A comparison of the antitumor effects of interferon-α and β on human hepatocellular carcinoma cell lines

Masayuki Murata a, Shigeki Nabeshima b, Kensuke Kikuchi a, Kouzaburo Yamaji b, Norihiro Furusyo a, Jun Hayashi a,b,*

* Department of Environmental Medicine and Infectious Disease, Internal Medicine, Faculty of Medical Science, Kyushu University, Fukuoka, Japan
b Department of General Medicine, Kyushu University Hospital, Fukuoka, Japan

Received 28 November 2004; received in revised form 20 April 2005; accepted 17 August 2005

Abstract

The antiviral, antiproliferative and immunomodulatory effects of type I interferons (IFNs) are well documented, however, few studies have been published concerning differences in the antitumor effects of IFN-α and β. In the present study, differences in antitumor effect, including the antiproliferative effect, cell cycle change, apoptosis, and the IFN-stimulated gene (ISG) were examined by flow cytometry between IFN-α and β on three human hepatocellular carcinoma (HCC) cell lines (HepG2, Huh7 and JHH4). The antiproliferative effect of both IFNs on the HCC cell lines was time- and dose-dependent, and IFN-β was significantly stronger than IFN-α. The cell cycle effect by both IFNs was an S-phase accumulation, with IFN-β having a tendency to increase the S-phase ratio more strongly than IFN-α, especially in Huh7. Apoptosis marker expression, Fas antigen and intracellular active caspase-3, was increased after the addition of IFNs, especially of IFN-β. The expression of human leukocyte antigen-class I molecules, ISG-encoded protein, was increased after the addition of IFNs, especially of IFN-β. These data suggest that IFN-β has a greater antitumor effect than IFN-α on HCC of a very early stage in patients with chronic hepatitis C.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: Antitumor effects; Hepatocellular carcinoma; Interferon-α; Interferon-β

1. Introduction

Hepatocellular carcinoma (HCC) is a common cancer worldwide. The hepatitis C (HCV), and hepatitis B viruses (HBV) have been directly linked to the development of HCC, especially in patients who have chronic active hepatitis with cirrhosis [1,2]. In Japan, the HCC incidence has increased, resulting in it becoming the third leading cause of death due to cancer.

Interferons (IFNs) are a family of cytokines that elicit a pleiotropic biological effect. IFNs have antiviral, antiproliferative and immunomodulatory effects, and are classified as type I (IFN-α, β and ω) and type II (IFN-γ) [3,4]. IFNs mediate their effects by binding to cell surface receptors (IFN receptors) and activating Janus kinases (JAK), resulting in the phosphorylation of the signal transducers and activators of transcription (STAT). STAT proteins homo- or heterodimerize and form complexes with other transcription factors to activate transcription of IFN-stimulated genes (ISGs) [3]. IFN actions are largely mediated by the proteins encoded by ISGs [5–7]. A number of IFN-related proteins, such as dsRNA-dependent protein kinase (PKR), the 2-5A system, human leukocyte antigen (HLA)-class I molecules and Mx proteins, mediate the antiviral actions of IFNs [3], and IFN-α and β are effective for the treatment of chronic hepatitis C [8,9].

In oncology, IFN-α and β are used for the treatment of a number of solid tumors and hematological malignancies,
such as malignant melanoma, renal cell carcinoma, and chronic myelogenous leukemia [4]. Recent reports showed that IFN-\(\alpha\) treatment reduced the risk for HCC in patients with chronic hepatitis C [10,11]. We previously reported that IFN-\(\beta\) treatment also reduced the risk of HCC in such patients [12]. Moreover, this study revealed that a reduction in HCC occurrence was independent of virological or biochemical responses of IFN-\(\beta\) [12]. Although the antitumor effect of IFN-\(\alpha\) on HCC cell lines has been reported in vitro [13,14], few studies have been published concerning differences in the antitumor effects of IFN-\(\alpha\) and \(\beta\) on HCC cell lines [15,16], as was done in this study.

2. Materials and methods

2.1. Cell lines and reagents

The three human HCC cell lines, HepG2, Huh7 and JHH4, were purchased from the Japanese Cancer Research Resources Bank (Tokyo, Japan). HepG2 was established by Aden et al. [17] from a liver tumor biopsy obtained from a 15-yr-old Caucasian male. The morphological characteristics and epithelial cell shape were compatible with that of liver parenchymal cells. Histology of the liver biopsy revealed well differentiated hepatocellular carcinoma with a trabecular pattern. Huh7 was established by Homma [19] from a hepatoma tissue of a 57-yr-old Japanese male with well differentiated hepatocellular carcinoma. JHH4 was established by Nakabayashi et al. [18] from a hepatoma tissue of a 15-yr-old Caucasian male. The morphological characteristics and epithelial cell shape were compatible with that of liver parenchymal cells. Histology of the liver biopsy revealed well differentiated hepatocellular carcinoma with a trabecular pattern. Huh7 was established by Nakabayashi et al. [18] from a hepatoma tissue of a 57-yr-old Japanese male with well differentiated hepatocellular carcinoma. JHH4 was established by Homma [19] from a hepatoma tissue of a 57-yr-old Japanese male with well differentiated hepatocellular carcinoma. These cell lines were maintained in Dulbecco’s modified Eagle medium (DMEM) supplemented with 25 mM HEPES, 50 units (U)/mL penicillin, 50 \(\mu\)g/mL streptomycin, and 10% heat-inactivated fetal calf serum (FCS) at 37 °C in a humidified incubator with 5% CO\(_2\) in air.

Human natural lymphoblast IFN-\(\alpha\) (Sumiferon), with a specific activity of 2.25 \(\times\) 10^6 IU/mg, was kindly supplied by Sumitomo Pharmaceutical Co., Ltd. (Osaka, Japan). Human natural fibroblast IFN-\(\beta\) (FERON), with a specific activity of 3.08 \(\times\) 10^6 IU/mg, was kindly provided by Daiichi Pharmaceuticals Co., Ltd. (Tokyo, Japan). Since these natural IFN-\(\alpha\) and \(\beta\) were of high purity, they did not contain other cytokines, that might have modulating effects such as tumor necrosis factor.

Fluorescein isothiocyanate (FITC)-conjugated mouse anti-human Fas antigen monoclonal antibodies (mAbs) and HLA-class I molecule mAbs were purchased from Beckman Coulter (Miami, FL). Phycoerythrin (PE)-conjugated polyclonal rabbit anti-active caspase-3 antibodies were purchased from BD Biosciences (San Jose, CA). DMEN, FCS, trypsin/EDTA, and penicillin/streptomycin were purchased from Gibco BRL (Life Technologies, Inc., Gaithersburg, MD).

2.2. Antiproliferative effect of IFN-\(\alpha\) and \(\beta\)

The antiproliferative effect of the IFNs was analyzed for three HCC cell lines, HepG2, Huh7 and JHH4. Cells (1 \(\times\) 10^5/well) were added in triplicate to a 6-well culture plate (Becton Dickinson). The medium was replaced 24 h later by 1.5 mL of fresh medium containing IFN-\(\alpha\) and \(\beta\). Concentrations of IFN-\(\alpha\) and \(\beta\) were 1 \(\times\) 10^4 IU/mL and cell lines cultured in medium alone were used as a control. Proliferation of HCC cell lines was determined over a period of 96 h after IFN addition. After the culture, the adhering cells were washed with PBS and detached using 0.25% trypsin/EDTA. The resulting single-cell suspension was washed in washing buffer (PBS containing bovine serum albumin and sodium azide), and the number of viable cells was counted by flow cytometer, CYTORON ABSOLUTE with ImmunoCount 2 software (Ortho Diagnostic Systems). In some experiments, the concentrations of IFN-\(\alpha\) and \(\beta\) ranged from 10^2 to 10^4 IU/mL. Cell viability was determined using the trypan blue dye exclusion method and exceeded 95% in all experiments. All assays were analyzed in at least three independent experiments.

2.3. Cell cycle

The effect of the IFNs on the cell cycle phase distribution of the HCC cell lines was analyzed by flow cytometry using the CycleTEST™ PLUS DNA reagent kit (Becton Dickinson Immunocytometry Systems, San Jose, CA) according to the manufacturer’s instructions. Briefly, cells (1 \(\times\) 10^7/well) were added in triplicate to a 6-well culture plate, and the medium was replaced 24 h later by 1.5 mL of fresh medium containing 10^3 IU/mL IFN-\(\alpha\) or 10^3 IU/mL IFN-\(\beta\). Cell lines cultured in medium alone were used as a control. The cultured cells were detached 24 h later using 0.25% trypsin/EDTA after washing with PBS. Cells were washed twice with PBS, 250 \(\mu\)L of Solution A (trypsin buffer) was added and the cells were incubated for 10 min at room temperature, followed by the addition of 200 \(\mu\)L of Solution B (trypsin inhibitor and RNase buffer) and incubation for a further period of 10 min at room temperature. Finally, 200 \(\mu\)L of cold Solution C (propidium iodide stain solution) was added and the cells were incubated on ice for 10 min in the dark. The samples were filtered through a 44-µm nylon mesh, and analyzed by flow cytometer, EPICS XL with EXPO32 software (Beckman Coulter).

2.4. Apoptosis-related markers

The expression of surface Fas antigen on the HCC cell lines was analyzed by flow cytometry. Cells (1 \(\times\) 10^5/well) were cultured with medium alone as a control, 10^3 IU/mL IFN-\(\alpha\) or 10^3 IU/mL IFN-\(\beta\). Twenty-four hours after the addition of IFNs, the cells were washed with PBS and detached using 0.25% trypsin/EDTA. Washed cells were incubated at 4 °C for 30 min in 10 \(\mu\)L of FITC-conjugated mouse anti-human Fas antigen mAbs. The samples were then washed with the washing buffer and analyzed by flow cytometer, EPICS XL with EXPO32 software.

Active caspase-3, a marker for cells undergoing apoptosis, consists of a heterodimer of 17 and 12 kDa subunits which are derived from the 32 kDa proenzyme. Caspase-3 is a key
protease that is activated during the early stages of apoptosis [20]. In this study, intracellular active caspase-3 in the HCC cell lines was analyzed. Cells (1 × 10^5/well) were cultured with medium alone as a control, 10^5 IU/mL IFN-α, or 10^5 IU/mL IFN-β. The cells were washed with PBS 24 h after the addition of IFNs and detached using 0.25% trypsin/EDTA. Washed cells were fixed and permeabлизated using Cytofix/Cytoperm kit (PharMingen) according to the manufacturer’s instructions, and were incubated in the dark at 4 °C for 30 min in 10 µL of PE-conjugated anti-active caspase-3 antibodies. The samples were then washed with a washing buffer and analyzed by flow cytometer, EPICS XL with EXPO32 software.

2.5. Expression of HLA-class I molecules on HCC cell lines

Cells (1 × 10^5/well) were cultured with medium alone as a control, 10^5 IU/mL IFN-α, or 10^5 IU/mL IFN-β. The cells were washed with PBS 24 h after the addition of IFNs and detached using 0.25% trypsin/EDTA. Washed cells were incubated in the dark at 4 °C for 30 min in 10 µL of FITC-conjugated mouse anti-human HLA-class I molecule mAbs. The samples were then washed with the washing buffer and analyzed by flow cytometer, CYTORON ABSOLUTE with ImmunoCount 2 software.

2.6. Statistical analyses

Statistical analysis was by the Stat View J-5.0 program (SAS Institute Inc., Cary, NC). Statistical differences between the control and IFN treatment groups were calculated by unpaired student’s t-test and considered significant at P < 0.05.

3. Results

3.1. Antiproliferative effect of IFN-α and β

As shown in Fig. 1, panels (a)–(c), the IFNs showed a significant time-dependent antiproliferative effect on HepG2 (control, IFN-α, IFN-β; 9.8 × 10^5 ± 0.5 × 10^5 cells, 8.4 × 10^5 ± 0.3 × 10^5 cells, 4.4 × 10^5 ± 0.2 × 10^5 cells, respectively), Huh7 (control, IFN-α, IFN-β; 8.8 × 10^5 ± 0.4 × 10^5 cells, 6.6 × 10^5 ± 0.7 × 10^5 cells, 3.3 × 10^5 ± 0.3 × 10^5 cells, respectively) and JHH4 (control, IFN-α, IFN-β; 17 × 10^5 ± 2.2 × 10^5 cells, 12 × 10^5 ± 0.4 × 10^5 cells, 9 × 10^5 ± 0.6 × 10^5 cells, respectively) compared with the control after the addition of the IFNs (P < 0.05). Furthermore, IFN-β was significantly stronger than IFN-α in time-dependent antiproliferative effect, with the first significant effect observed at 48 h in both HepG2 (IFN-α; 3.9 × 10^5 ± 0.3 × 10^5 cells, IFN-β; 3.0 × 10^5 ± 0.3 × 10^5 cells, P < 0.05) and Huh7 (IFN-α; 3.1 × 10^5 ± 0.1 × 10^5 cells, IFN-β; 2.2 × 10^5 ± 0.2 × 10^5 cells, P < 0.05).
and at 72 h in JHH4 (IFN-α: 9.6 x 10^5 ± 0.3 x 10^5 cells, IFN-β: 7.9 x 10^5 ± 0.1 x 10^5 cells, P < 0.05).

As shown in Fig. 1, panels (d)-(f), IFNs showed a dose-dependent antiproliferative effect on HepG2 (control, 10^2 U/mL IFN-α and β, 10^3 U/mL IFN-α and β, 10^4 U/mL IFN-α and β); 100%, 83 ± 6.5% and 71 ± 6.0% of control, 70 ± 6.5% and 50 ± 6.9% of control, 54 ± 4.7% and 48 ± 2.1% of control, respectively), Huh7 (control, 10^3 U/mL IFN-α and β, 10^3 U/mL IFN-α and β, 10^4 U/mL IFN-α and β; 100%, 79 ± 7.2% and 60 ± 2.1% of control, 74 ± 4.0% and 42 ± 3.5% of control, 61 ± 2.1% and 37 ± 3.2% of control, respectively) and JHH4 (control, 10^2 U/mL IFN-α and β, 10^3 U/mL IFN-α and β, 10^4 U/mL IFN-α and β; 100%, 91 ± 5.8% and 84 ± 1.7% of control, 79 ± 2.6% and 66 ± 1.5% of control, 65 ± 5.0% and 51 ± 5.9% of control, respectively) at 72 h after the addition of the IFNs. The antiproliferative effect of IFN-β was especially notable in Huh7, since the cell number in the culture with 10^2 IU/mL IFN-β was almost equal to 10^4 IU/mL of IFN-α.

3.2. Effect of IFN-α and β on the cell cycle distribution of HCC cell lines

We next analyzed the mechanism of the antiproliferative effect on HCC cell lines after the addition of IFNs. As shown in Fig. 2 and Table 1, at 24 h, the addition of IFNs significantly increased the S-phase ratio and slightly decreased the G2/M phase ratio compared with the controls. Furthermore, the increase of the S-phase ratio induced by IFN-β was significantly stronger than that induced by IFN-α in three HCC cell lines. These results suggest that the difference in effect on the cell cycle distribution is a mechanism contributing to the IFN-related antiproliferative effect.

3.3. Effect of IFN-α and β on the expression pattern of apoptosis-related markers of HCC cell lines

Apoptosis is thought to be related to another mechanism of IFN-related antiproliferative effect [3]. To examine the effect of IFN-α and β on apoptosis in HCC cell lines, the expression of surface Fas antigen, a protein encoded by ISGs, and intracellular active caspase-3 were analyzed by flow cytometry. As shown in Table 2, IFNs increased the mean fluorescence intensity (MFI) of Fas antigen on the three HCC lines. IFN-β significantly increased the cell surface expression of Fas antigen on HepG2 and Huh7 in comparison with IFN-α. Furthermore, both IFNs increased the MFI of intracellular active caspase-3 in the three HCC cell lines, and all had a tendency to be more strongly induced by IFN-β than IFN-α. These results suggest that apoptosis is another mechanism contributing to the antiproliferative effect of IFN-β as well as IFN-α.

3.4. Effect of IFN-α and β on the expression pattern of HLA-class I molecules of HCC cell lines

Ligation of IFNs with IFN receptors results in the upregulation of ISGs [5]. We compared the capacity of IFN-α and β to induce HLA-class I molecules, a protein also encoded by ISGs. As shown in Table 2, the expression of HLA-class I molecules on the three HCC cell lines was significantly increased by both IFNs compared with controls. The increase
### Table 1
Effect of IFN-α and β on the cell cycle distribution of HCC cell lines

<table>
<thead>
<tr>
<th></th>
<th>HepG2</th>
<th>Huh7</th>
<th>JHH-4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G₀/G₁ phase (%)</td>
<td>S phase (%)</td>
<td>G₂/M phase (%)</td>
</tr>
<tr>
<td>Control</td>
<td>65 (64 ± 1.5)</td>
<td>29 (29 ± 0.8)</td>
<td>7.3 (7.5 ± 1.1)</td>
</tr>
<tr>
<td>IFN-α</td>
<td>62 (61 ± 0.6)</td>
<td>33 asterisk (33 ± 0.9)</td>
<td>4.6 (6.1 ± 1.5)</td>
</tr>
<tr>
<td>IFN-β</td>
<td>57 asterisk (56 ± 2.9)</td>
<td>34 asterisk (38 ± 4.3)</td>
<td>6.3 (5.9 ± 1.8)</td>
</tr>
</tbody>
</table>

Variables were expressed as median (mean ± S.E.) in three independent experiments.  
* Significant difference (*P* < 0.05) compared with control.  
† Significant difference (*P* < 0.05) compared with IFN-α.

### Table 2
Effect of IFN-α and β on the expression pattern of apoptosis-related markers and HLA-class I molecules of HCC cell lines

<table>
<thead>
<tr>
<th></th>
<th>HepG2</th>
<th>Huh7</th>
<th>JHH-4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fas Caspase-3</td>
<td>HLA-class 1</td>
<td>Fas Caspase-3</td>
</tr>
<tr>
<td>Control</td>
<td>220 (216 ± 5.10)</td>
<td>170 (176 ± 4.00)</td>
<td>140 (137 ± 4.87)</td>
</tr>
<tr>
<td>IFN-α</td>
<td>283 asterisk (283 ± 19.1)</td>
<td>210 asterisk (233 ± 24.6)</td>
<td>280 asterisk (255 ± 36.9)</td>
</tr>
<tr>
<td>IFN-β</td>
<td>374 asterisk (367 ± 27.3)</td>
<td>280 asterisk (295 ± 24.6)</td>
<td>500 asterisk (445 ± 73.1)</td>
</tr>
</tbody>
</table>

Variables indicated mean fluorescence intensity, and were expressed as median (mean ± S.E.) in three independent experiments.  
* Significant difference (*P* < 0.05) compared with control.  
† Significant difference (*P* < 0.05) compared with IFN-α.
of the MFI of three HCC cell lines tended to be more strongly induced by IFN-β than IFN-α.

4. Discussion

There is accumulating evidence that IFN-β has a preferential antiproliferative effect on glioma, melanoma, and breast carcinoma cells, and that this effect is stronger than that by IFN-α [21−24]. The present study showed that IFN-β also had a superior antiproliferative effect on HCC cell lines than IFN-α. Type I IFNs exert their effects through the type I IFN receptor, which is composed of two major subunits, IFN-α receptor 1 (IFNAR-1) and 2c (IFNAR-2c) [3], which are potentially expressed in HCC cells [13]. IFN-α and β probably utilize a common receptor complex. Natural IFN-α (Sumiferon) was composed of approximately 20 subtypes, in which that contained α 2 subtype of 25%. IFN-α 2 is the subtype that is used as an antitumor and antiviral agent in the clinical setting, including chronic myelogenous leukemia, hairy cell leukemia, renal cell carcinoma and hepatitis C virus (HCV). Natural IFN-β (FERON) was composed of only one subtype, and has found clinical applications in several malignancies and viral diseases such as glioblastoma, melanoma, medulloblastoma and HCV. It was possible that the difference of component between natural IFN-α and β affected the antitumor effects on HCC cell lines in the present study. Previous reports showed that IFN-β had a greater antitumor effects on several cancer cell lines, such as melanoma cell, squamous cervical carcinoma cell, breast cancer cell compared with recombinant IFN-α 2 [21−25]. Because the α 2 subtype is the major subtype of which natural IFN-α is composed, the difference in the antitumor effects between both IFNs used may be involved with that of biological activity, rather than component, in the present study. Although it is still unknown why IFN-α and β have different biological effects, it is possible that IFN-α and β have different signaling events at the receptor level. IFN-β, but not IFN-α, formed a strong complex with IFNAR-1 and IFNAR-2c [26] and, alternatively, an IFN-β specific signaling domain within the cytoplasmic regions of the IFNAR chain was found in IFNAR-2c [27]. These reports suggest that the specific assembly of type I IFNAR leads to the differing biological responses to IFN-α and β. The present study showed that ISGs were more induced strongly by IFN-β than IFN-α since Fas antigen and HLA-class I molecules, proteins also encoded by ISGs, were more effectively upregulated by IFN-β. These results suggest that there are some differences in receptor interaction between IFN-α and β in HCC.

The present study showed that the antiproliferative effect of both IFNs on the HCC cell lines was time- and dose-dependent, and that IFN-β was significantly stronger than IFN-α. IFN-β showed a significantly stronger antiproliferative effect on Huh7 at any concentration examined than IFN-α, after 72 h of incubation, as shown in Fig. 1, panel (c). On other two cell lines, the antiproliferative effect at low (10^2 units/mL) as well as high (10^5 units/mL) concentrations had a tendency to be more strongly induced by IFN-β than IFN-α, although that was not statistically significant, as shown in Fig. 1, panels (d) and (f). It is possible that HCC cell lines differed in their sensitivity to IFNs, but we suppose that IFN-β has a stronger antiproliferative effect on HCC cell lines compared with IFN-α.

Type I IFNs are known to modify the cell cycle [3]. Although previous studies demonstrated that IFNs induced an inhibitory effect on G1−S phase transition [28,29], it was recently demonstrated that the S phase of HCC cell lines was delayed by IFN-α [13,14]. We showed a greater increase in the S phase population of HCC cell lines treated with IFN-β than with IFN-α. Qin et al. [30] has reported that IFN-β preferentially induced S phase accumulation in human transformed cells by losing or inactivating the normal G1 checkpoint conferred by the retinoblastoma protein, which acts as a cell cycle inhibitor. It is possible that IFN-β influences the normal G1 checkpoint of HCC cell lines.

Induction of apoptosis is a highly attractive mechanism of the antitumor effect of IFNs. Apoptosis plays a critical role in the elimination of cells that sustain DNA damage or undergo uncontrolled cellular proliferation [7,31], and probably occurs as an independent cell cycle arrest [32]. The mechanism of apoptosis has been shown to occur through the ligation of death receptors on the cell surface, such as Fas or tumor necrosis factor-related apoptosis inducing ligand (TRAIL). This leads to the activation of an adaptor protein, Fas associated death domain (FADD) and to the subsequent activation of caspase-8. Activated caspase-8 cleaves additional downstream caspases, including caspase-3, a major effector caspase, and elicits the morphological hallmarks of apoptosis [7,32]. While IFN-α has been shown to induce apoptosis in HCC cell lines [13,14], the present study demonstrated that IFN-β does the same. Previous studies reported that IFN-β preferentially induced apoptosis in non-HCC cell lines, which was correlated with a stronger induction of TRAIL by IFN-β [25,33,34]. The difference in the induction of apoptosis by IFN-β seen in the present study may be related to the more effective induction of ISGs with an apoptotic function, such as Fas and TRAIL.

Tatsumi et al. [35] reported that IFN-α increased the expression of HLA-class I molecules on HCC cell lines. We also showed that HLA-class I molecules were more effectively upregulated by IFN-β. The immunomodulatory effects of type I IFNs occurred by enhancing the expression of HLA-class I molecules, activating CD8+ cytotoxic T lymphocytes, natural killer cells and dendritic cells [3]. These data suggest a more effective antitumor immune response against HCC by IFN-β than by IFN-α.

It is still disputable if the prevention of HCC in patients with chronic hepatitis C treated with IFN-α and β is due to the direct antitumor effect on cancer cells. Several studies showed that the prevention of HCC would be associated with the virological or biochemical responses of IFNs [10,11]. Furthermore, our previous study [12] revealed that a reduction in the HCC development was independent of the biochemical response in natural IFN-β treated patients with chronic hepatitis C, but not in natural IFN-α treated patients, although similar rates of the HCC development were found.
in patients with chronic HCV viremia treated with either IFN-α or β. Thus, IFN-β, rather than IFN-α, may directly inhibit HCC growth at a very early stage in patients with chronic hepatitis C, as suggested in the present study, although the results obtained from this study have been done by in vitro model.

In conclusion, IFN-β had a stronger antiproliferative effect than IFN-α by inducing cell cycle change and apoptosis, and upregulated HLA-class I molecules more strongly than IFN-α in three HCC cell lines, indicating that ISGs would be more strongly induced by IFN-β than by IFN-α. These data suggest that IFN-β has a greater antitumor effect than IFN-α in the early stage of HCC in patients with chronic hepatitis C.

References

