Inhibition of Basic Fibroblast Growth Factor Expression, Angiogenesis, and Growth of Human Bladder Carcinoma in Mice by Systemic Interferon-α Administration

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ABSTRACT

The purpose of these studies was to determine whether systemic administration of IFN-α can inhibit the expression of basic fibroblast growth factor (bFGF) in human transitional cell carcinoma, reduce its angiogenesis, and thus inhibit its growth in the bladder wall of nude mice. In vitro incubation of the highly metastatic 253J B-V cells and the IFN-α-resistant 253J B-V IFNR cells with nontoxic concentrations of IFN-α downregulated the steady-state mRNA transcripts and protein production of bFGF. IFN-α-insensitive and IFN-α-resistant cells were implanted in the bladder wall of nude mice. Systemic administration of IFN-α decreased the in vivo expression of bFGF, decreased blood vessel density in the tumors, and inhibited tumor growth of both IFN-α-insensitive and IFN-α-resistant cells. These data suggest that in addition to its well-documented antiproliferative effects, IFN-α can inhibit the growth of human bladder cancer cells by inhibition of angiogenesis.

INTRODUCTION

Tumor growth and metastasis depend on adequate blood supply, i.e., angiogenesis (1), which is influenced by the balance between stimulatory and inhibitory molecules released by both tumor cells and host cells (2). Recent studies from our laboratory have shown that the invasive and angiogenic properties of human carcinomas can be modulated by specific cytokines released by host cells in specific organ environments (3–6) that can regulate the expression of angiogenic molecules (7, 8). For example, human melanomas growing s.c. in orthotopic organs of nude mice overexpress the angiogenic molecule IL-8 (9), whereas the same cells growing in ectopic organs do not. The overexpression of IL-8 by melanoma cells growing s.c. is caused by the production of IL-1 by keratinocytes (8). Similarly, human renal carcinomas implanted in the kidney of nude mice are highly vascularized and overexpress bFGF (mRNA and protein), whereas the same human renal cancer cells implanted s.c. do not. Immunohistochemical staining of the transplanted human renal carcinomas revealed that the expression of IFN-β was high in host cells surrounding the s.c. tumors and absent in the kidney (7). The subsequent demonstration that IFN-β and IFN-α can down-regulate the steady-state gene expression and protein production of bFGF by a mechanism that is independent of the growth factor’s antiproliferative effect (9) raised the possibility that bFGF production by the s.c. renal cancer tumors was down-regulated by IFN-β produced by keratinocytes and dermal fibroblasts (7).

Recent clinical observations have substantiated the antiangiogenic properties of the IFNs. The chronic systemic administration of IFN-α to patients with Kaposi’s sarcoma (10, 11), malignant hemangiopericytoma (12), and life-threatening hemangiomas of infancy (13) resulted in regression of these highly vascularized tumors. One hypothesis to explain these results is that bFGF gene transcription and protein production are suppressed in tumors that overexpress bFGF (14). However, experimental data to support this hypothesis have been lacking.

The importance of bFGF for the growth of human TCC is well recognized. Urine from patients with TCC was found to have angiogenic activity (15), attributed to high levels of biologically active bFGF (16). We have recently established an orthotopic model of human TCC in nude mice (17). After orthotopic implantation of parental TCC cells (into the bladder wall), we isolated metastases and reimplanted cells from these metastases into the bladder wall of nude mice, and that this inhibition is associated with inhibition of angiogenesis, and hence, inhibition of tumor growth.

MATERIAL AND METHODS

Animals. Male athymic BALB/c nude mice were purchased from the Animal Production Area of the National Cancer Institute, Frederick Cancer Research Facility (Frederick, MD). The mice were housed in laminar-flow cabinets under specific pathogen-free conditions and used at 8–12 weeks of age. Animals were maintained in facilities approved by the American Association for Accreditation of Laboratory Animal Care and in accordance with current regulations and standards of the United States Department of Agriculture, Department of Health and Human Services, and NIH.

Cell Culture. Highly metastatic 253J B-V human bladder tumor cells were grown as a monolayer culture in CMEM supplemented with 5% fetal bovine serum, vitamins, sodium pyruvate, L-glutamine, and nonessential amino acids (17). 253J B-V IFN-α is a cell line that was derived from the 253J B-V cells by prolonged culture with increasing concentrations of IFN-α. This cell line is resistant to the antiproliferative effects of IFN-α-2a at a concentration of more than 10,000 units/ml. HUVECs were maintained on 1% gelatin-coated flask in CMEM with 10% bovine serum albumin, vitamins, sodium pyruvate, L-glutamine, and nonessential amino acids in the presence of bFGF at a concentration of 10 ng/ml (19). Cell lines were free of Mycoplasma, reovirus type 3, pneumonia virus of mice, mouse adenovirus, murine hepatitis virus, lymphoectytic chorionvirus, ectromelia virus, and lactate dehydrogenase virus (Microbiological Associates, Bethesda, MD).

Orthotopic Implantation of Tumor Cells. Cultured 253J B-V and 253J B-V IFN-α cells (50% confluence) were given fresh medium 24 h before harvest and prepared for injection as described previously (17). Mice anesthetized with methoxyflurane were placed in the supine position. A lower midline abdominal incision was made, and the bladder was exteriorized. Tumor cells were injected into the dome of the bladder (all injection volumes ranged from
0.05–0.1 ml). A well-localized bleb was the sign of a technically satisfactory injection. The abdominal incision was closed in one layer using metal clips.

**Reagents.** Human IFN-α-2a (specific activity, 6 x 10^6 IU/mg protein) was purchased from Hoffman LaRoche (Nutley, NJ) and diluted to the desired concentration in saline. Rat antirat CD31 antibody was purchased from Pharmagen (San Diego, CA; Ref. 20).

**In Vivo Therapy with IFN-α-2a.** Experiments were designed to evaluate the effect of therapy with systemic administration of IFN-α-2a on the 253J B-V or 253J B-V IFNR tumors growing in the bladders of athymic nude mice and to determine whether this therapy down-regulated the expression of bFGF. In these experiments, 1 x 10^6 253J B-V or 253J B-V IFNR cells were implanted directly into the bladder wall of athymic nude mice (16). Therapy started on day 7 after tumor implantation. Groups of mice were injected s.c. with either saline (control) or differing doses of IFN-α-2a (1,000, 5,000, 10,000, or 25,000 units) every other day for 4 weeks (12 injections). On days 36–38, mice were killed by cervical dislocation, and the presence of tumor in the bladder and at metastatic sites was evaluated. The bladders were removed, weighed, and processed for histological and immunohistochemical assays.

**In Vitro Antiproliferative Assay.** Tumor cells (5 x 10^3) were seeded into 38-mm^2 wells of 96-well flat-bottomed plates in quadruplicate and allowed to adhere overnight. The cultures were then washed and refed with medium (control) or medium containing differing concentrations of IFN-α. After 96 h, the antiproliferative activity was determined by the MTT assay, which monitors the number of metabolically active cells (21). After incubation for 2 h in medium containing 40 mg/ml MTT, the cells were lysed in DMSO. The conversion of MTT to formazan was measured by a MR-5000 96-well microplate reader at 570 nm (Dynatech, Inc., Chantilly, VA). Growth inhibition was calculated from the formula:

\[
cytostasis (\%) = \left[ 1 - \left( \frac{A}{B} \right) \right] \times 100
\]

where A is the absorbance of the treated cells, and B is the absorbance of the control cells (21).

**Northern Blot Analysis.** Polyadenylated mRNA was extracted from both cultured cells and in vivo tumors using the FastTrack mRNA isolation kit (Invitrogen, San Diego, CA). mRNA was electrophoresed on a 1% denaturing formaldehyde/agarose gel, electrotransferred at 0.6 A to a GeneScreen nylon membrane (DuPont New England Nuclear, Boston, MA), and UV cross-linked with 120,000 mJ/cm^2 using a UV Stratalinker 1800 (Stratagene, La Jolla, CA). Hybridizations were performed as described previously (7). Nylon filters were washed three times at 60°C with 30 mM NaCl, 3 mM sodium citrate (pH 7.2), and 0.1% SDS (w/v).

The cDNA probes used in this analysis were a 1.3-kb PstI cDNA fragment corresponding to rat glyceraldehyde-3-phosphate dehydrogenase (22) and a 1.4-kb SstI cDNA fragment of bovine bFGF (23). Each cDNA fragment was purified by agarose gel electrophoresis, recovered using GeneClean (BIO 101, Inc., La Jolla, CA), and radioabeled with the random primer technique using [α-32P]deoxyribonucleotide triphosphate (24).

The level of bFGF mRNA transcripts was quantitated by densitometry readings of autoradiograms using the Image Quant software program (Molecular Dynamics, Sunnyvale, CA). Each sample measurement was expressed as the ratio of the average area under the curve of bFGF-specific mRNA transcripts: 1.3-kb glyceraldehyde-3-phosphate dehydrogenase mRNA transcripts.

**ELISA for bFGF.** Cellular, supernatant, and serum bFGF protein expression was analyzed by ELISA using the Quantikine bFGF ELISA kit (R&D Systems, Minneapolis, MN). Cells (5 x 10^3) were cultured, and the concentration of bFGF in unknown samples was determined by comparing the optical density of the samples to the standard curve. The minimal detectable level of bFGF by this assay is 1 pg/ml (9).

**Quantification of Microvessel Density.** Cryostat sections of tumors growing in the bladder were fixed with 2% paraformaldehyde in PBS (pH 7.5) for 10 min at room temperature, washed twice with PBS, and treated with 1% Triton X-100 for 5 min. The sections were washed three times, and endogenous peroxidase was blocked with 3% hydrogen peroxide in methanol for 12 min. The samples were then washed three times with PBS and incubated with a protein-blocking solution consisting of PBS containing 1% normal goat serum and 1% horse serum for 20 min at room temperature. Excess blocking reagent was drained off, and the samples were incubated for 15–18 h with the appropriate dilution of rat antirat CD31 antibody (Ref. 20; Pharmagen) at 4°C. The samples were rinsed four times with PBS and then rinsed with distilled water, counterstained with aqueous hematoxylin, washed, mounted with Permount, and examined in a bright-field microscope. The positive reaction was indicated by a reddish-brown precipitate. Negative controls were done using nonspecific IgG (25).

Microvessel density was determined by light microscopy according to the procedure of Weidner et al. (26). The areas with most intensely stained blood vessels were evaluated. Any brown-staining endothelial cell cluster distinct from adjacent microvessels, tumor cells, or other stromal cells was considered a single countable microvessel. The images were projected and recorded by digitizing the image in a cooled charge-coupled device Optronics Tec 470 camera (Optronics Engineering, Goleta, CA) linked to a computer and a digital printer (Sony Corporation, Tokyo, Japan; Ref. 27). Each count was expressed as the number of microvessels identified within selected fields x 200. All counts were performed on coded samples by two investigators.

**Immunohistochemical Assay of Human bFGF.** Cryostat sections of tumors growing in the bladder were treated sequentially with cold acetone (−20°C) for 5 min followed by cold chloroform:acetone (1:1) for 5 min and rinsed with cold acetone for 5 min followed by two rinses in PBS. Sections were processed for indirect immunoperoxidase assay, in which the primary antibody is a polyclonal rabbit anti-human bFGF (Sigma Chemical Co.), and the secondary antibody is a peroxidase-conjugated goat antirabbit IgG, F(ab')2 fragment (Jackson Immunoresearch Laboratory, Inc., West Grove, PA). The buffers and blocking solution used were as described above. The intensity of staining bFGF was quantitated in three different areas of each sample by an image analyzer using the Optimas software program (Biocantin, Edmonds, WA). Three different areas in each sample were quantified to yield an average measurement (27).

**ISH for mRNA.** ISH for mRNA of fixed tissue culture cells was performed as described previously (7, 28). Briefly, a bFGF-specific oligonucleotide probe was designed complementary to the 5' end of human bFGF mRNA transcript. The DNA oligonucleotide sequence 5'-CGGGAAGCCGCCTTGGCCCG-3' was in the antisense orientation and hence complementary to bFGF mRNA. A control sense oligonucleotide was 5'-GGCGGCAGCGGCGCCTTCCG-3'. A d(T)20 oligonucleotide was used to verify the integrity and absence of mRNA degradation in each sample. The oligonucleotide probes were synthesized with six biotin molecules (hyperbiotinylated) at the 3' end (Research Genetics). The specificity of the probe was confirmed by Northern blot analyses (27).

Cells were grown on dry sterilized ProbeOn slides (Fisher Scientific, Pittsburgh, PA), fixed in 4% paraformaldehyde in diethyl pyrocarbonate-PBS for 20 min at room temperature, and placed in the Microprobe slide holder. The slides were washed in Tris, 0.1% Triton, and 0.2 N HCl. Hybridization of the probe was carried out for 45 min at 45°C, and the samples were then washed with 2x SCC three times for 2 min at 45°C. The samples were incubated with alkaline phosphatase-labeled avidin for 30 min at 45°C, rinsed in 50 mM Tris buffer (pH 7.6), rinsed with alkaline phosphatase enhancer for 1 min, and incubated with a chromogen substrate for 15 min at 45°C. Additional incubation with fresh chromogen substrate was done if necessary to enhance a weak reaction. Red stain was a positive reaction in this assay. Control for endogenous alkaline phosphatase included treatment of the sample in the absence of the biotinylated probe and use of chromogen alone.

**In Vivo Morphogenesis of Vascular Endothelial Cells.** The surface of 24-well plates (Costar) was coated with Matrigel (5 mg/well), which was allowed to solidify for at least 1 h at 37°C. HUVECs (40,000 cells/well) were plated in either CMEM, CM collected from cultures of 253J B-V cells, CM and IFN-α (100 units/ml), or CM collected from cultures of 253J B-V cells pretreated for 96 h with IFN-α (100 units/ml). The plates were incubated at 37°C for 4–6 h and then examined under phase-contrast microscopy and photographed. The degree of tube branching was evaluated visually as described previously (29).

**Statistical Analysis.** The significance of the in vitro data was analyzed by Student’s t test (two-tailed), and the significance of the in vivo data was determined by the Wilcoxon χ2 test.
253J B-V cells with IFN-α-2a inhibits bFGF mRNA expression. The antisense bFGF probe (Fig. 3/4). whereas in sparse cultures of 253J B-V cells, the down-regulation of bFGF mRNA was confirmed by ISH. 253J B-V cells incubated with medium containing IFN-α-2a, the colorimetrie reaction was reduced (Fig. 3ß). The control sense bFGF probe (24, 27). All samples demonstrated an intense histochemical reaction, indicating that the mRNA was not degraded (data not shown). Sparse cultures of 253J B-V cells incubated in medium showed an intense reaction with the specific antisense bFGF probe (Fig. 3A), whereas in sparse cultures of 253J B-V cells incubated with medium containing IFN-α-2a, the colorimetrie reaction was reduced (Fig. 3B). The control sense bFGF probe did not produce a reaction (data not shown). Thus, the Northern blot and ISH analyses agreed that in vitro treatment of 253J B-V cells with IFN-α-2a inhibits bFGF mRNA expression. IFN-α therapy did not inhibit the in vitro expression of VEGF, IL-8, or angiogenin by the 253J B-V cells.

Measurement of bFGF in CM of 253J B-V. We next examined whether incubation of 253J B-V cells with IFN-α-2a would decrease the concentration of bFGF released into the CM of cells. The concentration of bFGF in the CM of 253J B-V cells was 28 pg/ml (Table 1), and it was not reduced by the direct addition of IFN-α (10 or 100 units/ml) to the medium. However, in the CM collected from 253J B-V cells incubated for 96 h in medium containing IFN-α-2a, the bFGF concentration was significantly reduced in a dose-dependent manner. IFN-α-2a at 10 units/ml reduced the bFGF to 9 pg/ml, and 100 units/ml reduced the bFGF to levels below the threshold for detection by the ELISA assay (<1 pg/ml). This reduction in the release of bFGF into the CM could not have been caused by cell lysis, which should have contributed to an increase in bFGF concentration in the CM.

Stimulation of in vitro Morphogenesis by HUVECs. HUVECs plated on Matrigel were incubated for 4–6 h in medium (negative control), CM from untreated 253J B-V cells (positive control), CM from 253J B-V cells pretreated for 96 h in medium containing 100 units/ml IFN-α-2a (test group), or CM from 253J B-V cells and 100 units/ml IFN-α-2a added immediately before the assay (IFN-α control). Endothelial capillary tube formation was assessed by light microscopy. Incubation of the HUVECs in CM from 253J B-V resulted in a dense tubular network (Fig. 4A). The addition of IFN-α to the CM at the time of the assay did not inhibit this capillary branching (Fig. 4B), indicating that IFN-α does not directly abrogate endothelial cell tube formation. However, significant inhibition of branching was found in HUVECs incubated in CM collected from 253J B-V cells cultured in medium containing 100 units/ml IFN-α-2a (Fig. 4C). Similarly, no significant branching was observed in HUVECs incubated in CMEM with 5% fetal bovine serum (Fig. 4D).

In Vivo Therapy of 253J B-V and 253J B-V IFNR Tumors by Systemic Administration of IFN-α-2a. We next evaluated the efficacy of systemic administration of IFN-α-2a against human TCC growing within the bladder wall of nude mice and determined whether this treatment was associated with inhibition of bFGF expression in the tumors. 253J B-V cells were implanted into the bladder wall of nude mice (n = 10). Therapy began on day 7 after tumor implantation. Mice received s.c. injections of saline or different doses of IFN-α-2a every other day for 4 weeks (12 injections). The mice were killed, and the concentration of bFGF released into the CM of cells. The concentration of bFGF in the CM of 253J B-V cells was 28 pg/ml (Table 1), and it was not reduced by the direct addition of IFN-α-2a (10 or 100 units/ml) to the medium. However, in the CM collected from 253J B-V cells incubated for 96 h in medium containing IFN-α-2a, the bFGF concentration was significantly reduced in a dose-dependent manner. IFN-α-2a at 10 units/ml reduced the bFGF to 9 pg/ml, and 100 units/ml reduced the bFGF to levels below the threshold for detection by the ELISA assay (<1 pg/ml). This reduction in the release of bFGF into the CM could not have been caused by cell lysis, which should have contributed to an increase in bFGF concentration in the CM.

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bladder tumors were evaluated. Tumor weights were significantly reduced by therapy with IFN-α over a wide dose range (Table 2). Maximum effect on tumor growth (size and weight) was observed after therapy with 5,000 and 10,000 units of IFN-α. This was accompanied by a significant reduction in tumor vascularization and serum bFGF levels (Table 2).

To rule out that the therapeutic effects were simply caused by the direct antiproliferative activity of IFN-α, we repeated the in vivo study using the 253J B-V IFNR cells. In vitro incubation of the 253J B-V IFNR cells with IFN-α-2a did not produce cytostasis but did inhibit bFGF protein (Fig. 5). These cells were implanted into the bladder of nude mice, which were treated systemically with 10,000 units/ml IFN-α-2a, as described. The mice were killed 48 h after the final treatment, and the presence of tumor in the bladder and at metastatic sites was evaluated. The bladders were removed and weighed. Tumor weight was significantly reduced from a median weight of 191 mg (range, 106–457 mg) in untreated control mice to 61 mg (range, 35–268 mg) in mice receiving the IFN-α therapy (Table 3). Significant reduction in tumor vascularization was revealed by immunohistochemical analysis using antibodies against CD31 (20). The number of blood vessels counted per field × 200 was reduced from 114 ± 6 in the tumors of untreated control mice to 66 ± 3 in the bladder tumors growing in IFN-α-2a-treated mice (Fig. 6, A and B). The intensity of bFGF immunostaining in the TCC cells was also significantly reduced after systemic therapy with IFN-α-2a (Fig. 6, C and D), and a 6.2-fold decrease in bFGF steady-state in RNA was demonstrated by Northern blotting with densitometry (Fig. 2B). The intensity of VEGF immunostaining in the TCC cells was not significantly reduced after therapy with IFN-α-2a (Fig. 6, E and F).
cells were pretreated with IFN-α-2a. D, CMEM with 5% fetal bovine serum (magnified from X40).

Minimal detectable level of bFGF is 1 pg/ml.

Table 1  Inhibition of bFGF release from 253J B-V cells by incubation with IFN-α-2a

<table>
<thead>
<tr>
<th>Treatment</th>
<th>bFGF (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% CMEM</td>
<td>0</td>
</tr>
<tr>
<td>5% CMEM + 10 units/ml IFN-α</td>
<td>0</td>
</tr>
<tr>
<td>5% CMEM + 100 units/ml IFN-α</td>
<td>0</td>
</tr>
<tr>
<td>B-V CM</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>B-V CM + 10 units/ml IFN-α</td>
<td>36 ± 5</td>
</tr>
<tr>
<td>B-V CM + 100 units/ml IFN-α</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>B-V CM/grown in 10 units/ml IFN-α</td>
<td>9 ± 2*</td>
</tr>
<tr>
<td>B-V CM/grown in 100 units/ml IFN-α</td>
<td>&lt;1 ± 0*</td>
</tr>
</tbody>
</table>

Table 2  Systemic therapy of 253J B-V bladder tumors in mice with IFN-α-2a

<table>
<thead>
<tr>
<th>IFN-α (units/dose)</th>
<th>Median tumor weight (mg)/median (range)</th>
<th>Vascular density</th>
<th>Serum bFGF (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>176 (60–413)</td>
<td>127 ± 18</td>
<td>154 ± 21</td>
</tr>
<tr>
<td>1,000</td>
<td>59 (32–160)*</td>
<td>82 ± 9*</td>
<td>41 ± 13*</td>
</tr>
<tr>
<td>5,000</td>
<td>41 (30–122)*</td>
<td>79 ± 8*</td>
<td>18 ± 9*</td>
</tr>
<tr>
<td>10,000</td>
<td>37 (29–71)*</td>
<td>67 ± 3*</td>
<td>19 ± 8*</td>
</tr>
<tr>
<td>25,000</td>
<td>67 (133–379)</td>
<td>93 ± 11</td>
<td>73 ± 16</td>
</tr>
</tbody>
</table>

Table 3  Systemic therapy of 253J B-V IFN-α bladder tumors in mice by IFN-α-2a

<table>
<thead>
<tr>
<th>IFN-α (units)</th>
<th>Median tumor weight (mg)</th>
<th>Vascularity index</th>
<th>bFGF optical density</th>
<th>Serum bFGF (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>190 (106–497)</td>
<td>114 ± 6</td>
<td>857 ± 46</td>
<td>101 ± 17</td>
</tr>
<tr>
<td>10,000</td>
<td>61 (37–268)*</td>
<td>66 ± 36*</td>
<td>86 ± 43</td>
<td>29 ± 9*</td>
</tr>
</tbody>
</table>

DISCUSSION

Our results demonstrate that the systemic administration of IFN-α-2a to nude mice bearing human TCC in the bladder wall (orthotopic implantation) decreased expression of bFGF, reduced vascular density, and inhibited tumor growth. That these effects occurred independently of the well-known antiproliferative effects of IFN-α (30) was shown by the fact that they were duplicated with tumors produced by IFN-α-resistant TCC cells.

Previous studies with IFN-α have attributed its antitumor effects to induction of cell differentiation, inhibition of cell proliferation, and modulation of host immunity (30). However, IFN-α can also down-regulate expression of proteases (5) and bFGF (9), and hence angiogenesis. Initial evidence for this mechanism was shown in studies with L1210 OR leukemia cells resistant to the antiproliferative effects of IFN, in which treatment of mice with IFN-β reduced tumor-induced vascularity (31). Treatment with IFN-α/β also reduced the neovascularization of human (RT4) and murine (MBT-2) TCC growing s.c. in mice (31). Systemic administration of IFN-α produced regression of life-threatening hemangiomas of infancy (12) and reduction of Kaposi’s sarcoma (10, 11). This effect of IFN-α may be mediated by endothelial cell injury, resulting in intensive tumor necrosis (32).

Because angiogenesis is influenced by the balance between stimulatory and inhibitory molecules released by the tumor and its microenvironment (3–9), any decrease in a stimulatory molecule or an increase in an inhibitory molecule should reduce the level of neovascularization within the tumor. The recent demonstration that IFN-α reduced the transcription and production of bFGF by human tumor cells provided a mechanism by which IFN-α or IFN-β (but not IFN-γ) inhibits tumor-induced neovascularization (9).

The present data confirm the finding that IFN-α can decrease expression of bFGF but not VEGF in human carcinoma cells (9). Exposure to 10–100 units/ml IFN-α down-regulated steady-state mRNA transcripts and protein expression of bFGF in the human TCC cell line 253J B-V. We also found that in vitro incubation with IFN-α inhibited the release of bFGF. Although IFN-α has been shown to inhibit the motility of capillary endothelial cells (29), it did not directly reduce morphogenesis of HUVECs on Matrigel (33). The growth of 253J B-V cells implanted in the bladder of nude mice was inhibited by therapy with a relatively low dose of IFN-α. This reduc-

![Fig. 4. Branching of HUVECs plated on Matrigel. A, CM from 253J B-V cells. B, CM and 100 units/ml IFN-α-2a added at the time of the assay; note the well-formed capillary network in both conditions under light microscopy. C, CM from 253J B-V cells grown in the presence of 100 units/ml IFN-α-2a. Note that capillary branching was abrogated when cells were pretreated with IFN-α-2a. D, CMEM with 5% fetal bovine serum (magnified from X40).](image)

![Fig. 5. IFN-α-2a inhibits bFGF protein production. 253J B-V IFN-α cells were cultured in the presence or absence of IFN-α-2a. Cell-associated bFGF production was measured by ELISA and inhibited by 100 units/ml IFN-α-2a.](image)
Collectively, the present data show that steady-state mRNA expression and protein production of bFGF can be down-regulated in vivo by systemic administration of IFN-α-2a. The exact mechanism by which this occurs remains unknown. IFN-α exerts most of its biological activity by altering the level of gene expression in target cells (7, 34–38). Oncogene expression in human lymphoblastoid cell lines has been shown to be down-regulated by IFN as a result of the regulation of both transcriptional and posttranscriptional events (39, 40). The bFGF promoter contains a single activator protein 1 site, a single
IN VIVO INHIBITION OF bFGF BY IFN-a

IFN-stimulating response element, two negative regulatory sequences, and several G-C boxes, but no TATA box, suggesting that bFGF is a housekeeping gene regulated at the transcriptional and posttranscriptional level (41).

IFN-α and IFN-β have been used to treat a myriad of human malignancies, with only marginal therapeutic benefit. In most of these studies, the IFNs were administered acutely at maximum tolerated doses for short durations (3–4 months; Refs. 35 and 42–44). In contrast, the chronic administration (>8 months) of low-dose IFN-α-2a to patients with malignant hemangiopericytoma, Kaposi’s sarcoma, or life-threatening hemangiomas of infancy has led to remarkable clinical regression of these tumors (10–13). This may be so because continuous local delivery may be required to inhibit angiogenesis (45) and tumor cell invasion (41, 46). Studies designed to determine the exact mechanisms by which IFN down-regulates bFGF could optimize the delivery of this cytokine and enhance its therapeutic efficacy.

REFERENCES


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