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# Interleukin-6 (IL-6) functions as an in vitro autocrine growth factor in renal cell carcinomas

Shunji Miki*, Masayuki Iwano°, Yoshitsugu Miki°, Masahiro Yamamoto*, Bo Tang°, Kiyoshi Yokokawa+, Takao Sonoda+, Toshio Hirano° and Tadamitsu Kishimoto°

*Third Department of Internal Medicine, Nissei Hospital, 6-3-8 Itachibori, Nishiku, Osaka 550, °Division of Immunology, Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamada-oka, Suita, Osaka 565 and +Department of Urology, Osaka University Hospital, 1-1-50, Fukushima, Fukushima-ku, Osaka 553, Japan

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Interleukin-6 (IL-6) was found to be a growth factor of renal cell carcinomas. Furthermore, renal cell carcinomas freshly isolated from the patients expressed mRNA of IL-6 and secreted biologically active IL-6 under the culture conditions where the tumor cells could grow, but they did not produce IL-6 nor proliferate in the absence of fetal calf serum. The production of IL-6 by the tumor cells was also demonstrated by immunostaining of the IL-6-producing cells utilizing anti-IL-6 antiserum. Moreover, anti-IL-6 antiserum specifically inhibited the in vitro tumor growth. All data indicated that IL-6 functions as an in vitro autocrine growth factor of renal cell carcinomas.

Interleukin-6; Autocrine growth factor; (Renal cell carcinoma)

## 1. INTRODUCTION

Interleukin-6 (IL-6) is a cytokine acting on a variety of cells including lymphocytes, hepatocytes, haematopoietic stem cells and nerve cells as either a growth factor, a growth inhibitor or a differentiation inducing factor [1,2]. Furthermore, IL-6 was demonstrated to be a growth factor for plasmacytoma, myeloma and certain T cell lymphoma cells [3-5] and was suggested to be involved in the oncogenesis of these tumors [2]. Since IL-6 is produced by a variety of cells and acts as a growth factor for various kinds of cells, there is the possibility that IL-6 acts as a growth factor for the other kinds of tumor and is involved in the oncogenesis of those tumors.

Certain patients with renal cell carcinoma show fever, an increase in serum acute phase proteins, secondary amyloidosis, hepatic disorder and leukemoid reactions [6,7], suggesting that tumor cells may produce substances which can induce acute phase protein synthesis. Since IL-6 was found to be a major factor which regulates the biosynthesis of acute phase proteins in hepatocytes [8-10], it was examined whether IL-6 is produced by renal cell carcinoma cells and acts as their own growth factor. In this report, we show that IL-6 functions as an autocrine growth factor for renal cell carcinomas in vitro.

*Correspondence address:* T. Kishimoto, Division of Immunology, Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamada-oka, Suita, Osaka 565, Japan

## 2. MATERIALS AND METHODS

### 2.1. *Reagents*

Recombinant IL-6 was produced in *E. coli* as described previously [11,12]. Goat anti-IL-6 antiserum was made by immunizing recombinant IL-6, and was kindly provided by Dr Poole, National Biological Standards Board, WHO. Monoclonal antibody, K 2.7 specific to renal cell carcinoma [13] was a kind gift from Dr Kinouchi, The Center of Adult Diseases, Osaka, Japan. Monoclonal antibody, M206, specific to macrophages was described previously [14].

### 2.2. *Cells and culture conditions*

Renal cell carcinoma cell line, ACHN, was obtained from the ATCC (Maryland, USA). The tumor mass obtained from the patients with renal cell carcinoma was minced and trypsinized to

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obtain single cell suspension. These cells were cultured in RPMI 1640 containing 10% fetal calf serum (FCS) (Flow Laboratories Inc., USA). The spindle shaped cells were eradicated by needle puncture. Adherent cells were harvested after treatment with 0.25% trypsin and 0.02% EDTA and used as renal cell carcinoma cells.

2.3. *Determination of IL-6 activity*

IL-6 activity was examined utilizing the IL-6-dependent murine hybridoma, MH60.BSF2, as described previously [15].

2.4. *Northern blot analysis*

RNA was purified by the guanidine method and subjected to Northern blot analysis utilizing $^{32}$P-labeled *TaqI-BanII* fragment of pBSF2.38 insert cDNA as described previously [11].

2.5. *Immunostaining of renal cell carcinoma cells*

Renal cell carcinoma cells were stained with anti-renal cell carcinoma antibody (K 2.7) and fluorescence-conjugated anti-mouse IgG (Tago, Inc.). IL-6-producing cells fixed in cold acetone were stained with goat anti-human IL-6 antiserum utilizing the avidin-biotin-peroxidase complex method (E.Y. Laboratories, Inc.) [16]. Goat anti-human IgG antiserum was used as a control antibody.

## 3. RESULTS AND DISCUSSION

Renal cell carcinoma cells freshly isolated from patients were found not to grow well in the culture medium without FCS. It was examined whether IL-6 can induce in vitro cell growth of renal cell carcinomas in the absence of FCS. IL-6 induced in vitro cell growth of renal cell carcinoma cells freshly isolated from all 18 patients. In 13 cases, the cell growth induced by IL-6 was almost equivalent to that induced by 10% FCS and in the other cases, it was approx. 50% of that exerted by 10% FCS. Fig.1 demonstrates representative data on 3 different renal cell carcinoma cells. To demonstrate whether the responding cells are in fact renal cell carcinoma cells, the growing cells were stained with periodic acid-Schiff (PAS) and monoclonal anti-renal cell carcinoma antibody, K2.7. Almost all cells were positively stained (not shown). The direct action of IL-6 was further confirmed, because IL-6 induced cell growth of the renal cell carcinoma cell line, ACHN (not shown).

IL-6 could replace the requirement for FCS in in vitro growth of renal cell carcinoma. Therefore, the possibility was examined whether FCS might induce IL-6 production in renal cell carcinoma cells. IL-6 activities equivalent to those exerted by 1–15 ng/ml of recombinant IL-6 were detected in the culture supernatants of ACHN cell line or freshly isolated renal cell carcinoma cells cultured in the presence of 10% FCS but not in the absence of FCS. The IL-6 activity was neutralized by goat anti-IL-6 antiserum (not shown). The amount of IL-6 in the culture supernatants was not changed by the treatment of the cell preparation with anti-macrophage monoclonal antibody, M206 and complement (not shown). Furthermore, IL-6-producing cells were directly identified by immunostaining

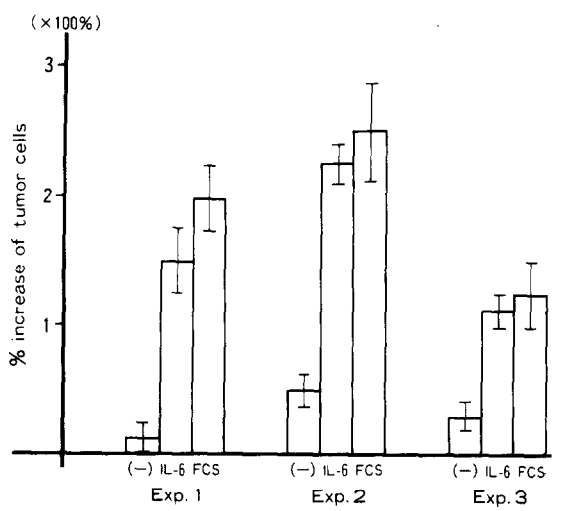


Fig.1. IL-6-induced cell growth of renal cell carcinoma cells isolated from the patients. Tumor cells ($4 \times 10^4$/well) were cultured in the presence or absence of 20 ng/ml recombinant IL-6 without FCS. They were also cultured in the presence of 10% FCS without IL-6. After 6 days, the number of recovered cells was determined. Data represents the percent increase of cell number and the mean ± SE of triplicate cultures.

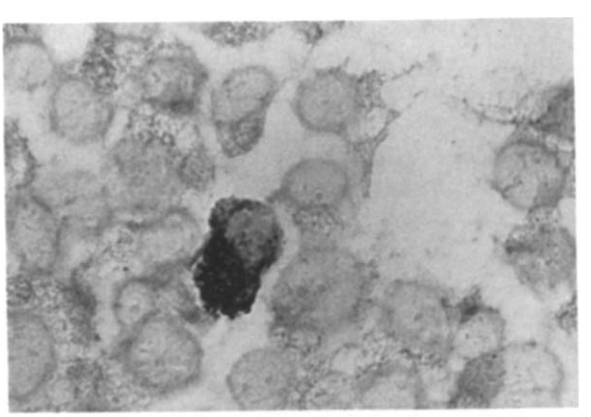

Fig.2. Immunostaining of cytoplasmic IL-6 in renal cell carcinoma cells.

utilizing goat anti-IL-6 antiserum. As shown in fig.2, approx. 2–9% of cultured renal cell carcinoma cells were positively stained by anti-IL-6 antiserum. The staining was inhibited by the presence of 20 $\mu$g/ml of recombinant IL-6, indicating the specificity of the staining. Moreover, IL-6 mRNA was detected in RNA preparations isolated from renal cell carcinoma cells cultured in the presence of 10% FCS for 2 days (fig.3a) or ACHN cell line (fig.3b).

As described, growing renal cell carcinoma cells produced IL-6 in the presence of 10% FCS, whereas they did not proliferate nor produce IL-6 in the absence of FCS. The data suggest that IL-6 functions as an autocrine growth factor of renal cell carcinoma cells in vitro. To prove this possibility, it was examined whether anti-IL-6 antibodies can inhibit the cell growth of renal cell carcinoma cells in the presence of FCS. As shown in fig.4, goat anti-IL-6 antiserum inhibited the cell growth of renal cell carcinoma cells isolated from 2 patients in a dose-dependent manner, but control goat anti-human IgG antiserum did not. The inhibitory effect of anti-IL-6 antiserum was specific for IL-6, because the addition of 20 ng/ml of recombinant IL-6 could recover the anti-IL-6-induced inhibition of tumor growth as shown in fig.4.

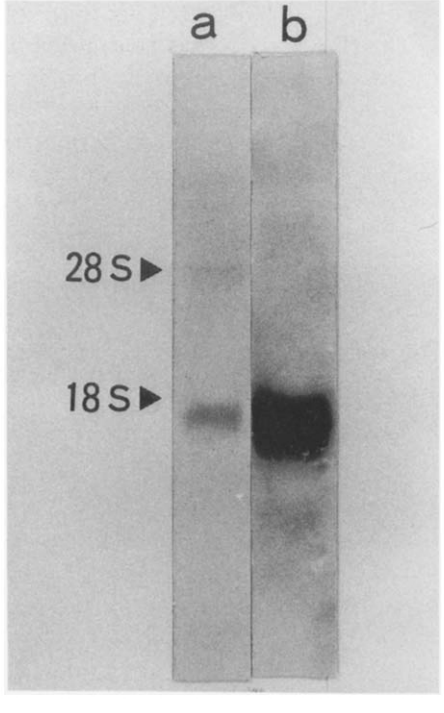


Fig.3. Expression of IL-6 mRNA in renal cell carcinoma cells obtained from the patients (a) and ACHN cell line (b).

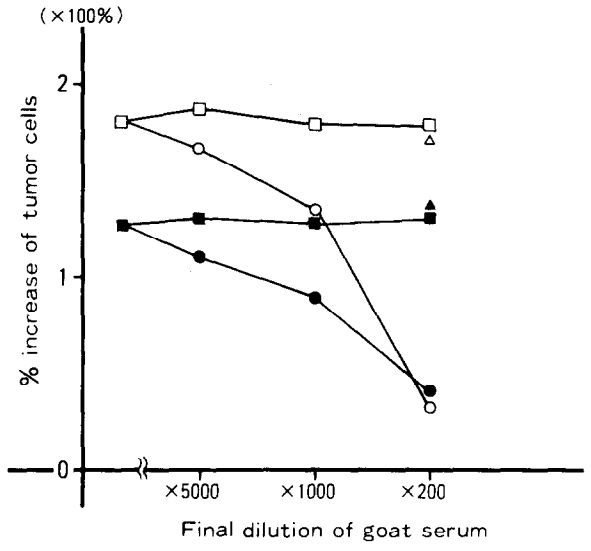


Fig.4. Inhibitory effect of anti-IL-6 antiserum on the in vitro growth of renal cell carcinoma cells. Tumor cells isolated from two patients were cultured ($4 \times 10^4$/well) in the presence of 10% FCS with various concentrations of goat anti-human IL-6 antiserum (●, ○) or goat anti-human IgG antiserum (■, □) for 6 days. (▲, △) data on the culture containing both goat anti-IL-6 antiserum (200-fold dilution) and 20 ng/ml of recombinant IL-6. Data represents the mean of triplicate cultures.

Since Sporn and Todaro [17] proposed the autocrine stimulation as a mechanism of transformation, circumstantial evidence has been accumulating to support the original hypothesis in a variety of growth factors and tumors [18]. We also demonstrated previously that IL-6 is an autocrine growth factor for human myeloma cells [4]. Therefore, this is another example of IL-6-mediated autocrine growth loop operating in renal cell carcinoma.

At present, it is not known whether IL-6 is actually produced by renal cell carcinoma cells in vivo. However, certain patients with aggressive type of renal cell carcinoma show an increase in serum acute phase proteins [6,7], suggesting that these rapidly growing renal cell carcinoma cells produce IL-6 in vivo also, because IL-6 is a major factor regulating acute phase protein biosynthesis

[8–10]. It remains to be elucidated what stimuli can induce IL-6 production in renal cell carcinoma cells in vivo. Future studies on the mechanism(s) leading to the IL-6 gene activation in renal cell carcinoma cells in vivo may clarify the oncogenesis of renal cell carcinoma.

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