Volume 278, number 2, 179-182 FEBS 09351 January 1991

ADONIS 001457939100057W

Suicide process of renal cell carcinoma cells encountering mumps virus

Shunji Miki[1], Hidekazu Yamada[2], Tomoaki Orita[2], Masahiro Yamamoto[1] and Yoshitsugu Miki[3]

[1]*The Third Department of Internal Medicine, Nissei Hospital, 6-3-8 Itachibori, Nishiku Osaka 550, Japan,* [2]*Department of Dermatology, Kinki University, School of Medicine, 377-2, Ohonohigashi, Osaka-Sayama, Osaka 589, Japan* and [3]*Miki Clinic, 2-2-1, Kuzuhara, Neyagawashi, Osaka 572, Japan*

Received 15 October 1990, revised version received 21 November 1990

Renal cell carcinoma cells produced the substance(s) which killed them (suicide factor(s)) after co-culture with mumps virus. The suicide factor(s) were heat-sensitive and were degraded with trypsin. Furthermore, actinomycin D inhibited the production of the substance(s) by cancer cells. Considering these facts, the substance(s) were thought to be protein(s) derived from de novo synthesis in cancer cells. It was demonstrated that renal cell carcinoma cells proliferated with the autocrine loop of interleukin-6 (IL-6). Mumps virus almost completely inhibited the IL-6 production in several hours. Because of these two facts, the suicide process might be initiated in renal cell carcinoma cells after encountering mumps virus, i.e. inhibition of the autocrine growth loop of IL-6 followed by the induction of an autocrine killing loop of unknown substance(s).

Renal cell carcinoma, Mumps virus, Interleukin-6, Suicide factor

1. INTRODUCTION

From the following, we could presume that cellular death might be derived from mechanisms existing intracellularly. Breast cancer cells which were induced to produce Transforming Growth Factor Beta (TGF-β) by an estrogen antagonist inhibited their own growth [1]. Steroid and Cytotoxic T lymphocytes (CTL) induced the same intracellular processes followed by DNA fragmentation in thymoma cells [2]. But details about the mechanisms of cellular death were still unknown.

Clinically, tumor regression was observed in patients injected with mumps virus intravenously or intrathecally [3]. However, the cellular mechanisms of tumor regression were not examined. Direct actions of mumps virus on cancer cells were investigated. Renal cell carcinomas were chosen for target cells because autocrine growth with IL-6 was demonstrated in them [4]. In this report, we show that mumps virus induced the growth inhibitory substance(s) (suicide factor(s)) in renal cell carcinoma cells and inhibited the production of the autocrine growth factor, IL-6.

2. MATERIALS AND METHODS

2.1 *Reagents*

Human TGF-β was obtained from Otsuka Pharmaceutical Co., Tokushima, Japan. Recombinant interferon-α-2b (IFN-α-2b) was a gift from Yamanouchi Pharmaceutical Co., Tokyo, Japan. Interferon-β (IFN-β) was from Daiichi Pharmaceutical Co., Tokyo, Japan. Sheep anti TGF-β antiserum was from BIOTX, Texas, USA. Goat anti human IFN-α antiserum was from BioNative AB, Sweden. Rabbit anti human IFN-β antiserum was from LEE Biomolecular Research Inc., USA. Rabbit anti mumps virus antiserum was provided by Dr Yamanishi (Research Institute for Microbial Disease, Osaka University, Japan). Dexamethasone was from BANYU Pharmaceutical Co. Tokyo, Japan. Fetal calf serum (FCS) was from Flow Laboratories Inc., USA.

2.2 *Cells and mumps virus*

Renal cell carcinoma cells were obtained from the patients as described previously [4]. The Urabe strain of mumps virus, isolated from human embryonic kidney (HEK) cells [5] was provided by Dr K. Yamanishi (Research Institute for Microbial Diseases, Osaka University, Japan). Mumps virus was completely inactivated by UV irradiation (3 min at a distance of 20 cm from a UV lamp of 15 W). UV inactivated mumps virus were seeded on Vero cells. After 7 days, virus replication was not detectable and cellular degeneration was not observed.

2.3 *Production of suicide factor(s)*

Renal cell carcinoma cells were usually cultured in RPMI 1640 medium containing 10% FCS for 2 days at 37°C in a humidified 5% CO_2 atmosphere and then the medium was discarded and replaced by fresh medium supplemented with 20 HAU/ml of mumps virus. After 24 h culture under the same condition, we obtained the culture supernatant. For elimination of mumps virus particles, it was centrifuged at 60000 × *g* at 4°C for 30 min, and finally clarified by passing it through a 0.2 μm filter. The filtrate was used for subsequent studies of suicide factor(s). Dexamethasone was also used as stimulant instead of mumps virus.

2.4 *Characterization of suicide factor(s)*

Renal cell carcinoma cells were incubated for 1 h at 37°C, alone or with actinomycin D. Actinomycin D treated cells were thoroughly washed and used as producers of suicide factor(s). Supernatants with suicide factor(s) were incubated for 1 h at 37°C with trypsin (1 mg/ml). This dose of trypsin alone did not have any influence on the growth of renal cell carcinoma cells.

2.5 *Northern blot analysis*

RNA was purified by the guanidine method and subjected to Nor-

Correspondence address Y. Miki, Miki Clinic, 2-2-1, Kuzuhara, Neyagawashi, Osaka 572, Japan

thern blot analysis utilizing ^{32}P-labeled *EcoI-BamH* I fragment of pBSF 2.3# insert cDNA (a gift from Dr T. Kishimoto, Institute for Molecular and Cellular Biology, Osaka University, Japan) as described previously [6]. β-actin cDNA (a gift from Dr K. Yamanishi, Kyoto Prefectural University of Medicine, Japan) was used as a control

3. RESULTS AND DISCUSSION

Various concentrations of mumps virus which induce regression of tumor in clinical use were added to the culture of renal cell carcinoma cells (Fig. 1). After 72 h culture, 100 HAU/ml of mumps virus led to complete cellular death. Mumps virus inactivated by UV irradiation had almost the same dose response curve as the native virus. This result shows that the cytotoxicity of mumps virus did not need the intracellular viral proliferation. At various intervals, 20 HAU/ml of mumps virus was added to renal cell carcinoma cells. After 24 h, most cells were viable. With each culture supernatant, from which mumps virus particles were eliminated, renal cell carcinoma cells were cultured for 72 h (Fig. 2). In the culture supernatant, cytotoxic soluble factor(s) which killed renal cell carcinoma cells (suicide factor(s)) appeared after 12 h. After 24 h, the culture supernatant included suicide factor(s) which had almost 85% lethality against cancer cells. Addition of rabbit anti mumps virus antiserum which completely neutralizes 20 HAU/ml of mumps virus had no effect on the cytotoxicity of the culture supernatant against renal cell carcinoma cells (data not shown). So, the cytotoxicity of the culture supernatant was not due to virus particles which might be contaminated. Cellular death by virus particles was caused by suicide factor(s) which the cancer cells themselves produced. When renal cell carcinoma cells treated with actinomycin D (an inhibitor of protein synthesis) were co-cultured with mumps virus, the culture supernatant after 24 h contained a small amount of suicide factor(s) (Table I). This showed that suicide factor(s) were metabolites produced by renal cell carcinoma cells. On the other hand, suicide factor(s) were not detectable in culture supernatant co-cultured with dexamethasone (1 mM), which had almost the same cytotoxicity as 20 HAU/ml of mumps virus (Fig. 2). It showed that suicide factor(s) were the substance(s) specifically produced in renal cell carcinoma cells encountering mumps virus. Physicochemical characteristics of suidice factor(s) are shown in Table I. Suicide factor(s) were stable when the culture supernatant was maintained at low temperature or at 56°C for 30 min. There was, however, a great loss of cytotoxicity at 70°C for 30 min and at 100°C for 5 min. Suicide factor(s) were not dialysable by using a dialysis tube with a molecular cut off of 10000 Da. When suicide factor(s) were treated with trypsin prior to an assay, subsequent lethal effect on renal cell carcinoma cells was reduced by 63%. Together with the result of the above experiment using actinomycin D, these results demonstrated that suicide factor(s) may have a protein

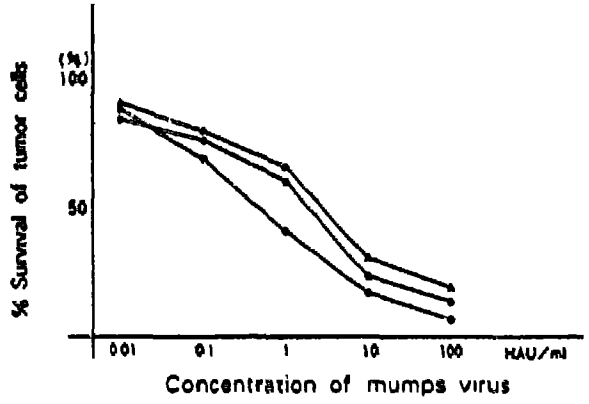

Fig. 1. Inhibitory effect of mumps virus on the in vitro growth of renal cell carcinoma cells. Renal cell carcinoma cells (1×10^4/well) were cultured in the presence of 10% FCS with various concentrations of native virus (○) or UV irradiated mumps virus (●), or in their own culture supernatants free of virus stimulated with various concentrations of native mumps virus for 24 h (▲). After 72 h, the number of recovered cells was determined. The data show percentage against the number under the conditions without the effect of mumps virus and represent the mean of triplicate cultures

nature and their molecular weight may be larger than 10000 Da. In Fig. 1, the cytotoxicity of the culture supernatant was blotted, which was harvested after 24 h stimulation with various concentrations of mumps virus. The extent of cytotoxicity was lower than in the case in which native or inactivated mumps virus particles were added directly to renal cell carcinoma cells. To understand this reason, the production of IL-6 which was an autocrine growth factor in renal cell carcinoma cells was examined. Fig. 3A shows IL-6 mRNA

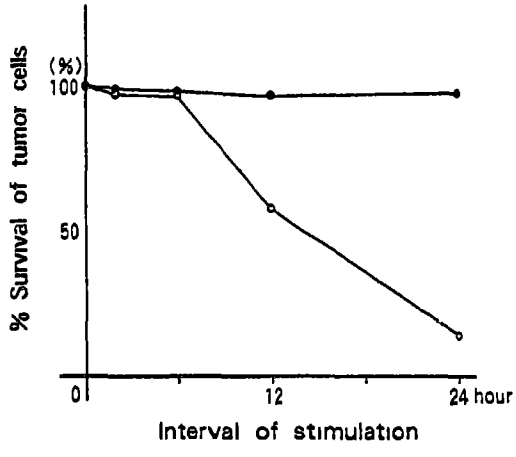

Fig. 2. Kinetics of production of suicide factor(s) in renal cell carcinoma cells with mumps virus. At various intervals, renal cell carcinoma cells were stimulated with mumps virus (20 HAU/ml) (○), or dexamethasone (1 mM) (●). The culture supernatant with elimination of mumps virus by centrifugation or dexamethasone by dialysis were added to the culture of renal cell carcinoma cells. After 72 h, the number of recovered cells was determined. The data represent the percent survival as shown in Fig. 1 and the mean of triplicate cultures

Table I

Physico-chemical characteristics of suicide factor(s)

Treatment	% survival[2]	% Inhibition[3]
No treatment	38.1	0
Dialysis	34.7	0
Heating at 56°C for 30 min	39.7	0
Heating at 70°C for 30 min	100.0	100
Heating at 100°C for 5 min	85.1	76
Incubation at −20°C for 1 month	32.2	0
Trypsin (1 mg/ml)	76.9	63
Derived from Actinomycin D (1 μg/ml) treatment[1]	52.9	24
Derived from Actinomycin D (5 μg/ml) treatment[1]	70.4	52

Supernatants containing suicide factor(s) produced by co-culture of renal cell carcinoma cells with mumps virus (20 HAU/ml) for 24 h were treated as shown and were added to the culture of renal cell carcinoma cells.

[1] Supernatants were derived from renal cell carcinoma cells pretreated with actinomycin D for an hour. [2] % Survival was calculated by the formula: percent survival = (the number of recovered cells in the culture supernatant with each treatment/the number of recovered cells in control culture medium) × 100. Values represent the mean of triplicate cultures. [3] % Inhibition was calculated by the formula: percent inhibition = [(percent survival with treatment − percent survival with no treatment)/(100 − percent survival with no treatment)] × 100

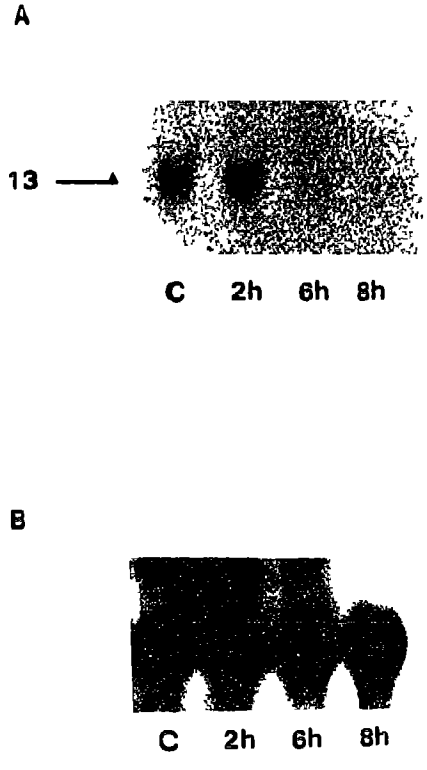

Fig 3 Kinetics of expression of IL-6 mRNA in renal cell carcinoma cells cultured with mumps virus. Renal cell carcinoma cells were cultured with mumps virus (20 HAU/ml) at various intervals. Expression of IL-6 mRNA (Fig. 3A) or β actin mRNA (Fig 3B) in renal cell carcinoma cells are shown by Northern blot analysis

in renal cell carcinoma cells by utilizing Northern blot hybridization after adjustment of cell numbers or RNA contents. Renal cell carcinoma cells increased IL-6 production after 1 or 2 h stimulation with mumps virus. But thereafter, the production of IL-6 was barely detectable. This result showed that the break of the growth mechanism in renal cell carcinoma cells took place at an early period of stimulation with mumps virus. Because mRNA of β-actin did not change (Fig. 3B), the production of IL-6 was specifically inhibited. These results demonstrated that intracellular metabolism was related to cellular death, i.e. early inhibition of the production of IL-6 (autocrine growth factor) is followed by the production of suicide factor(s). It has been demonstrated that TGF-β, which was produced by breast cancer cells treated with an estrogen antagonist, was able to inhibit

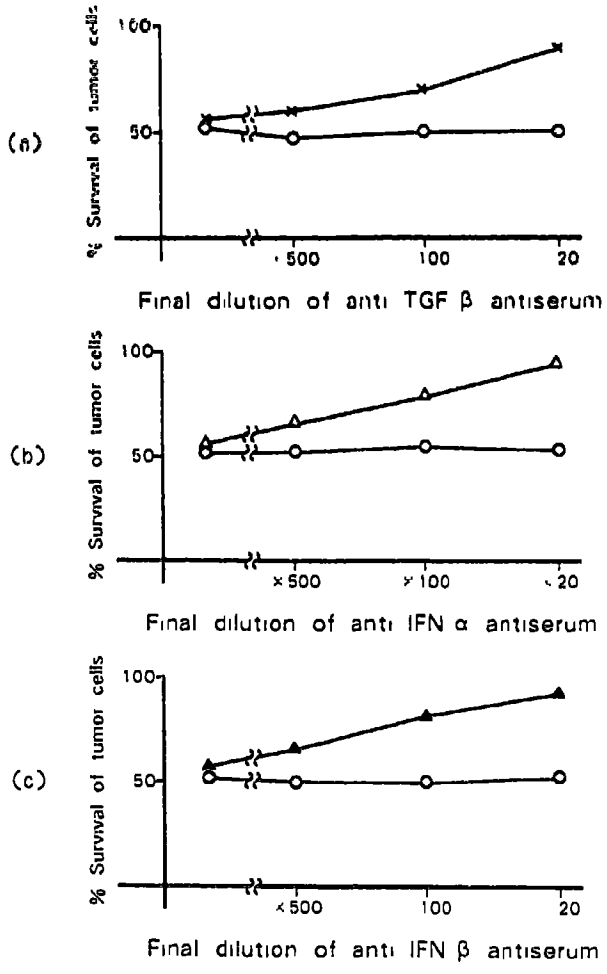

Fig 4 Effect of anti TGF-β antiserum, anti IFN-α antiserum and anti IFN-β antiserum on the cytotoxicity of suicide factor(s). Serially diluted anti TGF-β antiserum (Fig 4a), anti IFN α antiserum (Fig 4b) and anti IFN-β antiserum (Fig 4c) were added to the culture of renal cell carcinoma cells with suicide factor(s) (10-fold dilution) (○), TGF-β (3 ng/ml) (×), IFN-α (3000 U/ml) (△), or IFN-β (5000 U/ml) (▲). After 72 h, the number of recovered cells was determined. The data represent the percent survival as shown in Fig 1 and the mean of triplicate cultures

the growth of breast cancer cells [1]. It has also been reported that renal cell carcinoma cells could produce TGF-β and that TGF-β inhibited the growth of renal cell carcinoma cells [7,8]. On the other hand, IFN-α and IFN-β can induce tumor regression in some patients with renal cell carcinomas and inhibit the growth of renal cell carcinoma cells or cell lines [9,10]. These reports suggest that the suicide factor(s) were associated with TGF-β, IFN-α and IFN-β. In our preliminary experiments, mumps virus barely induced TGF-β, IFN-α and IFN-β in renal cell carcinoma cells. To ascertain whether TGF-β, IFN-α and IFN-β are not involved in the suicide process, anti TGF-β antiserum, anti IFN-α antiserum and anti IFN-β antiserum were added to the supernatants with the suicide factor(s) and a cytotoxicity assay was performed. As shown in Fig. 4, anti TGF-β antiserum, anti IFN-α antiserum and anti IFN-β antiserum failed to recover the cell growth of renal cell carcinoma cells in the culture supernatant with suicide factor(s) (10-fold dilution), although their antiserums were able to block the cytotoxicity of TGF-β (3 ng/ml), IFN-α (3000 U/ml) or IFN-β (5000 U/ml) which was to the same extent as suicide factor(s) (10-fold dilution). These results suggest that TGF-β, IFN-α and IFN-β are scarcely involved in the suicide process. Therefore, unknown substance(s) might be candidates for suicide factor(s). Dexamethasone inhibited the production of IL-6 as soon as it was added to the culture of renal cell carcinoma cells (data not shown), and led to cellular death. However, dexamethasone never induced the production of suicide factor(s). Thus, it was demonstrated that various intracellular mechanisms induce the common phenomenon, cellular death. It remains to be elucidated what substance(s) are suicide factor(s) in this report. Further study of cellular death at a molecular level will provide a useful tool for the regression of tumor growth.

Acknowledgements We wish to thank Dr T. Kishimoto (Institute for Molecular and Cellular Biology, Osaka University, Japan), Dr M. Takahashi and Dr K. Yamanishi (Research Institute for Microbial Diseases, Osaka University, Japan) and Dr K. Miyazono (Ludwig Institute for Cancer Research, Sweden) for helpful discussions

REFERENCES

[1] Knabbe, C., Lippman, M.E., Wakefield, L.M., Flanders, K.C., Kasid, A., Derynck, R. and Dickson, R.B. (1987) Cell 48, 417-428.
[2] Ucker, D.S. (1987) Nature 327, 62-64.
[3] Asada, T. (1974) Cancer 34, 1907-1928.
[4] Miki, S., Iwano, M., Miki, Y., Yamamoto, M., Tang, B., Yokokawa, K., Sonoda, T., Hirano, T. and Kishimoto, T. (1989) FEBS Lett. 250, 607-610.
[5] Yamanishi, K., Hosai, H., Ueda, S., Takahashi, M. and Okuno, Y. (1970) Biken J. 13, 127-132.
[6] Hirano, T., Yasukawa, K., Harada, H., Taga, T., Watanabe, Y., Matsuda, T., Kashiwamura, S., Nakajima, K., Koyama, K., Iwamatu, A., Tsunasawa, S., Sakiyama, F., Matsui, H., Takahara, Y., Taniguchi, T. and Kishimoto, T. (1986) Nature 324, 73-76.
[7] Gomella, L.G., Sargent, E.R., Wade, T.P., Anglard, P., Linehan, W.M. and Kasid, A. (1989) Cancer Res. 49, 6972-6975.
[8] Gomella, L.G., Sargent, E.R., Linehan, W.M. and Kasid, A. (1989) J. Urol. 141, 1240-1244.
[9] Ishii, Y. and Tsukagoshi, S. (1989) J. Pharmacobiodyn. 12, 299-304.
[10] Kinney, P., Triozzi, P., Young, D., Drago, J., Behrens, B., Wise, H. and Rinehart, J.J. (1990) J. Clin. Oncol. 8, 881-885.