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Interferons α and β down-regulate the expression of basic fibroblast growth factor in human carcinomas

(angiogenesis)

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ABSTRACT **We investigated the influence of interferons α, β, and γ (IFN-α, -β, and -γ) on the production of basic fibroblast growth factor (bFGF) by human renal carcinoma cells. The human renal carcinoma cell metastatic line SN12PM6 was established in culture from a lung metastasis and SN12PM6-resistant cells were selected *in vitro* for resistance to the antiproliferative effects of IFN-α or IFN-β. IFN-α and IFN-β, but not IFN-γ, down-regulated the expression of bFGF at the mRNA and protein levels by a mechanism independent of their antiproliferative effects. Down-regulation of bFGF required a long exposure (>4 days) of cells to low concentrations (>10 units/ml) of IFN-α or IFN-β. The withdrawal of IFN-α or IFN-β from the medium permitted SN12PM6-resistant cells to resume production of bFGF. The incubation of human bladder, prostate, colon, and breast carcinoma cells with noncytostatic concentrations of IFN-α or IFN-β also produced down-regulation of bFGF production.**

Angiogenesis, the formation of new blood vessels in and around tumors, is crucial for tumor growth and metastasis (1). Indeed, the intensity of angiogenesis in various human tumors—e.g., prostate and breast—has been correlated with their potential for invasion and metastasis (1, 2). Furthermore, the progression of tumors from benign to malignant is associated with a switch to the "angiogenic phenotype," which is influenced by the interaction of stimulatory and inhibitory molecules produced by the tumor cells and organ-specific environment (1, 3). The induction of vascularization in both cases is mediated by several angiogenic molecules that are released by tumor cells and host cells (1, 3). The level of these angiogenic molecules in tissues depends on the balance between inducers and inhibitors (4).

Studies from our laboratory have shown that the invasive and angiogenic properties of human tumor cells are modulated by specific organ environments (5). For example, the implantation of human renal cell carcinoma (HRCC) into the subcutis of nude mice yields localized noninvasive and poorly vascularized tumors, whereas the implantation of the same cells into the kidney of nude mice results in highly vascularized and invasive neoplasms (6). Moreover, the expression of the angiogenic mitogen basic fibroblast growth factor (bFGF) in primary human renal cancer has been shown to inversely correlate with survival (7) as do elevated levels of bFGF in the urine of patients with this tumor (8).

The expression levels of cytokines have been linked to the growth of blood vessels in lesions such as rheumatoid arthritis or corneal panus (9, 10). This has led us to studies of the effects of cytokines on certain tumors. In particular, we have found that the production of collagenase IV by renal cancer cells or colon cancer cells is down-regulated by skin fibroblasts by a mechanism involving the production of fibroblast interferon (IFN)—e.g., IFN-β (11, 12).

The IFN family consists of three major glycoproteins, IFN-α, IFN-β, and IFN-γ. Although IFNs were first developed as an antiviral agent, it is now clear that they also control cell growth and differentiation, limit oncogene expression, and modulate various aspects of host immunity (13). More recently, clinical data concluded that the systemic chronic administration of IFN-α or IFN-β can produce regression of vascular tumors, including Kaposi sarcoma (14), pulmonary hemangiomatosis (15), and hemangiomas (16). The mechanisms responsible for this remarkable clinical outcome remained unclear.

The purpose of this study was to determine a regulatory mechanism by which IFNs produce the regression of highly vascularized neoplasms. We report here that IFN-α and IFN-β, but not IFN-γ, down-regulate the steady-state gene expression and protein production of the angiogenic molecule bFGF by a mechanism that is independent of antiproliferative activity.

MATERIALS AND METHODS

Cells and Culture Conditions. The metastatic SN12PM6 cell line was established in culture from an HRCC as described (6). The cell line was maintained as a monolayer in Eagle's minimum essential medium (EMEM) (BioWhittaker) supplemented with 10% fetal bovine serum, sodium pyruvate, nonessential amino acids, L-glutamine, and a 2-fold vitamin solution (GIBCO). The SN12PM6-resistant ($SN12PM6^R$) cell line was established in culture by subjecting the SN12PM6 to increasing concentrations of human IFN-α or IFN-β. $SM12PM6^R$ cells are resistant to antiproliferative effects of >10,000 units (U) of either IFN per ml. The metastatic human KM12L4 colon carcinoma variant line was isolated from a Duke stage B2 carcinoma (17). The MDA-435 human breast carcinoma line was the gift of Janet E. Price (M. D. Anderson Cancer Center) (18). The 253-J human bladder carcinoma was the gift of Jerald J. Killion (M. D. Anderson Cancer Center) (19). The metastatic human prostate cancer PC-3M line was isolated as described (20). All cell lines were free of *Mycoplasma* and pathogenic mouse viruses (BioWhittaker).

Reagents. Human IFN-β (Betaseron) (specific activity, 32×10^6 U/mg of protein) was a gift from Berlex Biosciences (Richmond, CA). Human IFN-α-2a (specific activity, 6×10^6 U/mg of protein) was purchased from Hoffmann–La Roche. Human recombinant IFN-γ (specific activity, 2×10^7 U/mg of protein) was purchased from Boehringer Mannheim. The

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Abbreviations: bFGF, basic fibroblast growth factor; IFN, interferon; HRCC, human renal cell carcinoma; ISH, *in situ* hybridization; R, resistant; U, unit(s); GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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different IFNs were diluted to the desired concentrations in supplemented medium.

***In Vitro* Cytostasis Assay.** In all assays, 5×10^3 tumor cells 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 different concentrations of IFN. After 4 days, the antiproliferative activity was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (21) using an MR-5000 96-well microtiter plate reader (Dynatech) at 570 nm. Growth inhibition was calculated from the formula: cytostasis (%) = $[1 - (A/B)] \times 100$, where A is the absorbance of treated cells and B is the absorbance of the control cells.

Enzyme-Linked Immunosorbent Assay (ELISA) for bFGF. Cultures were frozen and stored at −70°C and then thawed. Following cell lysis, expression of cellular bFGF protein was analyzed by ELISA using the Quantikine bFGF ELISA kit (R & D Systems). The concentration of bFGF in unknown samples was determined by comparing the optical density of the samples to the standard curve.

Northern Blot Analysis. mRNA was extracted from 1×10^8 cells (semiconfluent cultures) and analyzed as described (22).

The cDNA probes used in this analysis were a 1.3-kb *Pst* I cDNA fragment corresponding to rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (23) and a 1.4-kb *Eco*RI cDNA fragment of bovine bFGF (24). Each cDNA probe was radiolabeled with the random primer technique using α-^{32}P-labeled deoxyribonucleotide triphosphate (25). The level of expression of the bFGF gene was quantitated by densitometry readings of autoradiograms using the IMAGE QUANT software program (Molecular Dynamics). Each sample measurement was expressed as the ratio of the average area under the curve of bFGF-specific mRNA transcripts to 1.3-kb GAPDH mRNA transcripts.

***In Situ* Hybridization (ISH) for mRNA.** ISH for mRNA in cultured cells was performed as described (26, 27). Briefly, a bFGF-specific oligonucleotide probe was designed complementary to the 5′ end of human bFGF mRNA transcript. The antisense DNA oligonucleotide sequence was 5′-CGG-GAA-GGC-GCC-GCT-GCC-GCC-3′ in the antisense orientation, and the control sense oligonucleotide was 5′-GGC-GGC-AGC-GGC-GCC-TTC-CCG-3′. A d(T)$_{20}$ oligonucleotide was used to verify the integrity and lack of degradation of mRNA in each sample. The oligonucleotide probes were synthesized with six biotin molecules (hyperbiotinylated) at the 3′ end (Research Genetics) (26, 27).

Statistical Analysis. The significance of the data was determined with the Student's t test (two-tailed).

RESULTS

Inhibition of bFGF Production by IFN-α and IFN-β. In the first set of experiments, we incubated SN12PM6 cells for 96 hr in medium (control) or medium containing 100 U of IFN-α or IFN-β or IFN-γ per ml. Cellular and released bFGF protein was analyzed by ELISA and cytostasis was determined by the MTT assay. The data are shown in Fig. 1*A*. At the concentration used, none of the three IFNs produced antiproliferative effects. In contrast, IFN-α and IFN-β, but not IFN-γ, significantly decreased the amount of bFGF protein ($P < 0.01$). All bFGF protein was associated with the cell lysate.

The decrease in bFGF protein was a consequence of inhibition of bFGF gene expression. SN12PM6 cells were incubated in medium or medium containing IFN-α or IFN-β or IFN-γ for 96 hr and then mRNA was isolated and analyzed for bFGF-specific mRNA transcripts by Northern blot analysis (Fig. 1*B*). Control SN12PM6 cells expressed specific transcripts for bFGF mRNA. IFN-α and IFN-β produced 60% and 70% less bFGF mRNA transcripts, whereas IFN-γ did not reduce the mRNA level.

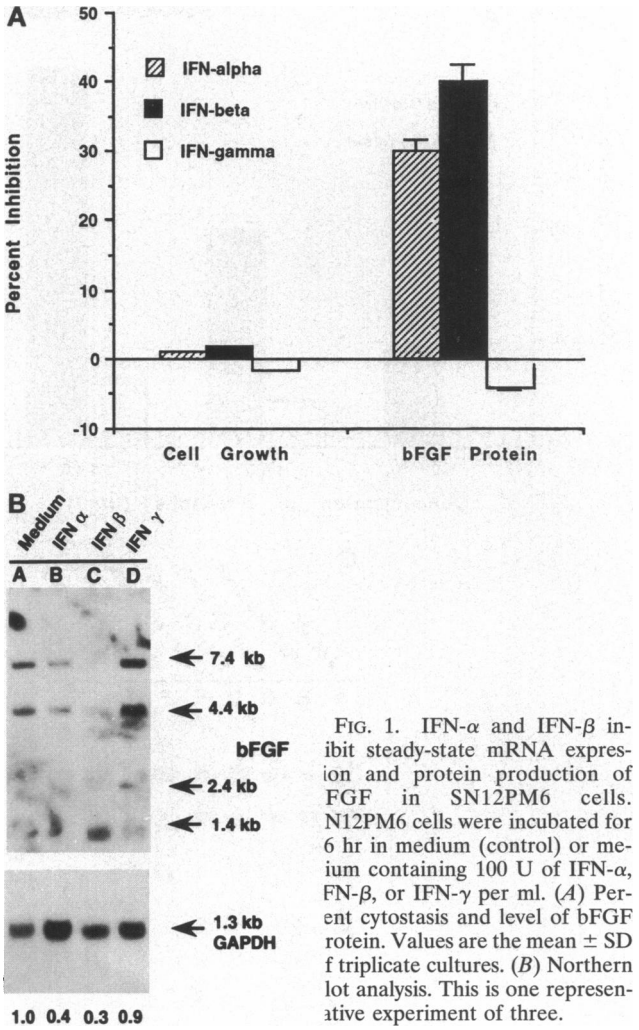

FIG. 1. IFN-α and IFN-β inhibit steady-state mRNA expression and protein production of FGF in SN12PM6 cells. N12PM6 cells were incubated for 6 hr in medium (control) or medium containing 100 U of IFN-α, FN-β, or IFN-γ per ml. (*A*) Percent cytostasis and level of bFGF rotein. Values are the mean ± SD f triplicate cultures. (*B*) Northern lot analysis. This is one representative experiment of three.

The inhibition of bFGF in SN12PM6 cells by IFN-α and IFN-β was dose-dependent (Fig. 2). At the concentrations tested, neither IFN-α nor IFN-β produced significant levels of cytostasis. In contrast, we found a concentration-dependent inhibition in bFGF protein (Fig. 2 *A* and *B*) and steady-state expression of bFGF mRNA (Fig. 2*C*). IFN-γ at 1000 U/ml produced significant cytostasis but did not inhibit bFGF protein (data not shown).

Next, we incubated SN12PM6 cells in medium (control) or medium containing 100 U of IFN-α or IFN-β per ml for different periods. The cultures were maintained in large dishes to avoid confluence and refed every 48 hr with fresh medium and IFNs. After 1, 2, 4, 7, and 14 days, cell growth was analyzed and parallel cultures were washed and frozen at −70°C. Cellular bFGF protein was analyzed by ELISA (Table 1). Neither IFN produced significant cytostasis. In contrast, both IFN-α and IFN-β inhibited bFGF protein after 96 hr of incubation (18% and 30%, respectively; $P < 0.01$). The inhibition in bFGF protein increased with the duration of treatment. By 14 days, it was 52% and 62% ($P < 0.001$) for IFN-α and IFN-β, respectively.

Inhibition of bFGF mRNA by IFN-α and IFN-β in SN12PM6 cells also required incubation exceeding 1 day. Densitometry (Fig. 3) demonstrated that IFN-α and IFN-β produced a respective 90% and 95% reduction in bFGF mRNA transcripts after 7 days of continuous incubation.

ISH for bFGF mRNA. We next examined the modulation of bFGF mRNA in SN12PM6 cells incubated with IFNs on the

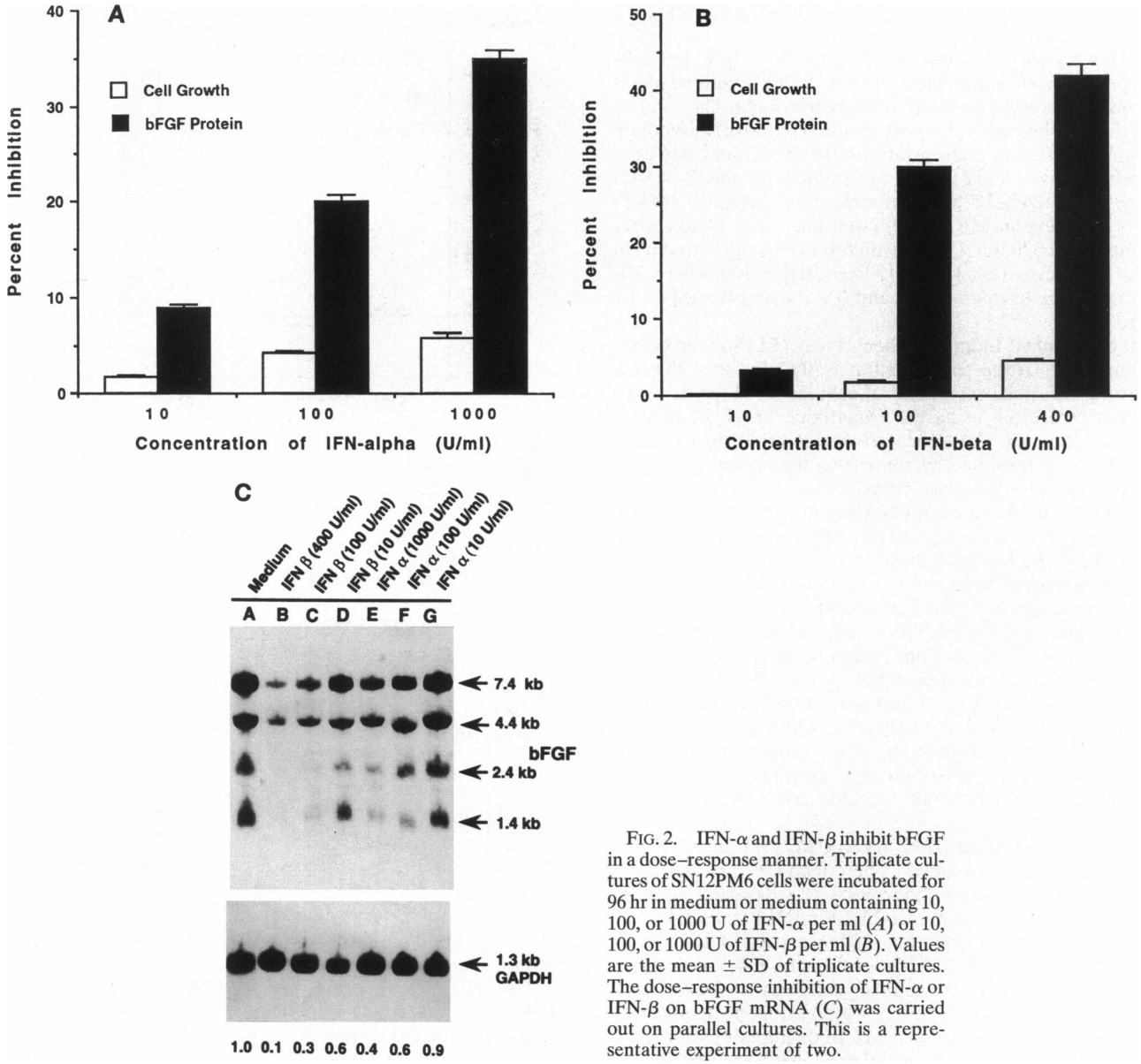

FIG. 2. IFN-α and IFN-β inhibit bFGF in a dose–response manner. Triplicate cultures of SN12PM6 cells were incubated for 96 hr in medium or medium containing 10, 100, or 1000 U of IFN-α per ml (*A*) or 10, 100, or 1000 U of IFN-β per ml (*B*). Values are the mean ± SD of triplicate cultures. The dose–response inhibition of IFN-α or IFN-β on bFGF mRNA (*C*) was carried out on parallel cultures. This is a representative experiment of two.

individual cell level (Fig. 4). Cells cultured in medium (Fig. 4 *A1* and *A2*) or medium containing IFN-α (Fig. 4 *B1* and *B2*) or IFN-β (Fig. 4 *C1* and *C2*) for 7 days were prepared for ISH for mRNA. The integrity of the mRNA in the samples was verified by using a polyd(T)$_{20}$ probe (Fig. 4 *A2, B2*, and *C2*). All three cultures demonstrated an intense histochemical reaction, indicating that the mRNA was not degraded.

SN12PM6 cells cultured in medium showed an intense reaction with the specific bFGF probe (Fig. 4*A1*), whereas the cells treated with IFN-α (Fig. 4*B1*) or IFN-β (Fig. 4*C1*) did not. The control sense bFGF probe did not produce a measurable reaction (data not shown).

Inhibition of bFGF mRNA and Protein by IFN-α and IFN-β Is Independent of Antiproliferative Effects. We next studied the effects of IFN-α and IFN-β on SN12PM6^R cells (selected for resistance to antiproliferative effects of IFN-α and IFN-β). SN12PM6^R cells were cultured in medium (control) or medium containing 0–10,000 U of IFN-β per ml. After 7 days,

Table 1. Inhibition of cellular bFGF in HRCC by IFN-α and IFN-β

Incubation, days	Medium	IFN-α*	IFN-β*
1	282.0 ± 5.0	274.0 ± 8.0 (2.9)	277.0 ± 5.0 (1.8)
2	280.0 ± 4.6	270.2 ± 5.0 (3.5)	229.0 ± 6.0 (7.2)
4	282.0 ± 8.6	261.6 ± 7.0 (18.0)†	197.4 ± 5.0 (30)†
7	265.2 ± 4.8	172.0 ± 8.0 (35.0)†	167.6 ± 10.0 (37)†
14	265.2 ± 4.8	126.0 ± 6.0 (52.0)‡	100.0 ± 5.0 (62)‡

Triplicate cultures of SN12PM6 were incubated in medium (control) or medium containing 100 U of either IFN-α or IFN-β per ml. The medium and IFNs were replaced every 48 hr. Cellular bFGF was assayed using ELISA. The values are mean concentrations ± SD of bFGF expressed as pg/ml per 10^4 viable cells. This is a representative experiment of three.

*Percent inhibition of bFGF protein in parentheses.

†$P < 0.01$, as compared to control cells incubated with medium.

‡$P < 0.001$, as compared to control cells incubated with medium.

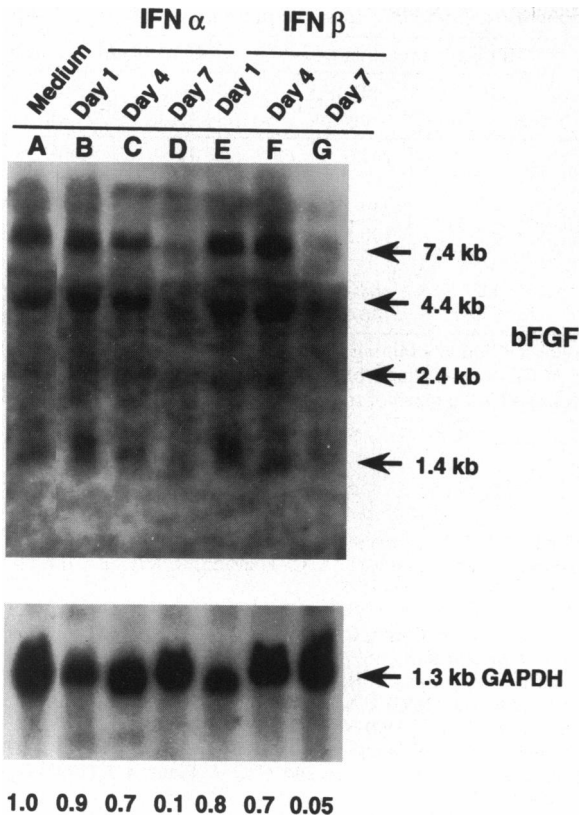

FIG. 3. Kinetics of bFGF mRNA inhibition by IFN-α or IFN-β. SN12PM6 cells were incubated with medium (control) or medium containing 10 U of IFN-α or IFN-β per ml. Cultures were harvested for Northern blot analysis after 1, 4, or 7 days of incubation. Lane A, continuously cultured SN12PM6 cells.

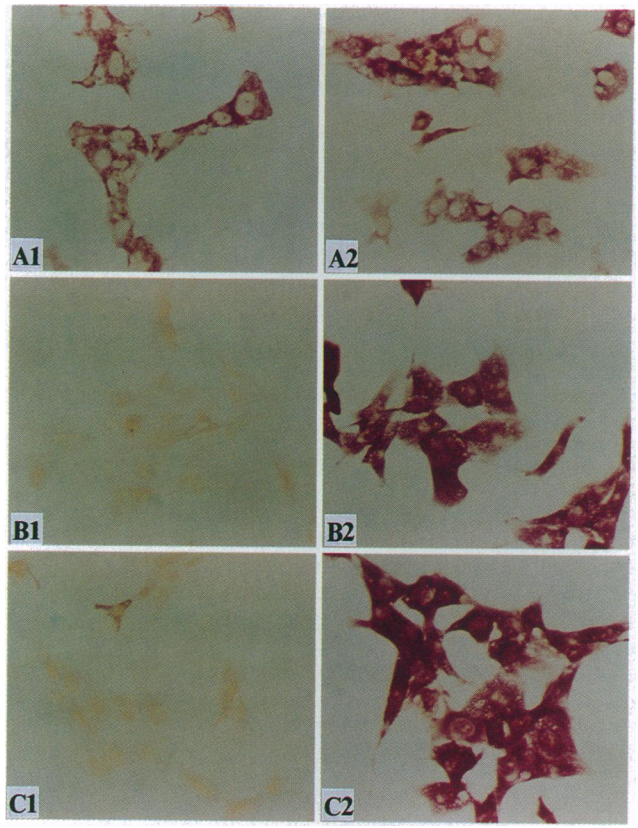

FIG. 4. ISH for bFGF mRNA transcripts in SN12PM6 cells incubated with IFN-α or IFN-β. SN12PM6 cells were incubated for 7 days in medium (*A*) or medium containing 10 U of IFN-α (*B1*) or IFN-β (*C1*) per ml. Hybridization of cells with hyperbiotinylated $d(T)_{20}$ oligonucleotide probe (*A2, B2, C2*) confirmed mRNA integrity and lack of degradation.

cytostasis and cellular bFGF protein were determined (Table 2). SN12PM6^R cells proliferated in the presence of even 10,000 U of IFN-β per ml. In contrast, at 1000 U/ml, IFN-β produced 95% cytostasis in SN12PM6 parental cells. IFN-β produced significant inhibition of bFGF protein in a dose-dependent manner. Once placed in medium devoid of IFN-β, SN12PM6^R cells produced a high amount (255 pg/ml per 10^4 viable cells) of bFGF protein.

Inhibition of bFGF Protein in Human Carcinoma Cell Lines Incubated with IFN-α or IFN-β. In the last set of experiments, we examined whether IFN-α, -β, or -γ could inhibit bFGF protein in human carcinoma cell lines established from prostate (PC-3M), colon (KM12L4), breast (MDA-435), or bladder (253-J) tumors. The cells were incubated in medium (control) or medium containing 10 or 100 U of the different IFNs per ml. After 6 days, IFN-γ did not inhibit bFGF protein (data not shown). At the concentrations used, IFN-α did not produce significant cytostasis but did significantly inhibit bFGF protein (Table 3). At 10 U/ml, IFN-β did not produce significant cytostasis but did significantly inhibit bFGF protein. At 100 U/ml, IFN-β was cytostatic against all cell lines and produced strong inhibition of bFGF protein (Table 3).

DISCUSSION

The outcome of cancer metastasis depends on the intrinsic properties of tumor cells and the responses of the host. Recent studies have suggested that organ-specific host factors can enhance or suppress the growth, vascularization, invasion, and metastasis of human tumors implanted into nude mice (5). Renal cell carcinomas, for example, are hypervascular tumors shown to produce high amounts of bFGF (7, 28). When we implanted HRCC cells into the kidney of nude mice, vascularized tumors producing high levels of bFGF mRNA and protein resulted, whereas their implantation into the subcutis resulted in poorly vascularized tumors that produced low levels of bFGF mRNA and protein. These data, demonstrating an association between the production of bFGF by tumor cells and vascularization, showed that the expression level of bFGF could be influenced by the specific organ microenvironment (29).

The systemic administration of IFN-α or IFN-β can produce regression of vascularized rodent tumors by a mechanism associated with endothelial cell damage leading to necrosis (30, 31). In the present study, the exposure of human carcinoma cells to

Table 2. Production of bFGF protein by SN12PM6^R cells growing in the presence or absence of IFN-β

IFN-β, U/ml	% cytostasis	Cellular bFGF protein,* pg/ml per 10^4 viable cells
0 (medium)	0	328 ± 4
10	−17	243 ± 5 (26)†
100	−3	207 ± 6 (37)†
1,000	2.3	121 ± 8 (63)‡
10,000	6.7	78 ± 4 (76)‡
Medium§		
1 week	0	92 ± 9 (72)‡
2 weeks	0	255 ± 12 (22)
3 weeks	0	295 ± 8 (10)

*Percent inhibition of bFGF protein in parentheses.
†$P < 0.01$.
‡$P < 0.001$.
§SN12PM6^R cells were cultured for 7 days in medium containing 10,000 U of IFN-β per ml and subsequently cultured in medium without IFN-β for the designated times.

Table 3. Antiproliferative effects and inhibition of bFGF protein in human carcinomas cultured *in vitro* in the presence of IFN-α or IFN-β

Treatment	PC-3M prostate cancer		KM12L4 colon cancer		MDA-435 breast cancer		253-J bladder cancer	
	% cytostasis	bFGF,* pg/ml	% cytostasis	bFGF,* pg/ml	% cytostasis	bFGF,* pg/ml	% cytostasis	bFGF,* pg/ml
Medium	0	315.6	0	225.4	0	243.6	0	344.7
IFN-α								
10 U/ml	0	288.0 (10)	6.0	162.8 (28)†	0	219.2 (10)	0	293.0 (15)
100 U/ml	2.4	237.0 (25)†	12.6	158.8 (30)†	6.2	170.5 (30)†	7.8	213.7 (38)†
IFN-β								
10 U/ml	5.6	248.0 (23)†	2.8	106.3 (53)‡	4.0	182.7 (25)†	8.6	279.2 (19)†
100 U/ml	14.0†	211.0 (34)‡	30.0†	62.2 (80)‡	18.0	146.1 (40)‡	21.0‡	172.3 (49)‡

Triplicate cultures of human carcinoma cells were incubated in medium (control) or medium containing IFN-α or IFN-β. The medium and IFNs were replaced every 48 hr. After 6 days, cell proliferation was determined by the MTT assay. Cellular bFGF was assayed using ELISA. Percent cytostasis is the mean of triplicate samples; cellular bFGF = pg/ml per 10^4 viable cells and is the mean of triplicate samples. This is a representative experiment of three.

*Percent inhibition of bFGF protein in parentheses.

†$P < 0.01$.

‡$P < 0.001$.

noncytostatic concentrations of IFN-α or IFN-β but not IFN-γ down-regulated the mRNA expression and protein production of the angiogenic factor bFGF. The inhibition of bFGF mRNA and protein production required long-term exposure (>4 days) of cells to IFNs. Moreover, once IFN was withdrawn, cells resistant to the antiproliferative effects of this cytokine resumed production of bFGF. These observations are also consistent with the clinical experience that IFN-α must be given for many months to bring about involution of hemangiomas (16).

In agreement with earlier reports (32–34), we did not find bFGF protein in the culture medium conditioned by human tumor cells. The nucleotide sequence encoding for bFGF lacks a signal sequence for secretion, and the mechanisms by which bFGF is released from cells, basement membranes, or extracellular matrix are unclear (33, 34). The decrease in bFGF production was not associated with the well-known antiproliferative effects of IFNs. We base this conclusion on the results of several experiments. (*i*) The concentration of IFN necessary to inhibit bFGF mRNA and protein was significantly lower than that necessary to produce cytostasis. (*ii*) Although IFN-γ produced cytostasis against the HRCC (SN12PM6) cells and other human carcinoma cells, it did not inhibit bFGF. (*iii*) The growth of $SN12PM6^R$ cells (selected for resistance to the antiproliferative effects of IFN-α or IFN-β) was unaffected by the presence of even 10,000 U of IFN-α or IFN-β per ml. IFN-α or IFN-β, however, significantly inhibited bFGF in these cells. The effects of IFN-α and IFN-β on bFGF production were not limited to HRCC. We found similar activity in cultures of human colon carcinoma (KM12L4 cells), prostate carcinoma (PC-3M cells), bladder carcinoma (253-J cells), and breast carcinoma (MDA-MB 231 cells).

In conclusion, we show that the steady-state mRNA expression and protein production of bFGF is down-regulated by IFN-α or IFN-β. Since IFN-α and IFN-β are constitutively produced by many host cells, their physiological role in limiting angiogenesis should be investigated.

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1. Folkman, J. (1995) *Nat. Med.* **1**, 27–31.
2. Weidner, N., Carroll, P. R., Flax, J., Blumenfeld, W. & Folkman, J. (1993) *Am. J. Pathol.* **143**, 401–409.
3. Folkman, J. & Klagsbrun, M. (1987) *Science* **235**, 444–447.
4. Folkman, J. (1992) *Semin. Cancer Biol.* **3**, 65–17.
5. Fidler, I. J. (1990) *Cancer Res.* **50**, 6130–6138.
6. Naito, S., von Eschenbach, A. C., Giavazzi, R. & Fidler, I. J. (1986) *Cancer Res.* **46**, 4109–4115.
7. Nanus, D. M., Schmitz-Drager, B. J., Motzer, R. J., Lee, A. C., Vlamis, V., Cordon-Cardo, C., Albino, A. P. & Reuter, V. E. (1994) *J. Natl. Cancer Inst.* **85**, 1597–1599.
8. Nguyen, M., Watanabe, H., Budson, A. E., Richie, J. P., Hayes, D. F. & Folkman, J. (1994) *J. Natl. Cancer Inst.* **86**, 356–361.
9. Harris, E. D., Jr. (1990) *N. Engl. J. Med.* **322**, 1277–1288.
10. Plouet, J. & Gospodarowicz, D. J. (1989) *Cell Physiol.* **141**, 392–399.
11. Fabra, A., Nakajima, M., Bucana, C. D. & Fidler, I. J. (1992) *Differentiation* **52**, 101–110.
12. Gohji, K., Nakajima, M., Fabra, A., Bucana, C. D., von Eschenbach, A. C., Tsuruo, T. & Fidler, I. J. (1994) *Jpn. J. Cancer Res.* **85**, 152–160.
13. Gutterman, J. U. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 1198–1205.
14. Real, F. X., Oettgen, H. F. & Krown, S. E. (1986) *J. Clin. Oncol.* **4**, 544–551.
15. White, C. W., Sondheimer, H. M., Crouch, E. C., Wilson, H. & Fan, L. L. (1989) *N. Engl. J. Med.* **320**, 1197–1200.
16. Ezekowitz, R. A. B., Mulliken, J. B. & Folkman, J. (1992) *N. Engl. J. Med.* **326**, 1456–1463.
17. Morikawa, K., Walker, S. M., Nakajima, M., Pathak, S., Jessup, J. M. & Fidler, I. J. (1988) *Cancer Res.* **48**, 6863–6871.
18. Zhang, R. D., Fidler, I. J. & Price, J. E. (1991) *Invasion Metastasis* **11**, 204–215.
19. Killion, J. J., Bucana, C. D., Frangos, D. N., Price, J. E. & Fidler, I. J. (1989) *J. Natl. Cancer Inst.* **81**, 1387–1391.
20. Stephenson, R. A., Dinney, C. P. N., Gohji, K., Ordonez, N. G., Killion, J. J. & Fidler, I. J. (1992) *J. Natl. Cancer Inst.* **84**, 951–957.
21. Alley, M. C., Scudieno, D. A., Monks, A., Hursey, M. L., Czerwinski, M. J., Fine, D. L., Abbott, B. J., Mayo, J. G., Schoemaker, R. H. & Boyd, M. R. (1988) *Cancer Res.* **48**, 589–602.
22. Radinsky, R., Kraemer, P. M., Raines, M. A., Kung, H. J. & Culp, L. A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5143–5147.
23. Fort, P., Marty, L., Piechaczyk, M., Sabrouty, S. E., Dani, C., Jeanteur, P. & Blanchard, J. M. (1985) *Nucleic Acids Res.* **13**, 1431–1442.
24. Rogelj, S., Weinberg, R. A., Fanning, P. & Klagsbrun, M. (1988) *Nature (London)* **331**, 173–175.
25. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6–13.
26. Radinsky, R., Bucana, C. D., Ellis, L. M., Sanchez, R., Cleary, U. R., Brigati, D. J. & Fidler, I. J. (1993) *Cancer Res.* **53**, 937–943.
27. Bucana, C. D., Radinsky, R., Dong, Z., Sanchez, R., Brigati, D. J. & Fidler, I. J. (1993) *J. Histochem. Cytochem.* **41**, 499–506.
28. Mydlo, J. H., Heston, W. D. & Fair, W. R. (1988) *J. Urol.* **140**, 1575–1579.
29. Singh, R. K., Bucana, C. D., Gutman, M., Fan, D., Wilson, M. R. & Fidler, I. J. (1994) *Am. J. Pathol.* **145**, 365–374.
30. Sidky, Y. A. & Borden, E. C. (1987) *Cancer Res.* **47**, 5155–5161.
31. Dvorak, H. F. & Gresser, I. (1992) *J. Natl. Cancer Inst.* **81**, 497–502.
32. Burgess, W. H. & Maciag, T. (1989) *Annu. Rev. Biochem.* **58**, 575–606.
33. Abraham, J. A., Mergia, A., Whang, J. L., Tumolo, A., Friedman, J., Hjerrild, K. A., Gospodarowicz, D. & Fiddes, C. (1986) *Science* **233**, 545–548.
34. Vlodavsky, I., Korner, G., Ishai-Michaeli, R., Bashkin, P., Bar-Shavit, R. & Fuks, Z. (1990) *Cancer Metastasis Rev.* **9**, 203–226.