Short Communications

Antiproliferation and Colony-forming Inhibition Activities of Recombinant Feline Interferon (rFeIFN) on Various Cells *in vitro*

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ABSTRACT

The antiproliferative and colony inhibiting activities of recombinant feline interferon (rFeIFN Type I) against various cells *in vitro* were examined. Feline and canine cells were both sensitive to rFeIFN. To inhibit the growth of feline cells by 50% approximately 5 × 10^2 to 1 × 10^3 U/mL rFeIFN was required and maximum activity was achieved at a concentration of 1 × 10^5 U/mL. Approximately 5 × 10^3 to 5 × 10^4 U/mL rFeIFN was necessary to inhibit the growth of canine cells by 50%. The antiproliferative and colony inhibiting activities of rFeIFN on canine cells appeared to be cell-specific and dose-dependent. However, human, monkey and hamster cells were resistant to rFeIFN. We suggest that rFeIFN might be useful for treatment of feline and some canine neoplastic conditions.

RÉSUMÉ

La présente étude *in vitro* a porté sur l'habilité de l'interféron félin recombinant (IFNFer) à inhiber l'établissement et la prolifération de diverses lignées cellulaires. Les cellules d'origine canine et féline étaient toutes deux sensibles à l'IFNFer. Une quantité approximative de 5 × 10^2 à 1 × 10^3 U/mL d'IFNFer était nécessaire pour inhiber de 50 % la croissance de cellules félines et une concentration de 1 × 10^5 U/mL permettait d'atteindre une activité maximale. Pour les cellules canines, environ 5 × 10^2 à 1 × 10^3 U/mL d'IFNFer étaient nécessaires pour inhiber de 50 % la croissance. Les effets de l'IFNFer envers les cellules canines semblaient être spécifiques aux types cellulaires et dépendants de la dose. Toutefois, les cellules d'humains, de singes et de hamsters étaient résistantes à l'IFNFer. Il se pourrait que l'IFNFer puisse être utile pour le traitement de conditions néoplasiques chez les chats et chiens. (*Traduit par Dr Serge Messier*)

It is well known that interferon (IFN Type I) has antiviral and antitumor effects both *in vitro* and *in vivo*. These activities have been recognized to be species-specific (4,13) and have been widely studied (5,13–16). The antitumor effect of IFN in combination with chemotherapy (2,19), other biological agents (7,10) and radiation (1) have been reported and may be promising for future treatment (12). Recently, attention has focused on the activities of animal IFN and some studies have investigated the antiviral activity of feline IFN (FeIFN) (9,22). Recombinant feline interferon (rFeIFN) is the only feline interferon produced in sufficient amounts for purification and amino acid sequence analysis and with a known nucleotide sequence (21,25). The rFeIFN has been characterized as type I IFN (24) and classified as a ω type (25). Antiviral activity of rFeIFN has been reported *in vitro* against feline calicivirus (FCV), feline herpesvirus (FHV), feline leukemia virus, feline infectious peritonitis virus and feline immunodeficiency virus; and clinical signs experimentally caused by FHV and FCV were markedly reduced (25,26). A therapeutic effect of rFeIFN against FCV infection (23), and against feline viral rhinotracheitis (FVR) has been recognized (18). However, to our knowledge, there have been no reports on the antiproliferative and colony inhibiting activities of rFeIFN either *in vivo* or *in vitro*. We therefore undertook *in vitro* studies of rFeIFN activities against various neoplastic cells. Our objectives were 1) to determine whether rFeIFN inhibited the proliferation and colony-forming ability of feline neoplastic cells, and 2) to compare the antiproliferative and colony-forming inhibition effects of rFeIFN against various cells other than feline cells.

Eight cell lines were used in this study: two canine cell lines (MCA-B1 derived from oral acanthomatous epulis, and MCM-B2 derived from a benign mixed tumor of the mammary gland), two hamster cell lines (HmLu-1 derived from lung, and BHK-21 derived from kidney) provided by the Institute of Animal Health, Japan, through the Miyazaki Prefecture Livestock Service, Sadowara, Miyazaki, Japan, the Vero cell line (derived from green monkey kidney) supplied by Tokyo University Institute of Medical Science, and the Chang cell line (derived from human liver) provided by the Department of Microbiology, Miyazaki University. Two feline cell lines derived from surgical specimens of squamous cell carcinoma were maintained by serial passage of cell cultures in our laboratory.

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Submitted May 4, 1994.

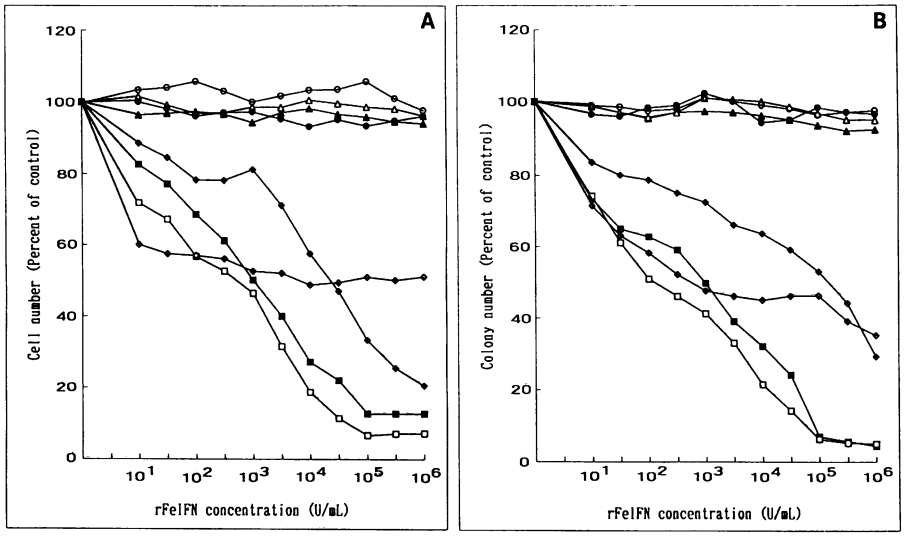

Fig. 1. Comparative effects of the dose-response relationship between rFeIFN and cell proliferation (A) and colony formation (B). —•— MCA-B1 cells, —○— MCM-B2 cells, —□— feline fibroblasts derived from squamous cell carcinoma, —■— feline squamous cell carcinoma, —▲— HmLu-1 cells, —●— Vero cells, —○— Chang cells, —△— BHK-21 cells. Each point represents the mean of three different determinations.

All cells were cultivated in 24-well tissue culture flasks (Falcon 3047; Becton Dickinson, New Jersey) at densities of 5×10^2 cells/mL in Dulbecco's modified Eagle's medium and Ham's nutrient mixture F-12 (Sigma, St. Louis, Missouri) for MCA-B1 and MCM-B2 cell lines; Eagle's minimum essential medium (Nissui, Tokyo, Japan) for HmLu-1 and BHK-21 cell lines, Eagle's basal medium (Sigma) for Chang cell line and Medium 199 (Nissui) for Vero cell line. All cell lines were supplemented with 10% fetal calf serum (FCS) except for Vero cell with 5% FCS, 100 IU/mL penicillin and 100 μg/mL streptomycin. Various concentrations from 10^1–10^6 U/mL of rFeIFN (KT-80, Toray Industries Inc., Tokyo, Japan) were added to each flask. Test and control flasks without rFeIFN were incubated at 37°C in a CO_2 incubator. After confluence had been attained in the control wells, all wells were trypsinized and the total number of cells per culture was counted with a hemacytometer using trypan blue dye, the results from triplicate wells being averaged.

Colony-forming inhibition assay was performed by plating a single-cell suspension at a density of 5×10^2 cells/mL onto 35 × 10-mm plastic dishes (Falcon, Becton Dickinson, New Jersey), supplemented with FCS, penicillin and streptomycin, and various concentrations of rFeIFN. After three to six days (depending on the cell line) of incubation at 37°C in a CO_2 incubator, cell colonies were counted directly using a phase-contrast microscope in triplicate dishes and the results averaged. Only colonies containing more than 50 cells were counted (4,6).

The average total cell number from control flasks for each cell type were 44.3×10^4 cells/mL for feline squamous cell carcinoma, 64.6×10^4 cells/mL for feline fibroblasts derived from squamous cells carcinoma, 308.3×10^4 cells/mL for MCM-B2, 112.6×10^4 cells/mL for MCA-B1, 88.3×10^4 cells/mL for HmLu-1, 51.3×10^4 cells/mL for BHK-21, 24.7×10^4 cells/mL for Vero and 12.3×10^4 for Chang cells. An antiproliferation effect of rFeIFN against feline and canine cells was observed (Fig. 1a). Fifty percent cell growth inhibition was achieved when approximately 5×10^2 to 1×10^3 U/mL rFeIFN was added to the cultures of feline cells, whereas the maximum inhibition (88–93%) occurred when the cells were exposed to 1×10^5 U/mL. Similar results were also obtained for canine MCM-B2 cells, although around 5×10^3 U/mL rFeIFN was required to inhibit the cell growth by 50%. However, for MCA-B1 cells, 50% inhibition occurred when about 5×10^4 U/mL rFeIFN was added. We were unable to determine the maximum concentration of rFeIFN necessary to inhibit MCA-B1 cell growth, because we did not test concentrations higher than 1×10^6 U/mL (Fig. 1a). In contrast, hamster, monkey and human cell lines were resistant to the antiproliferation activity of rFeIFN. The maximum degree of growth inhibition for these four cell lines were 6% for HmLu-1, 4% for BHK-21, 7% for Vero and 2.5% for Chang cells. A slight increase in cell number was detected on BHK-21, Vero cells, and Chang cells.

The average total colony number from control flasks for feline squamous cell carcinoma, feline fibroblast derived from squamous cell carcinoma, MCM-B2, MCA-B1, HmLu-1, BHK-21, Vero and Chang cells were 38.8, 54.3, 85.3, 83.6, 52.0, 32.7, 17.3 and 27.0, respectively. A colony-forming inhibition effect of rFeIFN was also recognized on feline and canine cells, showing a pattern similar to that of the antiproliferation effect. To inhibit 50% of the colony-forming capability of feline cells, approximately 1×10^2 to 1×10^3 U/mL rFeIFN was required, whereas 1×10^3 to 1×10^5 U/mL was necessary for canine cells. More than 95% of feline cells and 65% of canine cell colonies disappeared after exposure to 1×10^6 U/mL of rFeIFN. On the other hand, hamster, monkey and human cells showed insensitivity to the colony-forming inhibition activity of rFeIFN (Fig. 1b). Colony-forming capability was reduced by only 2.5–7% when 1×10^6 U/mL rFeIFN was added to the HmLu-1, BHK-21, Vero and Chang cells. A slight increase in colony number on BHK-21, Vero and Chang was also observed.

In this study rFeIFN showed very effective suppression of cell proliferation and colony-forming ability of feline squamous cell carcinoma *in vitro*. There are two major cellular mechanisms of the antitumor action of IFN: a direct antiproliferative effect and an immunomodulatory effect (3,4,17). Our present results indicated that rFeIFN directly inhibited the proliferation of tumor cells, thus supporting the first mechanism. A concentration of 1×10^5 U/mL seemed to be the maximum at which rFeIFN

inhibited the growth of feline cells, because concentrations higher than this gave the same response. Our comparative study showed that suppressive activities of rFeIFN also occurred in two canine neoplastic cells, although a slightly higher concentration was required. On the other hand, a suppressive effect of rFeIFN was not observed in hamster, monkey and human cells. The dose and target cell dependency of IFN activity was also observed in human interferon (2,15). Garbe and Krasagakis (10) reported that the interferon species also played an important role. The fact that antiproliferation and colony-forming inhibition activities of rFeIFN were slightly different between feline and canine cells and the slightly higher dose-dependency of canine cells suggests the possibility that some differences are dependent on the cell type, rather than the species of origin. Our observations indicate that the antiproliferation and colony-forming inhibition activities of rFeIFN are common to both feline (squamous cell carcinoma and fibroblast) and canine (mammary mixed tumor and canine acanthomatous epulis) cells. We suggest that rFeIFN might be useful for treatment of feline, but also some canine neoplastic cells. Further observation *in vitro* with large number of cell lines and also *in vivo* trial are required.

ACKNOWLEDGMENTS

We are grateful to Toray Industries Inc., Tokyo, Japan for supplying rFeIFN (KT-80). We thank Miyazaki Prefecture Livestock Service, Sadowara, Miyazaki, Japan for providing HmLu-1 and BHK-21 cell lines and to Tokyo University Institute of Medical Science for the kind gift of Vero cell line.

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