Chronic Inflammatory IFN-γ Signaling Suppresses Hepatocarcinogenesis in Mice by Sensitizing Hepatocytes for Apoptosis

Stefan Lütz, Jörg Schrader, Stefan Zander, Antonella Carambia, Juliane Buchkremer, Samuel Huber, Kurt Reifenberg, Ken-Ichi Yamamura, Peter Schirmacher, Ansgar W. Lohse, and Johannes Herkel

Abstract

Chronic liver inflammation is a critical component of hepatocarcinogenesis. Indeed, inflammatory mediators are believed to promote liver cancer by upholding compensatory proliferation of hepatocytes in response to tissue damage. However, inflammation can also mediate the depletion of malignant cells, but the difference between tumor-suppressive and tumor-promoting inflammation is not defined at the molecular level. Here, we analyzed the role of the major inflammatory mediator IFN-γ in chemical hepatocarcinogenesis of transgenic mice that overexpress IFN-γ in the liver; these mice manifest severe chronic inflammatory liver damage and lasting compensatory regeneration. We found that chronic exposure to IFN-γ suppressed chemical hepatocarcinogenesis, despite overt liver injury. Indeed, IFN-γ–transgenic mice had significantly fewer and significantly less advanced malignant lesions than nontransgenic mice. This tumor-suppressive effect of IFN-γ seemed to be mediated in part by its known immune activating function, indicated by infiltration of IFN-γ–transgenic livers with CD8 T cells, natural killer T cells, and natural killer cells. However, IFN-γ seemed to prevent carcinogenesis also by activating the cell-intrinsic p53 tumor suppressor pathway. Indeed, exposure to IFN-γ in vivo or in vitro was associated with accumulation of p53 in hepatocytes and the sensitization of hepatocytes to apoptosis induced by genotoxic stress. The IFN-γ–induced increase in apoptosis of hepatocytes seemed to be p53 dependent. Thus, chronic inflammation dominated by IFN-γ may prevent hepatocarcinogenesis, despite continued inflammatory liver injury and regeneration. Therefore, the carcinogenic potential of inflammation seems to be determined by type and composition of its mediators and manipulating the type of chronic inflammation may serve the prevention of cancer. Cancer Res. 71(11): 3763–71. ©2011 AACR.

Introduction

Chronic inflammation of tissue is believed to predispose to the formation of cancer (1). Hepatocellular carcinoma (HCC), which is one of the 5 most common and most lethal tumors worldwide (2, 3), may serve as a paradigm for inflammation-induced cancer. Indeed, HCC develops most frequently on grounds of cirrhosis after years of chronic hepatitis, independent of what had caused this condition (2, 3). However, how chronic inflammation is linked to carcinogenesis at the molecular level is not entirely clear. Recent findings indicate that inflammatory mediators, which cause tissue injury, may promote carcinogenesis by inducing compensatory proliferation and regeneration (4, 5), and the chronic stimulation of aberrant cells with inflammatory mediators, such as interleukin-6 (IL-6; refs. 6, 7) or TNF-α (8) was found to promote carcinogenesis.

A major inflammatory pathway that may modulate carcinogenesis is the IFN-γ/STAT1 pathway (9). However, the role of IFN-γ, which is secreted by inflammatory cells, in carcinogenesis is not clear. On the one hand, IFN-γ signaling has been shown to suppress carcinogenesis in vivo, because a lack of IFN-γ receptors or STAT1 molecules, which are the major signal transducers of IFN-γ, promotes carcinogenesis (10–13). It is believed that the anticarcinogenic activity of IFN-γ is mediated mainly by enhancing tumor cell immunogenicity and cancer immunosurveillance (12). On the other hand, however, sustained STAT1 signaling was reported to be associated with the development of liver cancer (14). Indeed, a lack of or silencing of suppressor of cytokine signaling (SOCS)-1, which is an endogenous inhibitor of the IFN-γ/STAT1 pathway (15), was found to promote IFN-γ–dependent carcinogenesis (16–18). However, SOCS-1 also...
interferes with other cytokine pathways, notably with the IL-6/STAT3 pathway; therefore, the exact role of sustained IFN-γ signaling in hepatocarcinogenesis is not established.

To explore the role of IFN-γ in hepatocarcinogenesis, we used the model of IFN-γ–transgenic mice that overexpress IFN-γ in the liver under control of the human serum amyloid P component gene promoter (19). These mice manifest significant hepatic IFN-γ levels, lifelong chronic hepatitis, continuous liver injury, and consecutive chronic regeneration (19); it is believed that such a chronic state of regenerative hepatocyte proliferation under inflammatory stress constitutes a major risk factor for hepatocarcinogenesis (4, 5). Because these mice are not prone to spontaneous liver cancer, we induced chemical hepatocarcinogenesis by application of diethylnitrosamine (DEN) and phenobarbital (4).

Despite chronic inflammation and sustained IFN-γ signaling, the IFN-γ–transgenic mice were resistant to chemically induced liver cancer. This protection from liver cancer was associated with IFN-γ–induced accumulation of the p53 molecule and the sensitization of damaged hepatocytes to apoptosis. Our findings indicate that (i) the activation of the p53 pathway and apoptosis of damaged hepatocytes by IFN-γ may be a relevant mechanism that limits hepatocarcinogenesis, and (ii) chronic inflammation dominated by IFN-γ can protect from liver cancer, despite continued compensatory regeneration of inflammatory liver damage. Thus, chronic inflammation need not be tumorigenic and the carcinogenic potential of hepatic inflammation may be determined by the type of its mediators rather than by mere chronicity.

Materials and Methods

Mice

SAP-IFN-γ–transgenic or nontransgenic C57BL/6 mice were bred and kept in a specific pathogen-free animal facility at the Johannes Gutenberg University (Mainz, Germany) or at the University Hospital Centre Hamburg-Eppendorf. The experiments were approved by the institutional animal experimentation committee. Liver carcinogenesis was induced in male mice by neonatal application of DEN (5 mg/kg at day 10 after birth) and subsequently promoted by phenobarbital (0.05% in drinking water). The p53+/− mice for the preparation of p53−/− hepatocytes were obtained from The Jackson Laboratory.

Histology

At the indicated time points, mice were sacrificed and the livers were fixed in 4% neutral-buffered formaldehyde, embedded in paraffin, sectioned at 2 μm, and stained with hematoxylin and eosin. At least 6 representative sections of each liver were evaluated by a senior hepatopathologist in a blinded fashion (P. Schirmacher). Tumor burden was determined as the number of lesions per centimeter (2) and as total lesion area (%). Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay was done with the in situ cell death detection kit (Roche); cells were counterstained with Hoechst 33258 nuclear dye (Invitrogen). Immunohistologic staining was carried out with antibody to p53, cleaved caspase-3 (Cell Signaling), or p21 (Santa Cruz Biotechnology).

Western blot

Whole cell lysates were prepared by lysis in HEPES buffered saline with 1% Triton X-100 containing protease/phosphatase inhibitors. Liver lysates were prepared from snap-frozen tissue in T-Per (Pierce) with protease/phosphatase inhibitors. Protein was separated in 12% SDS-polyacrylamide gels and blotted onto nitrocellulose membranes (Schleicher & Schuell). After incubation with 5% dry nonfat milk, membranes were probed with primary antibodies and appropriate horseradish peroxidase–conjugated secondary antibodies [all from Cell Signaling except antibodies to actin, p53, p21, Cyclin D1, and PCNA (proliferating cell nuclear antigen; Santa Cruz Biotechnology)]. Detection was done by using an ECL kit (Roth) and horseradish peroxidase.

Cell culture

Primary hepatocytes were isolated as described (20) and stimulated with 10 ng/mL recombinant mouse IFN-γ (Peprotech), 30 or 60 μmol/L cisplatin, 200 μmol/L DEN (both from Sigma), or 50 μmol/L Pifithrin-α (Calbiochem) as indicated. Cell survival was measured by adding 0.5 mg/mL MTT (Sigma) for 2 hours. The supernatant was removed, the tetrazolium dye dissolved in dimethyl sulfoxide, and absorbance was measured at 570 nm. Results shown are means of quadruple experiments. Alternatively, cell survival was determined by counting alive and dead cells after trypan blue staining in at least 6 microscope fields per cell culture sample.

Analysis of intrahepatic infiltrates

Nonparenchymal liver cells from perfused mouse livers were prepared as described (21), and the included hepatic inflammatory cells were then stained with fluorescence-labeled antibody to CD3, CD4, CD8, CD56, or NK1.1 (BD Biosciences) and analyzed by flow cytometry.

Results

Sustained activation of STAT1 suppresses chemical hepatocarcinogenesis

Liver protein extracts of 6- or 12-week-old nontransgenic or IFN-γ–transgenic mice were assessed by Western blot for activity of the IFN-γ/STAT1 pathway and markers of regenerative hepatocyte proliferation (Fig. 1A). The nontransgenic mice did not show STAT1 phosphorylation at the age of 6 or 12 weeks. In contrast, IFN-γ–transgenic mice showed continued upregulation and activation of STAT1, both at 6 and 12 weeks of age. The degree of STAT3 activation was considerably higher in the IFN-γ–transgenic mice than in nontransgenic mice, both at 6 or 12 weeks of age. Furthermore, the lyses from IFN-γ–transgenic livers, taken at 6 or 12 weeks of age, contained higher amounts of the proliferation markers PCNA and cyclin D1 than those of nontransgenic mice. These findings showed that IFN-γ signaling in the transgenic mice was continuously active and not silenced, and that the IFN-γ–transgenic livers were characterized by continued regenerative proliferation.
findings are in agreement with the continuously activated IFN-γ signaling found in vivo (Fig. 1A).

We then induced chemical hepatocarcinogenesis in nontransgenic or IFN-γ–transgenic mice by neonatal application of DEN, which was subsequently promoted by application of phenobarbital in drinking water. Cancer development in DEN-treated mice was assessed histologically after 40 weeks, because by then 2 mice in the group of nontransgenic mice already had died from liver cancer. The livers of IFN-γ–transgenic mice (n = 22) had significantly fewer (Fig. 2A; P < 0.0001) and smaller lesions (Fig. 2B; P < 0.0001) than those of the nontransgenic mice (n = 14). Representative histologic sections of IFN-γ–transgenic mice (Fig. 2C) show mild portal inflammation and mild perivenular fatty change, but lack of premalignant or malignant changes. Representative histologic sections of nontransgenic mice (Fig. 2D) show a premalignant eosinophilic (dysplastic) nodule without signs of cytologic atypia, increased proliferation, or invasive growth. Moreover, carcinogenesis seemed to be delayed in the IFN-γ–transgenic mice compared with the nontransgenic mice (Table 1). Indeed, significantly fewer IFN-γ–transgenic mice had premalignant dysplastic nodules (Table 1; P = 0.0017), and significantly fewer IFN-γ–transgenic mice had malignant lesions (HCC; Table 1; P = 0.0002), with an OR of 25 for the nontransgenic mice versus IFN-γ–transgenic mice to develop liver cancer (95% confidence interval; 3.89–160.57). Therefore, activation of the IFN-γ/STAT1 pathway in IFN-γ–transgenic mice seemed to protect from chemical hepatocarcinogenesis, despite continuous liver injury and regeneration.

**IFN-γ–induced regulation of p53 activity**

The question then was how IFN-γ induced protection from chemical hepatocarcinogenesis. Because IFN-γ has been shown to increase MHC expression and the immunogenicity of aberrant cells, IFN-γ may have indirectly activated cancer immunosurveillance by tumor-reactive lymphocytes (11, 12). Indeed, there was a strong and significant accumulation of both CD8 T cells and natural killer T cells (NKT) in the livers of IFN-γ–transgenic mice, with an increase of the CD8 T-cell percentage among the intrahepatic lymphocytes from 11% in wild-type mice to 24% in IFN-γ–transgenic mice (P = 0.0002), and an increase in the percentage of NKT cells from 4% in wild-type mice to 24% in IFN-γ–transgenic mice (P = 0.0002). To a lesser degree, there was also an increase in NK cells in the livers of IFN-γ–transgenic mice (2% in wild-type mice vs. 4% in IFN-γ–transgenic mice; P = 0.0002), whereas CD4 T cells did not accumulate (11% in wild-type mice vs. 9% in IFN-γ–transgenic mice; P = 0.3282). Thus, it was possible that IFN-γ–induced protection from malignancy was related to indirect activation of immune cells.

However, it has been observed in vitro that IFN-γ signaling in hepatocytes can induce cell-cycle arrest or apoptosis (22, 23). Thus, it was also possible that IFN-γ–induced protection from malignancy was related to direct effects of IFN-γ on hepatocytes. Indeed, it has been reported that activated STAT1 can regulate p53 activation (24, 25); the p53 molecule functions to inhibit the growth of aberrant cells by inducing cell-cycle arrest

To confirm these in vivo findings, we assessed in vitro the response of primary hepatocytes from nontransgenic mice to stimulation by IFN-γ (Fig. 1B). IFN-γ induced the rapid upregulation and activation of STAT1 and STAT3 within 1 hour. STAT3 activation was silenced after 12 hours; STAT1 activation, in contrast, continued for at least 48 hours. These.
or apoptosis in response to various stimuli (26). Thus, IFN-γ signaling may have induced p53 activation and p53-dependent apoptosis of DEN-treated hepatocytes. To address this possibility, we stimulated primary hepatocytes from nontransgenic

<table>
<thead>
<tr>
<th>Histologic stage</th>
<th>Nontransgenic mice (n = 14)</th>
<th>IFN-γ-transgenic mice (n = 22)</th>
<th>Significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysplastic foci (small cell dysplastic foci)</td>
<td>13/14 (93%)</td>
<td>16/22 (73%)</td>
<td>0.2093</td>
</tr>
<tr>
<td>Dysplastic nodules (adenomas)</td>
<td>11/14 (79%)</td>
<td>5/22 (23%)</td>
<td>0.0017</td>
</tr>
<tr>
<td>HCC</td>
<td>10/14 (71%)</td>
<td>2/22 (9%)</td>
<td>0.0002</td>
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mice with IFN-γ. Exposure to IFN-γ induced both the accumulation of p53 and its activation, indicated by upregulation of p21 and cleavage of caspase-3 (Fig. 3A). We then incubated primary hepatocytes from nontransgenic mice, which had or
had not been stimulated with IFN-γ, with DEN, or cisplatin (Fig. 3B); this application of genotoxic stress reduced the expression of p21, a marker of growth arrest, and induced cleavage of caspase-3, indicating apoptosis. These findings showed that IFN-γ signaling induced p53 activation in hepatocytes and additional genotoxic stress shifted p53 activity from predominant growth arrest to predominant apoptosis.

**IFN-γ–induced p53 accumulation and apoptosis in vitro**

To confirm these in vitro observations, we assessed the amounts of p53 and of p53-induced p21 and cleaved caspase-3 in liver extracts from DEN-treated or untreated nontransgenic or IFN-γ–transgenic mice (Fig. 4A). Indeed, IFN-γ–transgenic mice had accumulated p53 irrespective of DEN treatment; nontransgenic mice, in contrast did not exhibit p53 accumulation. DEN exposure itself did not seem to modulate STAT1 or STAT3 activation (Fig. 4A). The STAT1-induced p53 accumulation in IFN-γ–transgenic mice was associated with elevated p53 activity, as shown by the induction of the p21 molecule and cleavage of caspase-3 (Fig. 4A). IFN-γ–induced p53 activity seemed to be modulated by DEN. DEN-treated mice displayed higher amounts of cleaved caspase-3, indicative of apoptosis, whereas untreated mice showed higher levels of p21, indicative of growth arrest. Thus, IFN-γ signaling

Figure 3. IFN-γ–induced p53-activation in primary hepatocytes. Cell extracts of primary nontransgenic hepatocytes after IFN-γ treatment in vitro were assayed by Western blot. A, cell extracts were derived from primary nontransgenic hepatocytes that were stimulated for indicated time with IFN-γ. IFN-γ stimulation seemed to induce accumulation of p53 and its activation, indicated by increased expression of p21 and cleavage of caspase-3. B, cell extracts were derived from primary nontransgenic hepatocytes that were stimulated with IFN-γ and the DNA-damaging agents cisplatin or DEN, as indicated. Damaged primary hepatocytes incubated with IFN-γ showed reduced p21 expression and increased cleavage of caspase-3.

Figure 4. IFN-γ–induced activation of p53 pathway in DEN-treated mice. Nontransgenic or IFN-γ–transgenic mice were treated with DEN in vivo and, after 6 days, liver extracts (A) or histologic liver sections (B) were obtained and analyzed. A, liver extracts were analyzed by Western blot. In contrast to nontransgenic mice, the livers of IFN-γ–transgenic mice exhibited marked p53 accumulation, independent of whether the mice had been treated with DEN or not. Activation of p53 in the IFN-γ–transgenic mice was indicated by expression of p21 and cleavage of caspase-3; previous treatment with DEN reduced p21 expression and increased caspase-3 cleavage. B, histologic liver sections were quantified for apoptosis of hepatocytes by TUNEL assay, indicating that IFN-γ–induced apoptosis of hepatocytes, and exposure to DEN further increased apoptosis rates of IFN-γ–exposed hepatocytes.
may induce p53 accumulation and activation, and the additional presence of genotoxic stress may shift p53 activity from predominant growth arrest to apoptosis.

To confirm that apoptosis of hepatocytes was induced by IFN-γ signaling in combination with genotoxic stress, we conducted TUNEL assays on liver sections of nontransgenic and IFN-γ-transgenic mice 6 days after treatment with DEN (Fig. 4B). Hepatocytes of IFN-γ-transgenic mice displayed significantly higher rates of apoptosis than those of nontransgenic mice (Fig. 4B; $P < 0.0001$); moreover, exposure to DEN significantly further increased the incidence of apoptosis in hepatocytes of IFN-γ-transgenic mice ($P < 0.0001$). A representative TUNEL stain is shown in Figure 5A.

To further confirm that IFN-γ signaling had induced p53 activation in hepatocytes in vivo, we treated nontransgenic or IFN-γ-transgenic mice with DEN and obtained the livers after 6 days. We then stained liver sections by histochemistry for p53 (Fig. 5B), p21 (Fig. 5C), and cleaved caspase-3 (Fig. 5D). In contrast to nontransgenic hepatocytes, hepatocytes of IFN-γ-transgenic mice displayed considerable accumulation of p53 and cleavage of caspase-3, notably when treated with DEN. Moreover, there was considerable expression of p21 in IFN-γ-transgenic hepatocytes, which was reduced after DEN treatment; nontransgenic hepatocytes did not express p21. These in vivo findings are in full agreement with the in vitro findings of Fig. 3.

To further substantiate these in vivo findings, we analyzed the survival of primary hepatocytes from nontransgenic mice in vitro, after stimulation with IFN-γ and in response to DNA damage induction by treatment with DEN (Fig. 6A, white columns) or

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**Figure 5.** Histologic analysis of IFN-γ–induced p53-activity and apoptosis of hepatocytes after DEN treatment in vivo. Six days after treatment of nontransgenic or IFN-γ–transgenic mice with DEN in vivo, liver sections were assessed for apoptosis of hepatocytes by TUNEL assay (A), p53 accumulation (B), p21 expression (C), and cleavage of caspase-3 (D). Shown are representative findings. A, apoptotic hepatocytes stained by the TUNEL assay appear in green; the nuclei are counterstained in blue with Hoechst 33258. IFN-γ seemed to induce apoptosis of hepatocytes; exposure to DEN further increased apoptosis rates of IFN-γ–exposed hepatocytes. B, hepatocytes in IFN-γ–transgenic mice showed accumulation of p53, notably after previous DEN treatment in vivo. C, hepatocytes of IFN-γ–transgenic mice showed increased expression of p21, which was reduced after previous DEN treatment in vivo. D, hepatocytes in IFN-γ–transgenic mice showed increased cleavage of caspase-3, notably after previous DEN treatment in vivo.
cisplatin (Fig. 6B, white columns). In concordance with the in vivo findings, IFN-γ and genotoxic stress synergistically reduced the survival of hepatocytes ($P < 0.05$). The induced hepatocyte death seemed to be p53 dependent, because it was prevented by the p53 inhibitor Pifithrin-a (ref. 27; Fig. 6A and B; black columns). To further test the p53 dependence of the induced hepatocyte death, we analyzed in vitro the survival of primary hepatocytes from p53$^{-/-}$ mice or wild-type mice in response to incubation with IFN-γ and DEN (Fig. 6C) or cisplatin (Fig. 6D). In agreement with the findings above, IFN-γ and genotoxic stress synergistically reduced the survival of wild-type hepatocytes (white columns; $P < 0.02$) but not of p53$^{-/-}$ hepatocytes (black columns; $P > 0.5$). These findings indicated that IFN-γ/STAT1 signaling to hepatocytes may suppress liver cancer by p53-dependent inhibition of the outgrowth of premalignant cells.

**Discussion**

Inflammation has both the capacity to foster or to inhibit carcinogenesis, by either promoting the growth or the depletion of malignant cells (28). Whether inflammation is rather tumor promoting or tumor suppressive seems to depend on the cell type and cytokine composition of the tumor-associated inflammatory reaction (28). The role of IFN-γ in this respect has been controversial; both tumor-promoting and tumor-suppressive functions of IFN-γ have been reported (11, 18). On the one hand, IFN-γ may function as a tumor promoter by stimulating the release of inflammatory mediators that may advance carcinogenesis, such as TNF-α, reactive oxygen species, or nitric oxide (28, 29); on the other hand, IFN-γ may function as a tumor suppressor by enhancing tumor cell immunogenicity and cancer immunosurveillance (12). The role of IFN-γ in hepatocarcinogenesis is further complicated by its antifibrotic effects, which may also influence carcinogenesis. Indeed, a liver cancer model that is associated with severe liver fibrosis showed a mild reduction in lesion numbers in mice lacking the IFN-γ receptor (30).

To clarify the role of IFN-γ in hepatocarcinogenesis, we used liver-specific IFN-γ-transgenic mice; the livers of these mice are marked by continuous inflammation, injury, and compensatory regeneration in the absence of fibrosis. Regenerating hepatocytes under chronic inflammatory stress are believed to be prone to malignant transformation.
(4, 5). However, despite chronic inflammation and regeneration, the IFN-γ–transgenic mice were significantly less susceptible to chemical hepatocarcinogenesis (Fig. 2 and Table 1); the OR of nontransgenic mice versus IFN-γ–transgenic mice to develop liver cancer was 25. This is in agreement with the earlier finding that IFN-γ–insensitive mice, which either were IFN-γ receptor deficient or STAT1 deficient, exhibited increased development of chemically induced skin cancer (10). In skin cancer, IFN-γ seemed to act by increasing tumor immune surveillance by lymphocytes (10, 11). We find the livers of IFN-γ–transgenic mice strongly infiltrated mainly by CD8 T cells and NK T cells and, to a lesser degree, by CD4 T cells and NK cells. At least CD8 T cells and NK cells are capable of depleting malignant cells; indeed, it has been reported that IFN-γ–driven accumulation of NK cells in established tumors seems to correlate with survival (31). Thus, it is conceivable that the protection from malignancy mediated by IFN-γ was related to IFN-γ–enhanced cytotoxicity of immune cells.

However, in addition to its indirect effects on malignant cells by activating cancer immune surveillance, we find that IFN-γ could also directly induce depleton of premalignant cells by activating the cell-intrinsic p53 tumor suppressor pathway. Indeed, we find that IFN-γ signaling to damaged hepatocytes seemed to modify the acute damage response by activation of the p53 pathway, resulting in sensitization to apoptosis in response to genotoxic stress (Figs. 3, 4, and 5). Although damage-induced cell death of hepatocytes seemed to depend on p53, there was a consistent increase in cell death induced by IFN-γ, and this increase was consistently lost in the absence of p53 activity (Fig. 6). Thus, our findings indicate a tumor-suppressive role of IFN-γ at early stages of carcinogenesis by the induction of apoptosis in damaged cells. Because lymphocytes are the most important physiologic source of IFN-γ in nontransgenic mice and in humans, it is possible that IFN-γ secretion is an important cancer-preventing activity of lymphocytes, independent of their cytolytic activity.

It has been reported that IFN-γ may promote colorectal carcinogenesis in SOCS-1–deficient mice, which manifest hyperactivation of the STAT1 pathway, by inducing expression of COX-2 and inducible nitric oxide synthase (18). Indeed, colorectal carcinogenesis in SOCS-1–deficient mice could be abrogated by blockade of IFN-γ (18). Because silencing of the SOCS-1 gene by methylation has been observed in liver cancer (16, 17), a lack of SOCS-1 activity and STAT1 hyperactivity may also promote hepatocarcinogenesis. Indeed, SOCS-1–/– mice are more susceptible to chemical hepatocarcinogenesis than SOCS-1+/+ mice (17), suggesting that STAT1 hyperactivation due to a lack of SOCS-1 activity may promote carcinogenesis. However, this observation is seemingly in contrast to our finding that chronic STAT1 activation in IFN-γ–transgenic mice was associated with suppressed hepatocarcinogenesis. One explanation for this divergence could be that SOCS-1 does not only interfere with the STAT1 pathway but also with other cytokine pathways, such as the STAT3 pathway (15); therefore, the tumor-promoting effect of SOCS-1 deficiency may depend on STAT3 rather than STAT1 signals. Indeed, the STAT3 pathway has been identified as a critical driver of carcinogenesis (32, 33), and tumor progression may be determined by the balance of STAT1 and STAT3 signals (34). Although IFN-γ stimulated both STAT1 and STAT3 (Fig. 1B), the STAT1 pathway in hepatocytes stimulated with IFN-γ seemed to be continuously activated, whereas the STAT3 signal seemed to be rather transient (Fig. 1B).

Taken together, our findings indicate that chronic inflammation and compensatory regeneration are not necessarily oncogenic, and that the type of inflammation, notably the proportion of IFN-γ–secreting lymphocytes in chronic inflammatory tissue infiltrates, may determine the carcinogenic potential of chronic inflammation. Indeed, one may speculate that these findings could explain the clinical observation that some chronic inflammatory conditions, such as viral hepatitis, confer a high risk of progression to cancer, whereas in other inflammatory conditions, such as autoimmune hepatitis, progression to cancer is uncommon. Our findings further indicate that inflammatory IFN-γ/STAT1 signaling to damaged cells, in addition to indirectly activating cancer immune surveillance, may depend on STAT3 rather than STAT1 signals. Indeed, SOCS-1 activity may promote carcinogenesis. However, this explanation for this divergence could be that SOCS-1 does not only interfere with the STAT1 pathway but also with other cytokine pathways, such as the STAT3 pathway (15); therefore, the tumor-promoting effect of SOCS-1 deficiency may depend on STAT3 rather than STAT1 signals. Indeed, the STAT3 pathway has been identified as a critical driver of carcinogenesis (32, 33), and tumor progression may be determined by the balance of STAT1 and STAT3 signals (34). Although IFN-γ stimulated both STAT1 and STAT3 (Fig. 1B), the STAT1 pathway in hepatocytes stimulated with IFN-γ seemed to be continuously activated, whereas the STAT3 signal seemed to be rather transient (Fig. 1B).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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