Enhancement of the Growth of Human Osteosarcoma Cells by Human Interferon-γ

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ABSTRACT. It is well known that interferons inhibit cell growth. However, we found that human interferon-γ (HuIFN-γ) enhanced the growth of human osteosarcoma cells, HOS-Y1 cells, in a dose-dependent manner. This enhancing effect was found only under the following conditions: when the cells were precultured for 2 or 3 days and then treated with HuIFN-γ for 2, 3, or 4 days, and when the cells were seeded at a density of 1,000 or 2,000 cells/well. The degree of enhancement of cell growth was maximum when the cells were precultured at a density of 1,000 cells/well for 3 days and then treated with HuIFN-γ for 2 days. The enhancing effect of HuIFN-γ disappeared in the presence of anti-HuIFN-γ antibody. In addition, it was found that the conditioned medium from HOS-Y1 cells enhanced the growth of HOS-Y1 cells, and that the conditioned medium from HOS-Y1 cells cultured with HuIFN-γ enhanced the cell growth more than that from cells cultured without HuIFN-γ. Epidermal growth factor (EGF), acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), and transforming growth factor-β (TGF-β) did not enhance the growth of HOS-Y1 cells. These results suggest that HuIFN-γ enhanced the cell growth by augmenting the production of unknown growth factor(s) in HOS-Y1 cells via an autocrine mechanism.

Interferons (IFN), as well as having antiviral activity, also exhibit various biological activities, such as antitumor activity (1); suppression of antibody formation; suppression of delayed-type hypersensitivity; augmentation of the cytotoxicity of natural killer (NK) cells, killer (K) cells and killer T cells (11); activation of macrophages and neutrophils (8, 12); and the induction of cell differentiation (10).

The inhibitory effect of IFN on cell growth, first reported by Paucker et al. (7), is particularly well known and has been investigated in detail. It has also been recognized that IFN modulate fundamental cell function and physiology (15).

However, except for the study of Deshpande et al. (2), there are no reports of the enhancement of malignant cell growth by IFN. In the present study, since we found that human (Hu) IFN-γ enhanced HOS-Y1 cell growth, we examined the conditions under which this occurred and analyzed its underlying mechanism.

MATERIALS AND METHODS

Cell culture. The HOS-Y1 cell line, established from a human osteosarcoma by one of the authors (Yamaguchi) in 1982, was cultured in RPMI1640 supplemented with 10% fetal bovine serum (Cleveland, Ohio, U.S.A.), 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin in 75-cm² flasks.

IFN and anti-HuIFN-γ antibody. Recombinant HuIFN-αA with a specific activity of 2 × 10⁶ international units (IU)/mg protein was provided by Takeda Chemical Industries Ltd., Osaka, Japan. Recombinant HuIFN-β and HuIFN-γ, with a specific activity of 4 × 10⁶ IU/mg protein, were supplied by Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan.

Mouse monoclonal antibody against HuIFN-γ (provided by Green Cross Co. Ltd., Osaka, Japan), at more than 3,000 neutralizing units/ml, was used.

HOS-Y1 cells were precultured in 96-well microplates (Corning Laboratory Sciences Co., N.Y., U.S.A.) at a density of 1,000 cells/0.1 ml/well for 3 days, after which 20 or 200 IU/0.05 ml HuIFN-γ and/or 0.05 ml anti-HuIFN-γ antibody were added to the culture. After incubation at 37°C for a further 2 days, the growth of HOS-Y1 cells was determined by the methylene blue assay method described below.

Preparation of conditioned medium. HOS-Y1 cells were precultured in 96-well microplates (1,000 cells/0.1 ml/well) for 3 days, and incubated with 0.1 ml of fresh medium containing HuIFN-γ at 2,000 IU/ml, or with fresh medium alone, for 2 days. The supernatants were harvested and then centrifuged at 10,000 rpm for 30 min, and were used as the conditioned medium.
Growth factors. Epidermal growth factor (EGF), extracted from human urine (Japan Chemical Research Co. Ltd., Kobe, Japan) was dissolved in distilled water (100 mg/ml). Acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor (bFGF) were purchased from Peninsula Laboratories Inc., CA., U.S.A.. Transforming growth factor-β1 (TGF-β1) was purchased from King Brewing Co. Ltd., Tokyo, Japan.

Cell growth assay. The growth of HOS-Y1 cells was determined by the methylene blue assay method (14). Briefly, the cells, seeded at a density of 500, 1,000, or 2,000 cells/0.1 ml/well into 96-well microplates, were incubated for 1, 2, or 3 days, after which 0.1 ml/well of fresh medium containing 0 to 4,000 IU/ml of IFN was added to the culture. After 2- to 5-day incubation, 0.02 ml of 25% glutaraldehyde was added to each well for 15 min. After the cells were washed, fixed cells were stained with 0.1 ml of 0.05% methylene blue solution for 15 min. Excess methylene blue was removed, and the plates were dried. To extract the methylene blue, 0.2 ml of 0.33 N HCl was added to each well, and the plates were agitated with a mixer. The absorbance at 665 nm was then measured with an ELISA microplate reader (Titertek Multiskan Plus MK II,
Cell Growth Enhanced by Interferon-γ

Table I. Influence of different culture conditions on the enhancement of HOS-Y1 cell growth by HuIFN-γ.

<table>
<thead>
<tr>
<th>Preculture duration (days)</th>
<th>Viable cell numbers (% of control)</th>
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<tr>
<td></td>
<td>Days after treatment with HuIFN-γ (500 IU/ml)</td>
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<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>77.4±15.9</td>
</tr>
<tr>
<td>2</td>
<td>158.0±10.1***</td>
</tr>
<tr>
<td>3</td>
<td>206.9±16.6***</td>
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a) HOS-Y1 cells were precultured at a density of 1,000 cells/well.
*p<0.05, **p<0.01, ***p<0.005, ****p<0.001 (compared with control).

RESULTS

Enhancing effects of interferons on the growth of HOS-Y1 cells. HOS-Y1 cells were precultured in 96-well microplates for 3 days and then treated with 500 IU/ml HuIFN-γ for 2, 3, 4, or 5 days. It was found that HuIFN-γ significantly enhanced the growth of HOS-Y1 cells before the cells became confluent (Fig. 1). HOS-Y1 cells were treated with HuIFN-αA, HuIFN-β, or HuIFN-γ at doses of 7.8, 15.6, 31.3, 62.5, 125, 250, 500, 1,000 and 2,000 IU/ml for 2 days; the viable cells were then counted. HuIFN-γ at concentrations of more than 7.8 IU/ml, significantly enhanced the growth of HOS-Y1 cells in a dose-dependent manner, compared with the HuIFN-γ-untreated control group, and the degree of enhancement was maximal at 500 IU/ml (p<0.001). On the other hand, HuIFN-αA and HuIFN-β did not affect the cell growth (Fig. 2).

HOS-Y1 cells (1,000 cells/well) were precultured for 1, 2, or 3 days, and then incubated with HuIFN-γ at 500 IU/ml for 2, 3, or 4 days. HuIFN-γ significantly enhanced the growth of HOS-Y1 cells, compared with the HuIFN-γ-untreated control group, when the cells were precultured for 2 or 3 days and treated with HuIFN-γ for 2, 3, or 4 days (Table I).

When HOS-Y1 cells were precultured for 3 days at a cell density of 500, 1,000, or 2,000 cells/well and cultured with HuIFN-γ at 1,000 IU/ml for 2 days, the cell growth was 110.6%, 208.9% (p<0.001), and 161.2% (p<0.001), respectively, of that in the HuIFN-γ-untreated control group.

Effects of anti-HuIFN-γ antibody on enhancement of cell growth by HuIFN-γ. HOS-Y1 cells were precultured, at a density of 1,000 cells/well, for 3 days, and 20 or 200 IU/0.05 ml of HuIFN-γ and/or 0.05 ml of anti-HuIFN-γ antibody were added before cell growth was examined. The enhancement of cell growth by HuIFN-γ completely disappeared in the presence of anti-HuIFN-γ antibody (Table II).

Enhancing effects of the conditioned media on HOS-Y1 cell growth. HOS-Y1 cells were cultured at a density of 100 cells/well (at which their growth was not enhanced by IFN-γ [data not shown]) with conditioned media (50%) from HuIFN-γ-untreated and treated cells for 7 days, and cell growth enhancement was examined. Both conditioned media significantly enhanced (p<0.001) the growth of HOS-Y1 cells. The conditioned medium from HuIFN-γ-treated HOS-Y1 cells enhanced this growth to a greater extent than the conditioned medium from the HuIFN-γ-untreated cells (Fig. 3).

Effects of several growth factors on the growth of HOS-Y1 cells. HOS-Y1 cells (100 cells/well) were cultured with 0 to 10,000 pg/ml of EGF, aFGF, bFGF, or TGF-β1 for 7 days. Acidic FGF and basic FGF did not affect HOS-Y1 cell growth at any doses (Fig. 4), while, on the other hand, high concentrations of EGF and TGF-β1 inhibited HOS-Y1 cell growth, although low concentrations (less than 100 pg/ml) had no effect.

DISCUSSION

IFN plays an important role in the modulation of cell growth and differentiation, inhibiting cell growth (7) and inducing cell differentiation (4). It has been reported that IFN inhibited or did not affect the growth of osteosarcoma cell (3); IFN has also been shown to suppress osteosarcoma growth in mice (13) and to sup-
Fig. 3. Enhancement of cell growth by conditioned media obtained from HOS-Y1 cells cultured in the presence or absence of HuIFN-γ. The cells (100 cells/well) were incubated with fresh medium (control), 1,000 IU/ml of HuIFN-γ, 50% conditioned medium from HuIFN-γ-untreated HOS-Y1 cells, 50% conditioned medium from HuIFN-γ-untreated HOS-Y1 cells plus 1,000 IU/ml of HuIFN-γ, or 50% conditioned medium from HuIFN-γ-treated HOS-Y1 cells. **p<0.01 (growth enhancing effects of conditioned media from HuIFN-γ-untreated or -treated HOS-Y1 cells compared), ****p<0.001 (compared with control).

Fig. 4. Effects of various growth factors on the growth of HOS-Y1 cells. The cells (100 cells/well) were cultured with 0 to 10,000 pg/ml of EGF, aFGF, bFGF, or TGF-β, for 7 days.
press metastasized osteosarcoma in the lung (5). In contrast, however, Kelly et al. showed that pretreating malignant cells with IFN enhanced their metastatic potential in mice (6, 9). Thus, IFN does not always suppress tumor development, but rather, can enhance it under certain conditions.

In the present study, we found that HuIFN-γ enhanced the growth of HOS-Y1 cells. This effect may have been due to IFN itself, since the HuIFN-γ used in this study was highly purified (specific activity: 4 × 10⁷ IU/mg protein) and the enhancement was inhibited by anti-HuIFN-γ antibody.

Similar results have been reported by Deshpande et al., who found that HuIFN-γ stimulated the growth of human prostatic hyperplasia cells in primary culture (2), although the mechanism underlying this effect was not clarified.

In the present study, by focusing on known growth factors, i.e., EGF, FGF, and TGF, we analyzed the mechanism underlying the enhancing effect of HuIFN-γ on osteosarcoma cells. As TGF is an autocrine growth factor, it is possible that HuIFN-γ enhances cell growth by affecting the secretion and/or the action of TGF or other growth factors. However, our results showed that EGF, FGF, and TGF did not enhance the growth of HOS-Y1 cells, but rather that EGF and TGF inhibited it at high concentrations, which results suggest that these growth factors are not related to the enhancing effects of HuIFN-γ on cell growth.

We also examined the effects of unknown growth factor(s) secreted in the conditioned medium from HOS-Y1 cells. The conditioned medium obtained from the HOS-Y1 cell culture enhanced the growth of HOS-Y1 cells, which suggests that this unknown substance(s) might be an autocrine growth factor(s). We also found that the conditioned medium obtained from HOS-Y1 cells treated with HuIFN-γ enhanced the cell growth more strongly than the conditioned medium of HOS-Y1 cells not treated with HuIFN-γ. Moreover, when HuIFN-γ was added together with the conditioned medium to HOS-Y1 cells, cell growth was not enhanced, compared with that in cultures incubated with the conditioned medium alone, and HuIFN-γ alone did not enhance cell growth at an initial density of 100 cells/well (data not shown). These results suggest that IFN enhanced cell growth by augmenting the production of unknown growth factor(s), but not by enhancing its activity.

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REFERENCES


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