

Chronic hepatitis C virus (HCV) infection is a major public health problem, infecting 3.3% of the world’s population, and now HCV is the leading indication for liver transplantation (LTx) worldwide [1]. If HCV infection is not eradicated before LTx, reinfection occurs in 100% of patients, and recurrent disease affects the long-term graft survival. Recurrent infection leading to cirrhosis occurs in 10–25% of transplant recipients within 5–10 years of LTx, and once cirrhosis occurs, the 1-year actual risk of hepatic decompensation is ~40% [2].

Therefore, it seems reasonable to treat HCV reinfec-
tion after LTx, particularly since the introduction of interferon (IFN) and ribavirin (RBV) has resulted in high rates of sustained virological response (SVR) in the nontransplanted population. The SVR rates of PEGylated IFN monotherapy are as low as 0–17%. The addition of RBV appears to increase the SVR rate to 50–80% [3, 4]. Furthermore, the patients with SVR after LTx show no progression of liver fibrosis [5]. These reports suggest that the combination therapy of PEGylated IFN and RBV may contribute to improve the outcome in HCV-related transplantation. The optimal time to begin therapy after LTx is still unknown. Some authors start when the chronic lesion is already established, while others start in a pre-emptive fashion. Therefore, this study attempts to evaluate the influences of liver regenerative stimulating on IFN signaling in human hepatocytes. After binding to their receptors, IFN stimulates the intracellular IFN-signaling cascade including the Janus kinase-signal transducers and activators of transcription (Jak-STAT)-1 tyrosine kinases, the phosphoelylation of STAT-1 and -2, and the formation of IFN-stimulated gene factor 3 (ISGF-3), which consists of STAT-1, STAT-2 and p48 [6].

The aim of this study is to analyze the relationships between signaling by IFN, liver regeneration, and immunosuppressive drugs in human hepatocytes in vitro.
Materials and Methods

Reagents and cell culture

NhHeps (Lonza, Switzerland), which were isolated from single donors. Each donor was tested and found to be nonreactive by a Food and Drug Administration-approved method to detect the presence of Human Immunodeficiency Virus-I, Hepatitis B virus and HCV. NhHeps were cultured in Roswell Park Memorial Institute (RPMI) (Invitrogen, Grand Island, NY), supplemented with 10% fetal bovine serum and r-hHGF (Acros Antibodies GmbH, Germany) for 48 h. The cells were cultured in 10% RPMI containing varying concentrations of Tac, CyA and PLS for 16 h, and then the medium was exchanged and the cells were treated with 100 IU/mL IFN in 24 h, to determine the effect of calcineurin inhibitors.

Recombinant human IFN-α2b, Tac and CyA were provided by Schering Plough KK (Tokyo, Japan), Astellas Co. (Tokyo, Japan) and Novartis Pharma Co. (Basel, Switzerland), respectively.

Bromodeoxyuridine cell proliferation assay

A bromodeoxyuridine (BrdU) (Exalpha Biologicals, MA) cell proliferation assay was performed to determine whether the stimulation by HGF affects NhHeps or not. BrdU is incorporated into newly synthesized DNA strands of actively proliferating cells. Various concentrations of the NhHeps and HGF were plated and cultured with 10% RPMI and BrdU label for 48 h. Subsequently, the amount of BrdU incorporation in the proliferating cells was measured by using a spectrophotometric microtiter plate reader set at a dual wavelength of 450/550 nm.

Western blotting

Western blotting with anti-PKR (Santa Cruz Biotechnology, Santa Cruz, CA) was performed as described previously [7]. Cells were briefly incubated with 10% RPMI containing 5 µg/mL HGF for 48 h. Then cells were removed by centrifugation at 14,000 rpm for 30 min at 4°C. The same amount of protein from each lysate (20 µg per well) was analyzed by electrophoresis on 8–12% sodium dodecyl sulfate polyacrylamide gel. Protein were transferred onto nitrocellulose membranes which were then blocked for 1.5 h using 5% nonfat dried milk in phosphate-buffered saline (PBS) containing 0.1% Tween 20 (PBS-T), washed with PBS-T and incubated at 4°C overnight in the presence of each primary antibody. The membranes were washed with PBS-T and incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulin G, and the immunoreactive bands were visualized by the ECL[AU1] chemiluminescence system (Amersham Life Science, Buckinghamshire, England). The density of each band was quantified using the National Institutes of Health image analysis software program.

Statistical analysis

Categorical variables were expressed as the value (%) and compared with the Mann-Whitney’s U test. Continuous data are presented as the mean ± SD or median and analyzed with a 1-way analysis of variance. Statistical significance was set as P < 0.05 for all analyses. The statistical package used was StatMate III (ATMS Co., Ltd, Tokyo).

Results

The effects of HGF in NhHeps

NhHeps in number were increased under stimulation of HGF in various concentrations (data not shown). Above all, the concentration of HGF 5 ng/mL represented the largest cells proliferations inducer. Therefore, we decided to use 5 ng/mL concentration of HGF in this study, and this HGF’s hepatocyte growth stimulation was regarded as the liver regeneration in early phase after living-donor liver transplantation.

HGF increased the expression of PKR

To evaluate if HGF influences the expression of IFN-induced antiviral proteins, NhHeps were incubated in the absence or presence of IFN-α with or without HGF for 48 h and then were harvested for the Western blotting (Fig. 1). Pretreated HGF demonstrated an enhancement of the effect on IFN-α-induced PKR in our experiments (Fig. 2). Data were representative examples of three similar experiments.

Differential effects of immunosuppressant on IFN-induced antiviral protein expression

To investigate the influence of HGF and immunosuppression, NhHeps treated by IFN-α were incubated with or without immunosuppressive drugs (Tac, CyA and PLS) for 16 h, after pretreatment with HGF for 48 h or in a combination of both, and then analyzed by Western blotting (Fig. 3). In the absence of HGF, the expression of PKR was specifically suppressed by immunosuppressive drugs, with the effect of Tac > CyA > PLS (Fig. 4). Data were representative examples of three similar experiments. In cells pretreated with HGF, PKR expression was higher than that without nontreatment. Moreover, the expression of PKR was suppressed at levels similar to those observed in the nontreated group.

Discussion

HCV infection is a major concern after LTx due to the universal recurrence, more rapid progression of fibrosis,
HGF upregulates IFN signaling in human hepatocytes

**Fig. 1.** The effects of HGF on IFN-induced PKR expression. NhHeps were incubated with or without of 5 ng/mL HGF for 48 h, and 100 IU/mL IFN for 24 h or both. Thereafter, PKR expression was determined by Western blotting.

**Fig. 2.** The relative PKR expression in NhHeps treated with HGF and/or IFN. Density index represented the ratio to the control (no treatments of HGF and IFN). Data are expressed as the means ± SD and were representative examples of three similar experiments.

**Fig. 3.** Effects of calcineurin inhibitors, methylprednisolone and IFN on the IFN-induced expression of PKR. NhHeps were incubated with or without 5 ng/mL HGF for 48 h, and the effect of immunosuppressive drugs was investigated with each concentration or both. The expression of PKR was determined by Western blotting.

**Fig. 4.** The relative PKR expression in NhHeps treated with HGF and/or IFN and immunosuppressive drugs. The density index represents the ratio to the control (no IFN or immunosuppression). The data are expressed as the means ± SD and are representative examples of three similar experiments.
new option for this therapy to improve the rates of SVR, and to reduce the rates of treatment discontinuation.

HGF stimulated IFN-induced PKR expression in a cell culture system, and that mechanism does not appear to act via the standard IFN pathways. HGF interacts with the mitogen-activated protein kinase (MAPK)-signaling cascade and phosphoinositide 3-kinase (PI3K)-Akt pathway [17]. This study, did not investigate the interaction between IFN and MAPK-signaling and the PI3K-Akt pathway. This study demonstrated that liver regeneration induced by HGF, did not interfere with the signaling by IFN.

In conclusion, these experiments demonstrated that HGF serves as potent modulator of IFN-α-induced antiviral protein expression in NHHeps, although many questions remain unanswered. Understanding the interactive effects of HGF on IFN-signaling pathways may lead to the development of more effective therapeutic approaches to the control of viral clearance and liver inflammation in patients with HCV. Hepatocyte proliferation induced by HGF did not interfere with IFN signaling. However, the presence of immunosuppressive drugs negatively affects the IFN signaling.

References