Interferon therapy as chemoprevention of hepatocarcinogenesis in patients with chronic hepatitis C

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Hepatocellular carcinoma (HCC) is currently a very common malignancy and its incidence is increasing, both in Japan and the USA. Persistent hepatitis C virus (HCV) infection is a major risk factor for the development of HCC. A number of large-scale retrospective cohort studies have demonstrated that interferon therapy reduces the incidence of HCC not only in sustained virological responders but also in transient biochemical responders without the elimination of HCV. We also demonstrated that retreatment with interferon at certain intervals reduced the incidence of HCC in patients with chronic hepatitis C, even if eradication of HCV was not achieved by retreatment. We cannot, however, explain how a transient normalization of serum alanine aminotransferase levels induced by a maximum 6 months of interferon treatment reduces the incidence of HCC during the progression of chronic hepatitis to cirrhosis or HCC, which requires dozens of years. In this article, we discuss how interferon treatment might reduce the incidence of HCC even in transient biochemical responders, especially in view of antiproliferative or antioxidative activity of interferon-α.

Keywords: cell cycle, hepatocellular carcinoma, MEK/ERK pathway, oxidative stress

Introduction

Hepatocellular carcinoma (HCC) is currently a very common malignancy and its incidence is increasing, both in Japan and the USA. Persistent hepatitis C virus (HCV) infection is a major risk factor for the development of HCC. Approximately 80% of Japanese HCC patients are also diagnosed with HCV-associated cirrhosis or chronic hepatitis C. It has also been shown that the risk of HCC increases with the degree of liver fibrosis. Thus, HCV patients are a high-risk group for the development of HCC, and inhibition of hepatocarcinogenesis remains a crucial issue in treating patients with HCV-related chronic liver disease.

A number of large-scale, retrospective, cohort studies conducted in Japan have demonstrated that interferon therapy reduces the incidence of HCC, not only in sustained virological responders but also in transient biochemical responders, without eliminating HCV (Table 1). On the other hand, the incidence of HCC has been shown to increase 5 years or more after interferon therapy in transient biochemical responders, suggesting that, in this population, interferon’s effects are time sensitive. In this respect, we demonstrated that re-treatment with interferon at certain intervals reduced the incidence of HCC in patients with chronic hepatitis C, even if eradication of HCV was not achieved by re-treatment. It seems plausible that eradicating HCV would result in a reduced incidence of HCC. We cannot, however, explain how a transient normalization of serum alanine aminotransferase (ALT) levels, induced by a maximum 6 months of interferon treatment, reduces the incidence of HCC during the progression of chronic hepatitis to cirrhosis or HCC, which requires dozens of years. We discuss herein how interferon treatment might reduce the incidence of HCC even in transient biochemical responders.

Hypercarcinogenic condition in HCV-associated chronic hepatitis or liver cirrhosis

We need to find out the molecular mechanism of hepatocarcinogenesis in HCV infection, which remains unclear, to understand how interferon therapy reduces the incidence of HCC in transient biochemical responders. In persistent HCV infection, hepatocarcinogenesis is closely related to the presence of chronic hepatitis with advanced liver fibrosis or liver cirrhosis, which represents a precancerous state accompanied by increased DNA synthesis. In fact, it has been shown in a prospective manner that cirrhotic patients with high liver cell proliferative activity, estimated by proliferating cell nuclear antigen staining, are more likely to develop HCC as compared with those without it. It has been suggested that HCV core protein enhances cell proliferation via activation of mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK). Activation of MAPK/ERK has also been reported in human HCC tissues. The mitogen-activated protein kinase kinase (MEK)/ERK signalling pathway is fundamental in controlling cell development, proliferation and the cell cycle. There is clear evidence that the MEK/ERK pathway is essential for the activation of the molecular events regulating cell cycle progression, such as degradation of
mitotic inhibitors (p21WAF1 and p27KIP1) and the induction of the cyclin–
cyclin dependent kinase (Cdk) complexes.14,15

A recent study has revealed that protein levels and kinase activities of cyclin D1, Cdk4, cyclin E, cyclin A and Wee1 are significantly elevated in HCV-associated HCC compared with surrounding cirrhotic tissues.16 More importantly, these kinases are already activated in cirrhosis before the development of HCC, compared with normal liver tissues, suggesting that this activation is an early event in hepatocarcinogenesis and that HCV-associated cirrhosis is a pre-cancerous condition. Thus, in a chronic hepatitis state, the cell cycle progresses out of control, and hepatocytes divide rapidly. As a result, irregular regeneration is bound to happen, accelerating genomic instability. Of course, the MEK/ERK pathway is not the only pathway potentially leading to the development of HCC. For, instance, there is considerable interest in the wnt/β-catenin pathway, specifically in the context of HCV-associated HCC.17 The complexity of all the biochemical pathways implicated in HCC development is well described in a broad review on the genetics of HCC.18

Another scenario for hepatocarcinogenesis in HCV infection is the involvement of oxidative stress, which can produce genetic mutations as well as gross chromosomal alterations. HCV core protein has been shown to produce reactive oxygen species (ROS) derived from mitochondria in inducible cell culture systems.19 A positive feedback effect of ROS on mitochondrial ROS generation further sensitizes cells to other oxidative insults, which may finally cause both mitochondrial and chromosomal DNA damage. In a transgenic mouse model for HCV-associated hepatocarcinogenesis, it is also demonstrated that HCV core protein causes a state of oxidative stress in the absence of inflammation.20

Although these results suggest the direct induction of oxidative stress by HCV proteins, the consequences of impaired mitochondrial function and abnormal ROS generation would be exacerbated by the immune-mediated inflammatory process present in patients with chronic hepatitis C, and the additional oxidant load it would present to the HCV-infected liver. Continuous ROS generation is likely to cause 8-hydroxy-2′-deoxyguanosine (8-OHdG) to accumulate in DNA. Kato et al.21 reported that lowering levels of 8-OHdG by phlebotomy potentially decreased the risk of hepatocarcinogenesis in patients with chronic hepatitis C. According to them, hepatic 8-OHdG levels decreased significantly in the short-term (initial iron reduction phase) and were almost completely normalized by the end of therapy (6 years later) by keeping a state of mild iron deficiency, defined by either <10 µg/L serum ferritin and/or 11 g/dL blood haemoglobin concentration. Thus, oxidative stress appears to be responsible, in part, for the development of HCV-associated HCC.

Clinical evidence suggesting the anti-
hepatocarcinogenic effect of interferon in patients
with HCV-associated chronic liver disease

As mentioned above, chronic hepatitis C with advanced liver fibrosis or liver cirrhosis is hypercarcinogenic at the molecular level. This is supported clinically in Japan by the high annual incidences of HCC in non-treated chronic hepatitis C patients with F3 staging22 of liver fibrosis (2%–3%) and those with liver cirrhosis (6%–7%). The rate of recurrence of HCC after complete surgical resection is much higher than these figures, suggesting that the post-operative state is more hypercarcinogenic.23

A recent randomized study has shown that interferon-β prevents the recurrence of HCC after complete resection or ablation of the primary tumour in patients with HCV-associated cirrhosis.24 This inhibitory effect on HCC recurrence by interferon was not associated with biochemical and virological improvement. Therefore, these results clearly suggest that interferon acts as an anti-hepatocarcinogenic agent in patients with HCV-associated chronic liver diseases.

Molecular mechanism by which interferon prevents
hepatocarcinogenesis in patients with chronic
hepatitis C

Taking into account the molecular mechanisms underlying the hypercarcinogenic condition in HCV infection, we focused on the mechanism responsible for the antiproliferative or antioxidative activity of interferon-α. The regulatory signals triggered by interferon-α are transduced to the nucleus through the Janus tyrosine kinase/signal

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**Table 1. Reduction in the development of hepatocellular carcinoma in sustained virological responders and transient biochemical responders: characteristics of Japanese selected studies**

| Reference     | Number of patients | Observation period (years) | Estimated cumulative incidence of HCC at the 5th year (%) | Risk ratio
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<tbody>
<tr>
<td>Kasahara et al.2</td>
<td>1022</td>
<td>3.1 ± 12.9</td>
<td>4.3 - 4.7</td>
<td>0.13</td>
</tr>
<tr>
<td>Imai et al.3</td>
<td>563</td>
<td>4.0</td>
<td>0.9 - 6.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Ikeda et al.4</td>
<td>1643</td>
<td>5.1 (0.1–11.3)</td>
<td>1.4 - 1.9</td>
<td>0.32</td>
</tr>
<tr>
<td>Yoshida et al.1</td>
<td>2890</td>
<td>4.4</td>
<td>NA - NA</td>
<td>0.25</td>
</tr>
<tr>
<td>Tanaka et al.5</td>
<td>738</td>
<td>4.8 ± 1.2</td>
<td>1.2 - 3.7</td>
<td>0.16</td>
</tr>
<tr>
<td>Okanoue et al.6</td>
<td>1370</td>
<td>5.6</td>
<td>NA - NA</td>
<td>0.10</td>
</tr>
</tbody>
</table>

SVR, sustained virological responders; TBR, transient biochemical responders; NR, non-responders; HCC, hepatocellular carcinoma; NA, not assessed.

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1Adjusted risk ratios for development of HCC were calculated compared with untreated patients in references 1, 3, 4 and 5, and with non-responders in references 2 and 6.
2These figures represent the 4 year incidence of HCC.
3This risk ratio was for the groups that responded; these included SVR and TBR.
4This risk ratio was for the patients who had normal serum ALT levels and were positive for HCV RNA.
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A schematic diagram illustrating the proposed mechanisms of action by which interferon might inhibit carcinogenesis through the regulation of the MEK/ERK pathway. Interferon has been shown to inhibit MEK/ERK function without affecting Ras and Raf-1 activity. Mechanisms by which interferon regulates MEK phosphorylation may involve interference with de novo protein synthesis and/or reexpression of a specific gene(s). Analysis of downstream events controlled by the MEK/ERK pathway showed reduced activity of Cdk2 and Cdk4, high levels of mitogenic inhibitors (p21Waf1 and p27Kip1), and decreased cyclin D and E expression. Cdk4 and Cdk2 are activated by binding to cyclin D and cyclin E, respectively. Cyclin D–Cdk4 complexes are required for G1 phase progression. Cyclin E–Cdk2 complexes are required for the G1/S transition. Cdk activity is curtailed by p21Waf1 and p27Kip1.

transducers of activation and transcription (JAK/STAT) pathway, whereby ligand-activated JAK kinases phosphorylate STAT proteins, which subsequently dimerize and migrate to the nucleus to regulate gene expression. Interferon-α was initially described as an antiviral cytokine able to inhibit viral replication, and thereafter additional properties of this multifunctional cytokine that can affect the growth, differentiation and function of many cell types were discovered. The cross-talk between the JAK/STAT and the MEK/ERK pathways was demonstrated by the observation that interferon-β can directly activate ERK, which associates with STAT1. A study revealed that interferon-α dose-dependently increased the protein levels of copper-, zinc- and manganese-dependent superoxide dismutase, as well as the enzyme activities of glutathione peroxidase, and decreased the lipid peroxidation product levels in oxidative-stressed rat hepatocytes. As HCV RNA levels are usually decreased or undetectable, even though uncommonly unchanged, during interferon therapy in transient biochemical responders, such antioxidative actions of interferon may be amplified in a condition where oxidative stress is attenuated due to decreased HCV load. In fact, 2 months interferon therapy has been shown to decrease the serum lipid peroxidation products (thiobarbituric acid reactive substances) of hepatitis C patients, whose serum ALT levels fall to the normal range.

Conclusions

Needless to say, eradication of HCV and normalization of the serum ALT level by interferon are the most important issues for chemoprevention of hepatocarcinogenesis in patients with chronic hepatitis C. However, the antiproliferative activity of interferon-α also seems to play a critical role in preventing chronic hepatitis C patients from developing HCC. Furthermore, interferon may have a direct anti-tumour effect on clinically undetectable HCC, since combination therapy with interferon-α and intraarterial 5-fluorouracil has been shown to be effective in reducing tumours in patients with HCC. With respect to this issue, a very recent study demonstrated the integration of interferon-α/β signalling to p53 responses in tumour suppression, which resulted in enhancement of cancer cell apoptosis by interferon. We need to ascertain whether the anti-proliferative action of interferon is actually elicited in transient biochemical responders, not in non-responders, and induces a normo- or hypo-carcinogenic condition in those patients.

There is no strong clinical evidence linking the antioxidative action of interferon to the inhibition of HCC development in patients with chronic hepatitis C. Thus, further studies are required to determine how the antioxidative activity of interferon is involved in reducing the HCC incidence in HCV infection.

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References


