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Authors: Kubohara, Yuzuru, Kabeya, Kenji, Matsui, Hirofumi, and Ishikawa, Koichi

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# Effects of DIF-1, an Anti-Tumor Agent Isolated from *Dictyostelium discoideum*, on Rat Gastric Mucosal RGM-1 and Leptomeningeal Cells

Yuzuru Kubohara<sup>1\*</sup>, Kenji Kabeya<sup>1</sup>, Hirofumi Matsui<sup>2,3</sup>  
and Koichi Ishikawa<sup>1</sup>

<sup>1</sup>Department of Molecular Physiology, Institute for Molecular and Cellular Regulation (IMCR), Gunma University, Maebashi 371, Japan

<sup>2</sup>Institute of Physical and Chemical Science (RIKEN) Cell Bank, and

<sup>3</sup>Institute of Clinical Medicine, University of Tsukuba, Tsukuba 305, Japan

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**ABSTRACT**—DIF-1 is a putative morphogen that induces stalk cell formation in the cellular slime mold *Dictyostelium discoideum*. We have previously discovered that DIF-1 suppresses cell growth and induces cell differentiation *in vitro* in some tumor cells, and also that relatively low concentrations of DIF-1 promote retinoic acid-induced cell differentiation in the human myeloid leukemia HL-60 cells. In this study, to verify cell biological and therapeutic potential of DIF-1, we have examined whether and how DIF-1 affects normal mammalian cells *in vitro*, using rat leptomeningeal (RLM) cells and rat gastric mucosal RGM-1 cells. In growing phase of both cells, DIF-1 at 5–40  $\mu\text{M}$  suppressed cell growth in a dose-dependent manner. High concentrations (15–40  $\mu\text{M}$ ) of DIF-1 were toxic to the growing cells so that the cells showed unusual morphology, but many of them were still alive even at Day 3–4. Withdrawal of DIF-1 allowed the cells to grow. In confluent phase of the cells, DIF-1 at more than 15  $\mu\text{M}$  promoted medium acidification that resulted in cell death, but DIF-1 itself did not markedly affect cell viability for 3 days. DIF-1 increased  $[\text{Ca}^{2+}]_i$  in RLM cells but did not affect  $\beta$ -trace secretion (an index of cell function in RLM cells). A low concentration (5  $\mu\text{M}$ ) of DIF-1 in combination with retinoic acid (0.1  $\mu\text{M}$ ) showed no marked effects on cell growth, cell viability, cell morphology and  $\beta$ -trace secretion in RLM cells. The present results indicate that DIF-1 may be utilized as a tool for cell biology. Also, since these concentrations of DIF-1 kill some tumor cells within 2–3 days, the present results suggest that DIF-1 might be utilized in the treatment of some sorts of cancer with a bearable degree of side-effects.

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## INTRODUCTION

The cellular slime mold *Dictyostelium discoideum* is a simple eukaryote that forms a fruiting body consisting of spores and stalk cells at the end of its development. DIF-1 (differentiation-inducing factor-1), 1-(3,5-dichloro-2,6-dihydroxy-4-methoxyphenyl)-1-hexanone (Fig. 1) (Morris *et al.*, 1987), is a putative morphogen that induces stalk cell formation (Kay *et al.*, 1989). This signal molecule has been suggested to function at least partly via an increase in  $[\text{Ca}^{2+}]_i$  in *D. discoideum* (Kubohara and Okamoto, 1994; Schaap *et al.*, 1996; Azhar *et al.*, 1997).

Recently, it was found that DIF-1 suppresses cell growth and induces erythroid differentiation in murine and human leukemia (B8 and K562) cells (Asahi *et al.*, 1995). It was also found that DIF-1 raises  $[\text{Ca}^{2+}]_i$  and suppresses cell growth in

rat pancreatic tumor, AR42J cells (Kubohara *et al.*, 1995b), and that high concentrations of DIF-1 induce apoptosis in the tumor cells (Kubohara *et al.*, 1995a). Moreover, it was shown with the human myeloid leukemia, HL-60 cells (Collins *et al.*, 1977), that DIF-1 suppresses cell growth at least partly by arresting the cells in G1 phase of cell-cycle, and that relatively low concentrations of DIF-1 promote retinoic acid-induced cell differentiation (Kubohara, 1997). These findings indicate that DIF-1 may have therapeutic potential in the treatment of a wide range of cancer. In particular, since retinoic acid has already been utilized in differentiation-therapy for human acute promyelocytic leukemia (APL) (Huang *et al.*, 1988), co-administration of retinoic acid and DIF-1 (or its artificial analogs) may improve chemoprevention/differentiation-therapy for APL and the other types of leukemia. However, it has not been examined if/how DIF-1 affects normal mammalian cells. Detailed analyses on the effects of DIF-1 on normal cell functions should be necessary for the therapeutic use of this agent. It should also be of great importance to verify the

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\* Corresponding author: Tel. +81-27-220-8847;  
FAX. +81-27-220-8849.

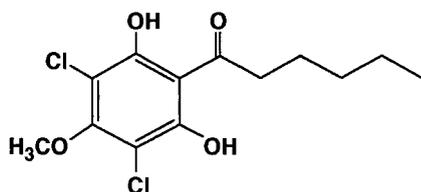


Fig. 1. Chemical structure of DIF-1.

cell biological potential of DIF-1 in many types of cells, in terms of basic cell science.

In our laboratory, Ishikawa and co-workers have established an *in vitro* primary culture system with rat leptomenigeal cells and have been analyzing the functions of the cells (Ishikawa *et al.*, 1995a,b). Leptomeninges are the cerebrospinal fluid-blood barrier covering the brain parenchyma (Smith and Shine, 1992; Tanno *et al.*, 1993). *In vitro* studies have revealed that these cells secrete many interesting proteins such as  $\beta$ -trace protein (prostaglandin D synthase), IGF-II, IGF-binding protein-2, apolipoprotein E,  $\beta$ 2-microglobulin and many others (Ishikawa *et al.*, 1995b; Ohe *et al.*, 1996) which are seen in cerebrospinal fluid *in vivo*, suggesting that meningeal cells play pivotal roles at least as an origin of the cerebrospinal fluid proteins. Since the meningeal cells can be cultured *in vitro* and also because the assay system for cell biological function ( $\beta$ -trace secretion) have been established (Kabeya *et al.*, 1998), these cells should provide an ideal *in vitro* system suitable for the analysis of the effects of DIF-1 on normal cell functions.

RGM-1 is an epithelial cell line established from gastric mucosa of adult Wistar rats and is well characterized (Hassan *et al.*, 1996; Okada *et al.*, 1996). This cell line also provides a useful model system for the analysis of the effects of DIF-1 on normal cells.

In this study, in order to verify both cell biological and therapeutic potential of DIF-1 and thus to elucidate if/how this agent affects normal cells, we examined the *in vitro* effects of DIF-1 on RGM-1 and rat meningeal cells. We show here that DIF-1 suppresses cell growth but is less toxic to the normal cells than to some tumor cells. It is also shown that cell growth, viability and  $\beta$ -trace secretion were not markedly affected in the presence of both retinoic acid and a low concentration of DIF-1, an expected combination for leukemia therapy. The potential of DIF-1 in mammalian cells will be discussed.

## MATERIALS AND METHODS

### Chemicals

DIF-1 was purchased from both Molecular Probes (USA) and Affiniti Research Products (UK) and stored as a 10 mM solution in EtOH at  $-20^{\circ}\text{C}$ . All-*trans* retinoic acid was from Wako Pure Chemical Industries (Osaka, Japan) and stored as a 100 mM solution in EtOH at  $-20^{\circ}\text{C}$ .

### Examination of the effects of DIF-1 on growing cells

Rat leptomenigeal (RLM) cells and rat gastric mucosa RGM-1 cells were used in this study. Meningeal cells were prepared from the

brains of 1-2-day-old rats as described previously (Ishikawa *et al.*, 1995a,b; Ohe *et al.*, 1996). The RLM cells and RGM-1 cells were plated on poly-D-lysine-coated multi(12)-well plates at a density of  $2-4 \times 10^3$  cells/cm<sup>2</sup>, and incubated with 1 ml of DMEM-FBS (Dulbecco's modified Eagle's medium containing 10% fetal bovine serum) at  $37^{\circ}\text{C}$  (5% CO<sub>2</sub>) for 1-2 days. The incubation media were replaced each with 1 ml of fresh DMEM-FBS containing 0-40  $\mu\text{M}$  DIF-1 and/or 0.1  $\mu\text{M}$  RA, and incubated at  $37^{\circ}\text{C}$  (5% CO<sub>2</sub>). Cell growth and morphology were observed by a phase-contrast microscope. At 24 hr of incubation, cell density was counted; living cell numbers in 3-4 different microscopic fields of each well were counted and the mean of the numbers was used as a cell density. Round cells detached from the bottom of the wells were regarded as dead cells.

### Examination of the effects of DIF-1 on confluent state of cells and $\beta$ -trace secretion

Cells were incubated at  $37^{\circ}\text{C}$  (5% CO<sub>2</sub>) in multi(12)-well plate as described above for 5-7 days until they reached a confluent state. The incubation media were replaced each with 1 ml of fresh DMEM-FBS containing 0-40  $\mu\text{M}$  DIF-1 and/or 0.1  $\mu\text{M}$  RA, and cells were incubated at  $37^{\circ}\text{C}$  (5% CO<sub>2</sub>) continuously, or during incubation, the media were replaced with fresh ones containing the reagents at every 24 hr. For the assay for  $\beta$ -trace secretion, confluent RLM cells were incubated with DIF-1/RA-containing media, and the incubation media were collected and replaced with 1 ml of fresh DMEM-FBS containing the reagents at every 12 or 24 hr.  $\beta$ -trace contents of the media thus collected were assayed using anti- $\beta$ -trace protein antibody as described by Kabeya *et al.* (1998).

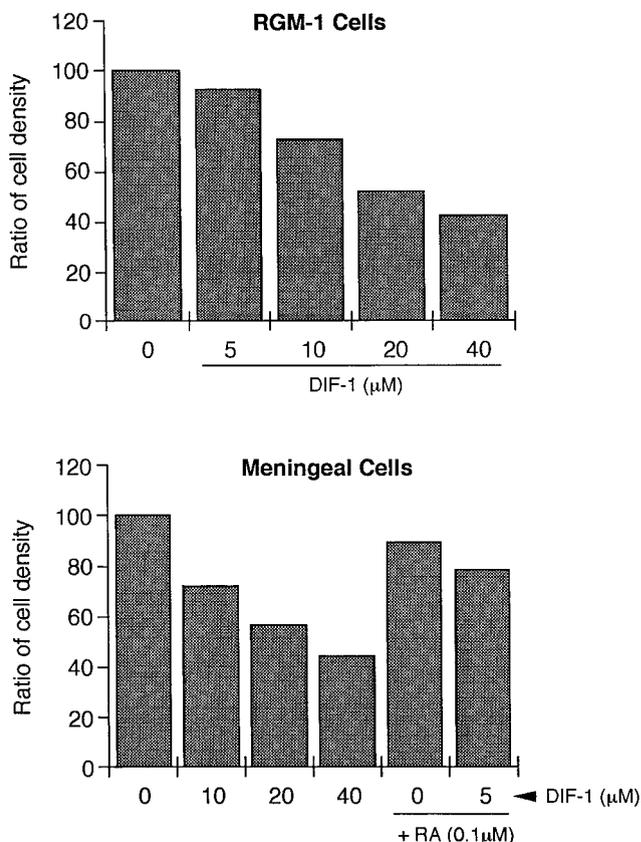


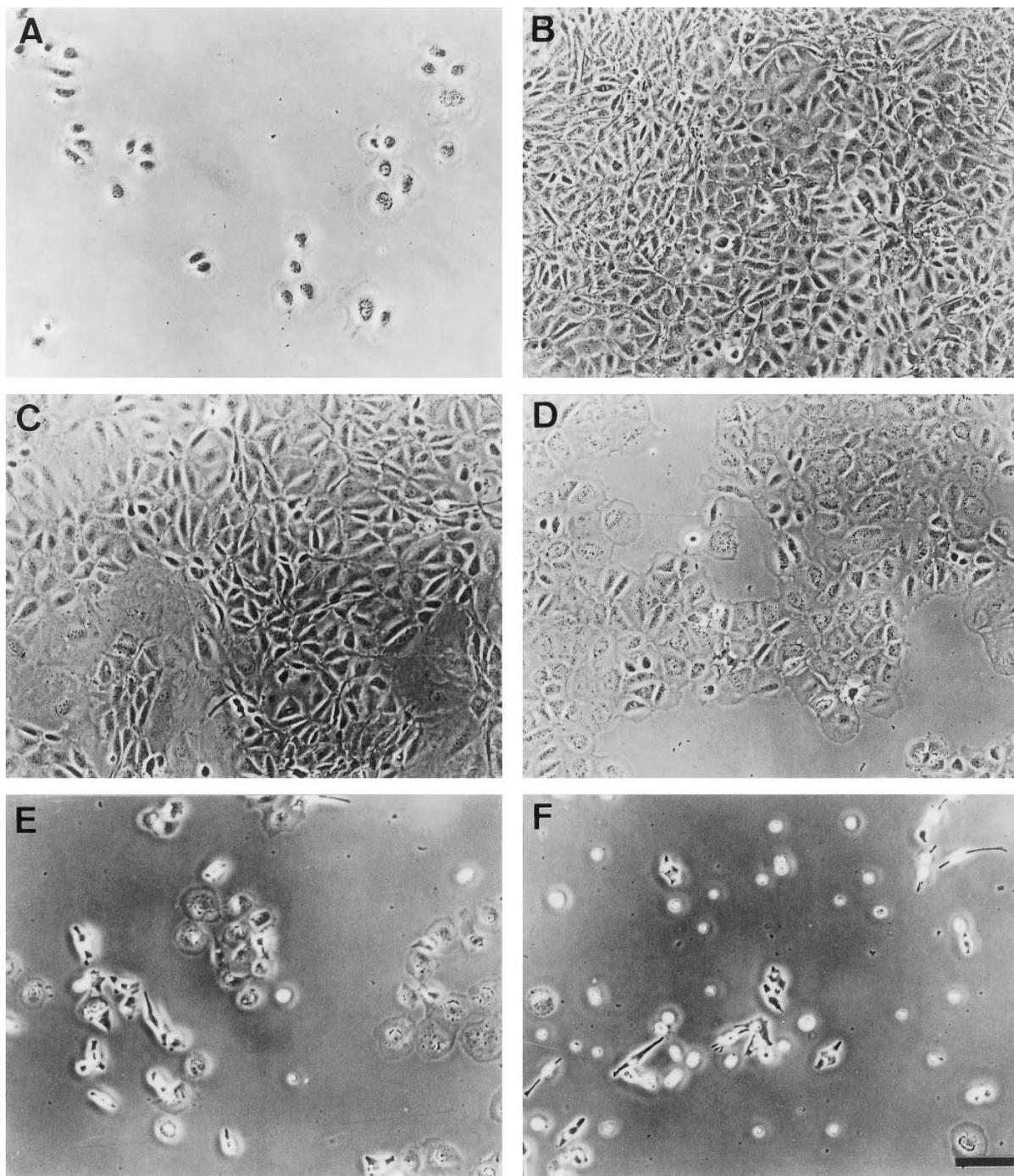
Fig. 2. Effects of DIF-1 on cell growth in RGM-1 and RLM cells. Cells were incubated for 1 day without or with 5-40  $\mu\text{M}$  of DIF-1 and/or 0.1  $\mu\text{M}$  of retinoic acid (RA), and cell density was counted microscopically.

**Assay for  $[Ca^{2+}]_i$** 

RLM cells were incubated in culture flasks until cells reached a confluent state. The cells were collected by trypsin treatment (Ohe *et al.*, 1996), and incubated with FuraPE3/AM (Wako Pure Chemical Industries, Osaka, Japan). FuraPE3 fluorometry was performed as described previously (Kubohara, 1997).

**RESULTS****Effects of DIF-1 on RGM-1**

We first examined the effects of DIF-1 on growing phase of the rat gastric mucosa cell line RGM-1 (Figs. 2 and 3). In the absence of DIF-1, RGM-1 cells grew as shown in Fig. 3

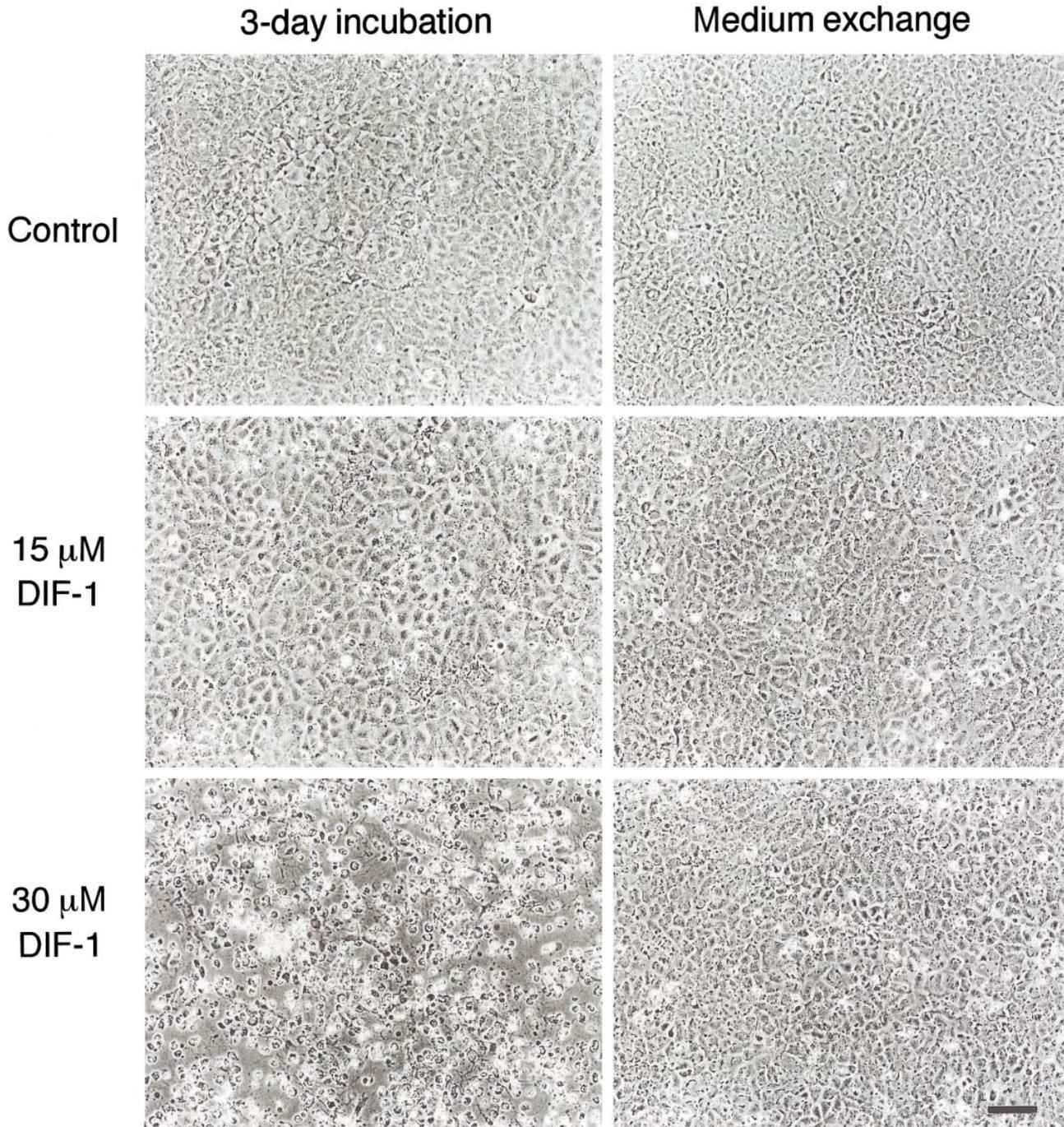


**Fig. 3.** Effects of DIF-1 on growing RGM-1 cells. RGM-1 cells (**A**: Day 0) were incubated without (**B**) or with 5 (**C**), 10 (**D**), 20 (**E**), and 40  $\mu$ M (**F**) of DIF-1, and cell growth and morphology were observed microscopically at Day 3. Control cells grew and reached a confluent state in 3-4 days. In the presence of 5  $\mu$ M DIF-1, cell growth and morphology were not markedly affected. DIF-1 at 10  $\mu$ M slightly suppressed cell growth but did not affect morphology. In the presence of 20-40  $\mu$ M DIF-1, cell growth was greatly suppressed, and there appeared some vesicle-containing cells and bi-polar cells. Bar; 100  $\mu$ m.

(Control) and reached a fully confluent state in 3-4 days. In the presence of 5  $\mu\text{M}$  DIF-1 (Fig. 3), cell growth and cell morphology were not much affected so that cells reached a confluent state as control cells did. DIF-1 at 10  $\mu\text{M}$  suppressed cell growth slightly but did not affect cell morphology (Fig. 3). Cell growth was greatly suppressed in the presence of 20-40

$\mu\text{M}$  of DIF-1 where the cells showed characteristic morphology; there appeared some flattened vesicle-containing cells and bi-polar cells (Fig. 3). The cells started growing after withdrawal of DIF-1 (data not shown), which indicates that the effect of DIF-1 is reversible.

We then examined the effects of DIF-1 on cell viability



**Fig. 4.** Effects of DIF-1 on confluent RGM-1 cells. Confluent RGM-1 cells were incubated without (control) or with 15-30  $\mu\text{M}$  DIF-1 continuously (left photos), or with exchanging the incubation media with fresh ones at every 24 hr (right photos), and at Day 3, cell morphology and viability were observed microscopically. DIF-1 at 15  $\mu\text{M}$  was not markedly toxic to the cells. Continuous incubation with 30  $\mu\text{M}$  DIF-1 caused medium acidification, which was toxic to the cells so that most died in 2-3 days. However, confluent RGM-1 cells survived for 3 days when incubation medium was exchanged with fresh one at every 24 hr. Bar; 100  $\mu\text{m}$ .

and morphology in a confluent state of RGM-1 cells. In the absence of DIF-1, cells kept confluent and they were alive at Day 3 (Fig. 4); cell morphology did not change for more than a week (data not shown). In the presence of 5-15  $\mu\text{M}$  DIF-1, they showed normal morphology at Day 3 (data not shown). In contrast, confluent cells were killed within 3 days when 30  $\mu\text{M}$  of DIF-1 was present in the media (Fig. 4). However, repeated experiments showed that in the presence of 30  $\mu\text{M}$  DIF-1, the culture media became yellow (indication of acidification) before cells died (data not shown). This seemed to indicate that DIF-1 would promote cell metabolism in some way resulting in the accumulation of metabolites and the acidification of the culture media, which might cause cell death; that is, the cytotoxic effect of DIF-1 was exerted at least in part via the acidification of the media and/or the accumulation of cytotoxic metabolites. To assess this possibility, the culture media were exchanged with fresh ones containing DIF-1 at every 24 hr and cell viability was observed. As expected, cells were viable at Day 3 even in the presence of 30  $\mu\text{M}$  of DIF-1 (Fig. 4).

#### Effects of DIF-1 on rat leptomeningeal cells

The effects of DIF-1 on growing phase of the rat leptomeningeal (RLM) cells were examined (Figs. 2 and 5). In the absence of DIF-1, RLM cells grew as shown in Fig. 5 (control) and reached a full confluent state in 3-4 days. In terms of the inhibitory effect of DIF-1 on cell growth, we obtained almost the same results with RLM cells as seen with RGM-1 (Figs. 2, 3 and 5). Again, withdrawal of DIF-1 allowed the cells to grow (data not shown). It should be noted however that in the presence of 20-40  $\mu\text{M}$  DIF-1, there appeared many bi-polar cells with long cytoplasmic projections distinct from normal (Fig. 5).

We then examined the effects of DIF-1 on cell viability and cell morphology in confluent RLM cells. We obtained almost the same results as with RGM-1, except that DIF-1 at 15  $\mu\text{M}$  was much toxic to RLM cells (data not shown) than to RGM-1 (Fig. 4); confluent RLM cells were killed by continuous incubation with 15-30  $\mu\text{M}$  DIF-1. But this toxicity was again reduced by medium exchange (data not shown).

#### Combinatory effects of DIF-1 and retinoic acid on rat leptomeningeal cells

Retinoic acid (RA) has been used in differentiation-therapy for APL (Huang *et al.*, 1988). Recently, we have shown with myeloid leukemia HL-60 that relatively low concentrations of DIF-1 promote RA-induced cell differentiation, suggesting that co-administration of RA and DIF-1 may improve differentiation-therapy for leukemia (Kubohara, 1997). We therefore examined the effects of RA and DIF-1 on RLM cells (Figs. 2 and 5). RA at 0.1  $\mu\text{M}$  showed no marked effects on both cell growth and morphology in RLM (Figs. 2 and 5). RA (0.1  $\mu\text{M}$ ) in combination with 5  $\mu\text{M}$  DIF-1 slightly suppressed cell growth but did not affect cell morphology in RLM (Figs. 2 and 5).

Neither RA (0.1  $\mu\text{M}$ ) nor the combination of RA (0.1  $\mu\text{M}$ ) and DIF-1 (5  $\mu\text{M}$ ) affected cell viability and morphology in

confluent RLM cells at least for 3 days (data not shown).

#### Effects of DIF-1 on $\beta$ -trace secretion and $[\text{Ca}^{2+}]_i$

To assess if DIF-1 affects normal cell functions, we examined the effects of DIF-1 on the secretion of  $\beta$ -trace protein in confluent RLM cells, using the medium-exchange-system described above. When the culture media were exchanged at every 24 hr, low concentrations (5-10  $\mu\text{M}$ ) of DIF-1 and/or RA (0.1  $\mu\text{M}$ ) did not affect  $\beta$ -trace secretion but high concentrations (20-30  $\mu\text{M}$ ) of DIF-1 inhibited  $\beta$ -trace secretion (data not shown). In this case however, since the culture media had become yellow in 24 hr in the presence of high concentrations of DIF-1, it seemed possible that the medium acidification and/or the accumulation of some metabolites affected  $\beta$ -trace secretion although the cells were alive. We therefore assessed  $\beta$ -trace secretion by exchanging the culture media with fresh ones at every 12 hr (Fig. 6). Under the conditions, DIF-1 and/or RA did not affect  $\beta$ -trace secretion at least for 2 days (Fig. 6).

In order to elucidate the mechanism of DIF-1-action in RLM cells, we examined the effect of DIF-1 on  $[\text{Ca}^{2+}]_i$ . Expectedly, DIF-1 (20  $\mu\text{M}$ ) increased  $[\text{Ca}^{2+}]_i$  in RLM cells, suggesting that DIF-1 might exert its effects at least in part via an increase in  $[\text{Ca}^{2+}]_i$  (Fig. 7).

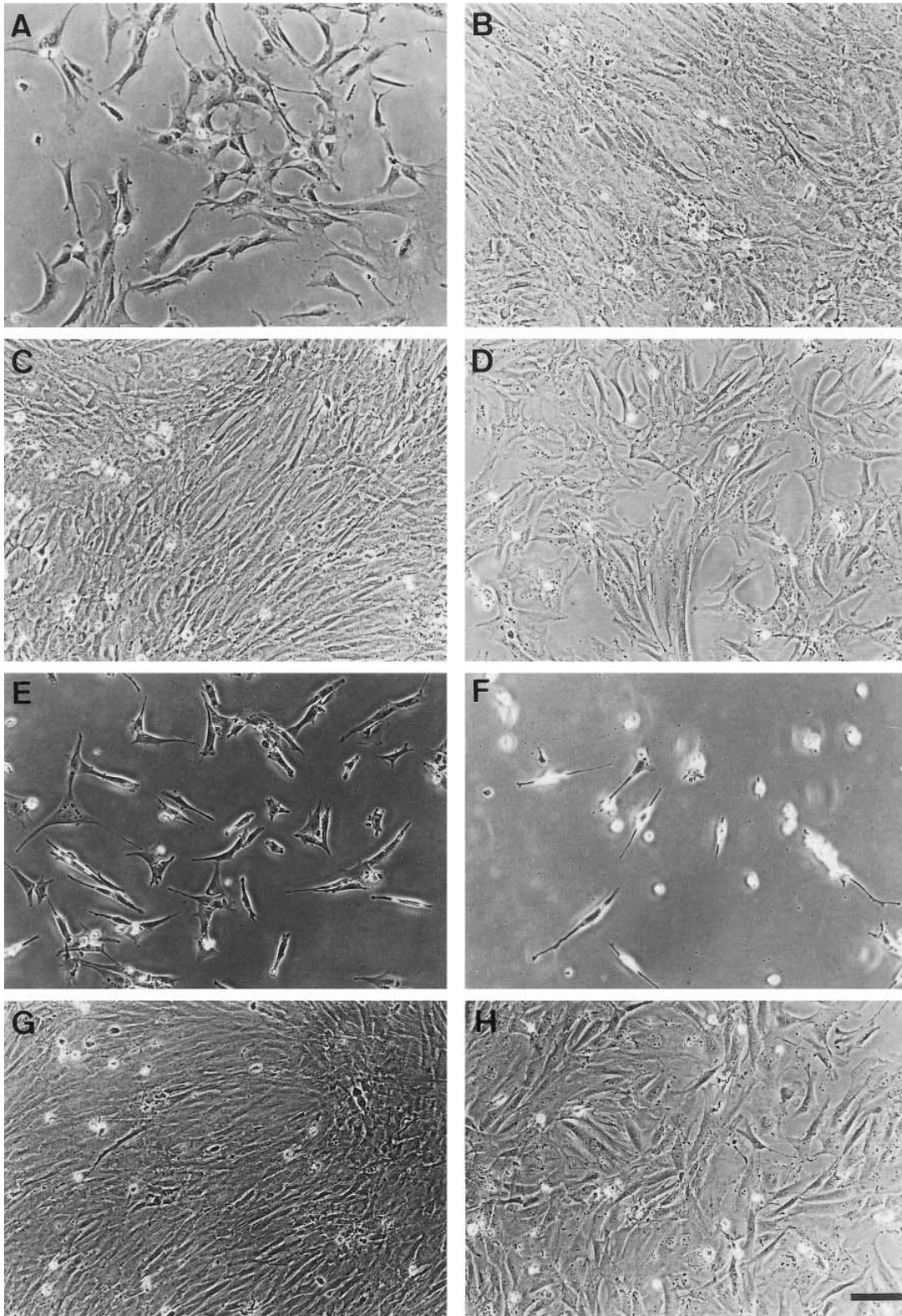
## DISCUSSION

We have previously shown that DIF-1, a putative morphogen in *D. discoideum*, has anti-tumor activities *in vitro* in several tumor cells tested so far (Asahi *et al.*, 1995; Kubohara *et al.*, 1995a,b; Kubohara, 1997). However, it has not been verified whether and how DIF-1 affects normal cells. In the present study, to assess this question, we have examined the effects of DIF-1 on normal cell functions, using RGM-1 and RLM cells; in this study, we have observed the DIF-1-treated cells for 3-4 days, since DIF-1 shows cytotoxic effects on tumor cells within 2-3 days (Kubohara *et al.*, 1995a,b; Kubohara, 1997). Table 1 summarizes the present and previous results on the effects of DIF-1.

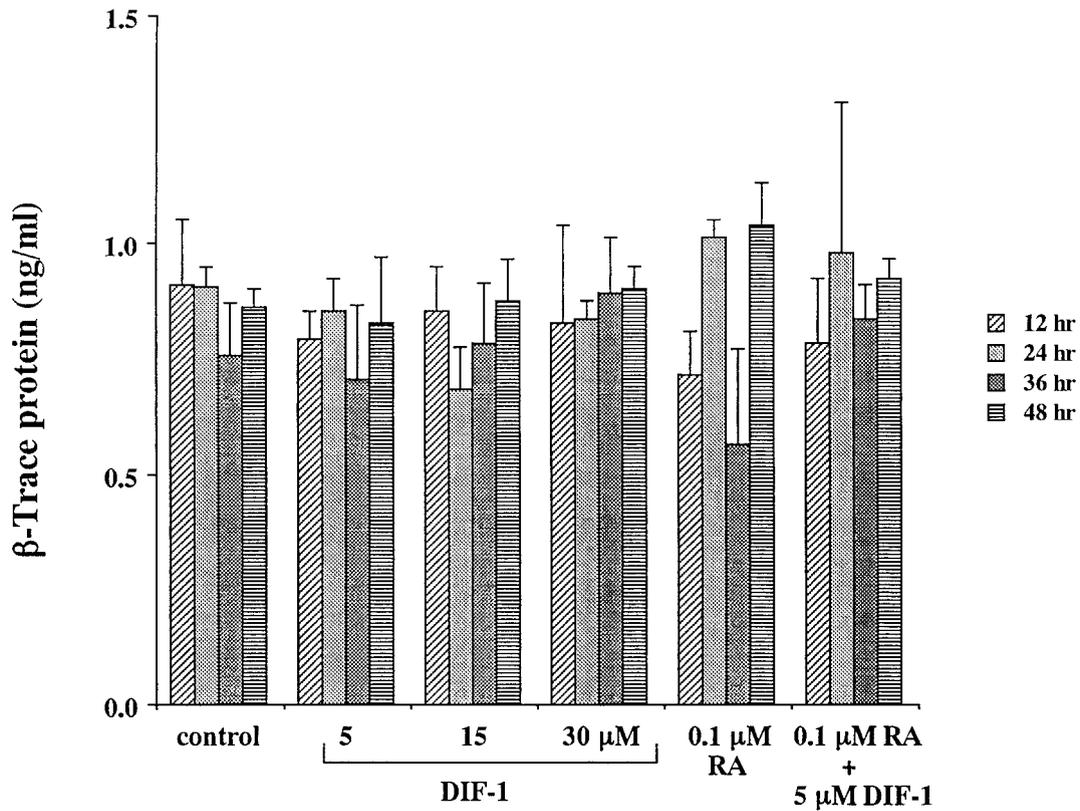
#### Effects of DIF-1 on normal cells

DIF-1 at 5-40  $\mu\text{M}$  suppressed cell growth in a dose-dependent manner (Figs. 2, 3 and 5) in both RLM and RGM-1 cells. High concentrations (20-40  $\mu\text{M}$ ) of DIF-1 affected cell morphology but the cells survived at Day 3-4; there appeared many bi-polar cells with long cytoplasmic projections, indicating that DIF-1 induced re-arrangement of cytoskeletal proteins possibly via a signalling system (Figs. 3 and 5). Characterization of the bi-polar cells should be of interest for the elucidation of DIF-1-signalling system (if any) in the mammalian cells.

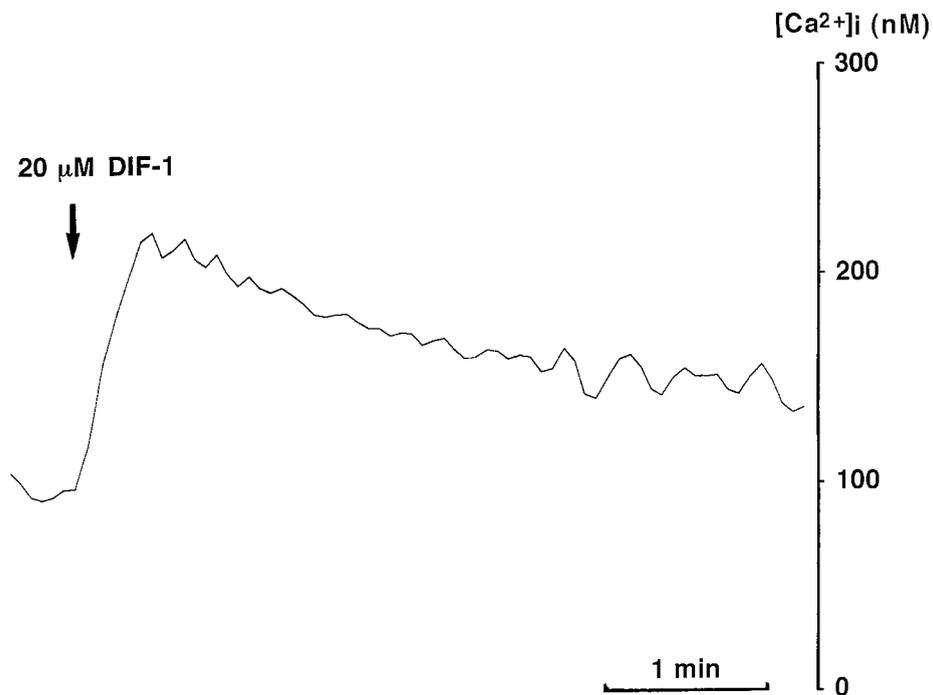
In confluent RLM and RGM-1 cells, high concentrations of DIF-1 promoted the acidification of the culture media, which might result in cell death. Although it is unknown how DIF-1 promotes medium acidification and what triggers cell death under the *in vitro* conditions, it should be of great importance to note that both RLM and RGM-1 cells survived at least for 3



**Fig. 5.** Effects of DIF-1 and retinoic acid on growing RLM cells. Growing RLM cells (**A**: Day 0) were incubated without or with 5-40  $\mu\text{M}$  of DIF-1, or 0.1  $\mu\text{M}$  retinoic acid (RA), and cell growth and morphology were observed microscopically at Day 4 (**B**: control, **C**: 10  $\mu\text{M}$  DIF-1, **D**: 20  $\mu\text{M}$  DIF-1, **E**: 40  $\mu\text{M}$  DIF-1, **G**: 0.1  $\mu\text{M}$  RA, **H**: 0.1  $\mu\text{M}$  RA and 5  $\mu\text{M}$  DIF-1). Control cells grew to reach a confluent state in 3-4 days. DIF-1 at 5  $\mu\text{M}$  showed no marked effect on both cell growth and morphology. DIF-1 at 10  $\mu\text{M}$  slightly suppressed cell growth but did not affect morphology. At 20-40  $\mu\text{M}$ , DIF-1 suppressed cell growth greatly and converted many cells to bi-polar cells with long cytoplasmic projections. RA showed no marked effect on both cell growth and morphology. RA in combination with 5  $\mu\text{M}$  DIF-1 slightly suppressed cell growth but did not affect cell morphology. Bar; 100  $\mu\text{m}$ .



**Fig. 6.** Effects of DIF-1 on  $\beta$ -trace secretion in RLM cells. Confluent RLM cells were incubated for 2 days without or with 5-30  $\mu$ M DIF-1 and/or 0.1  $\mu$ M retinoic acid (RA), and the incubation media were exchanged with fresh ones at every 12 hr.  $\beta$ -trace contents in the withdrawn media were assayed. Data are the means  $\pm$  S.E. of  $\beta$ -trace proteins secreted during each 12 hr (The experiment was done by 3-well determination ( $n = 3$ )).



**Fig. 7.** Effect of DIF-1 on  $[Ca^{2+}]_i$  in RLM cells. FuraPE3-loaded RLM cells were stimulated with 20  $\mu$ M of DIF-1 at the indicated time point. Fluorometry was performed as described under Materials and Methods.

**Table 1.** Effects of DIF-1 and retinoic acid on RLM and RGM-1 cells

	DIF-1 ( $\mu\text{M}$ )						RA	RA+ DIF-1
	5	10	15	20	30	40		
<b>RLM</b>								
Growing cells	-	$\pm$	$\pm$	+	+	++	-	$\pm$
Confluent cells	-		-		$\pm$		-	-
$\beta$ -trace secretion	-		-		-		-	-
<b>RGM-1</b>								
Growing cells	-	$\pm$	+	+	++	++	nd	nd
Confluent cells	-		-		-		nd	nd
AR42J (Growing cells)	-	$\pm$		++		+++	nd	nd
HL-60 (Growing cells)	-	$\pm$	+	++	+++	+++	+,cd	+,cd

Note: Growing cells were incubated with DIF-1 (5-40  $\mu\text{M}$ )/RA (0.1  $\mu\text{M}$ ). Confluent cells were incubated with DIF-1/RA and during incubation, the culture-media were exchanged with fresh ones containing the reagents at every 24 hr (for viability test) or every 12 hr (for the assay for  $\beta$ -trace secretion). Cell growth, viability and morphology were microscopically observed for 3 days.  $\beta$ -trace secretion was assayed as described in the text. Data on rat pancreatic tumor AR42J cells and human myeloid leukemia HL-60 cells are from our previous reports (Kubohara *et al.*, 1995a,b; Kubohara, 1997).

-, no marked effect;  $\pm$ , slight inhibition and slight changes in morphology; +, great inhibition and great changes in morphology; ++, great inhibition, great changes in morphology, and partial cell death; +++, great inhibition and a large number of cell death (> 90%); cd, cell differentiation; nd, not determined.

days (Fig. 4 and not shown data) and RLM cells secreted  $\beta$ -trace proteins normally when the culture media were exchanged with fresh ones (Fig. 6). These results would rather reflect *in vivo* situation where the blood-lymph systems circulate continuously, and the present results thus lead to an expectation that DIF-1 may not seriously damage normal confluent cells *in vivo*.

Considering that the high concentrations (20-40  $\mu\text{M}$ ) of DIF-1 kill human myeloid leukemia HL-60 (Kubohara, 1997) and rat pancreatic tumor AR42J cells (Kubohara *et al.*, 1995a,b) within 2-3 days, DIF-1 seems to be much more toxic to the tumor cells than to normal cells (Table 1).

It seems likely that DIF-1 functions via similar machinery involving increases in  $[\text{Ca}^{2+}]_i$  in both normal cells (Fig. 7) and tumor cells (Kubohara *et al.*, 1995a,b; Kubohara, 1997) as well as in *Dictyostelium* cells (Kubohara and Okamoto, 1994; Schaap *et al.*, 1996; Azhar *et al.*, 1997). However, the fine mechanisms of DIF-1-action in both mammalian and *Dictyostelium* cells are still unclear.

### Combinatory effects of retinoic acid and DIF-1 on normal cells

RA has been used in differentiation-therapy for APL which results in a high score of complete remission (Huang *et al.*, 1988). We have recently shown with human myeloid leukemia HL-60 cells that DIF-1 promotes RA-induced cell differentiation (Kubohara, 1997), suggesting that co-administration of RA and DIF-1 may improve chemoprevention/differentiation therapy for APL and other leukemia. In this study, we have therefore verified the effects of DIF-1/RA on the normal cells.

RA (0.1  $\mu\text{M}$ ) or DIF-1 (5  $\mu\text{M}$ ) alone showed no marked effect on all the indexes examined here (Table 1). DIF-1 (5  $\mu\text{M}$ ) in combination with RA slightly suppressed cell growth, but did not affect cell viability, morphology (Figs. 2 and 5), and  $\beta$ -trace secretion (Fig. 6) in RLM cells (Table 1). These results suggest that RA and DIF-1 may not damage normal cells.

### Conclusion

Most importantly, it was suggested here that DIF-1 alone or DIF-1 in combination with RA might be utilized in the treatment of some sorts of cancer cells with a bearable degree of side-effects, although the *in vivo* effects of DIF-1 should be examined in the future. Apart from the therapeutic potential of DIF-1, since DIF-1 affected cell growth and morphology in RGM-1 and RLM cells, it is also expected that DIF-1 may be a useful tool for the analysis of normal mammalian cell functions.

### ACKNOWLEDGMENT

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### REFERENCES

- Asahi K, Sakurai A, Takahashi N, Kubohara Y, Okamoto K, Tanaka Y (1995) DIF-1, morphogen of *Dictyostelium discoideum*, induces the erythroid differentiation in murine and human leukemia cells. *Biochem Biophys Res Commun* 208: 1036-1039
- Azhar M, Kennady PK, Pande G, Nanjundiah V (1997) Stimulation by

- DIF causes an increase of intracellular  $Ca^{2+}$  in *Dictyostelium discoideum*. *Exp Cell Res* 230: 403–406
- Collins SJ, Gallo RC, Gallagher RE (1977) Continuous growth and differentiation of human myeloid leukemic cells in suspension culture. *Nature* 270: 347–349
- Hassan S, Kinoshita Y, Min D, Nakata H, Kishi K, Matsushima Y, Asahara M, He-yao W, Okada A, Maekawa T, Matsui H, Chiba T (1996) Presence of prostaglandin EP4 receptor gene expression in a rat gastric mucosal cell line. *Digestion* 57: 196–200
- Huang ME, Ye YI, Chen SR, Chai JR, Lu JX, Zhou L, Gu LJ, Wang ZY (1988) Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. *Blood* 72: 567–572
- Ishikawa K, Kabeya K, Shinoda M, Katakai K, Mori M, Tatemoto K (1995a) Meninges play a neurotrophic role in the regeneration of vasopressin nerves after hypophysectomy. *Brain Res* 677: 20–28
- Ishikawa K, Ohe Y, Tatemoto K (1995b) Synthesis and secretion of insulin-like growth factor (IGF)-II and IGF binding protein-2 by cultivated brain meningeal cells. *Brain Res* 697: 122–129
- Kabeya K, Ishikawa K, Katakai K, Watanabe M, Ohe Y, Wakabayashi K, Mori M (1998) Prostaglandin-D-synthase ( $\beta$ -trace protein) levels in rat cerebrospinal fluid are decreased by peripheral injection of retinoic acid. *Neuroreport* 9: 915–919
- Kay RR, Berks M, Traynor D (1989) Morphogen hunting in *Dictyostelium discoideum*. *Development Suppl*: 81–90
- Kubohara Y (1997) DIF-1, putative morphogen of *D. discoideum*, suppresses cell growth and promotes retinoic acid-induced cell differentiation in HL-60. *Biochem Biophys Res Commun* 236: 418–422
- Kubohara Y, Okamoto K (1994) Cytoplasmic  $Ca^{2+}$  and  $H^+$  concentrations determine cell fate in *Dictyostelium discoideum*. *FASEB J* 8: 869–874
- Kubohara Y, Kimura C, Tatemoto K (1995a) Putative morphogen, DIF, of *Dictyostelium discoideum* induces apoptosis in rat pancreatic AR42J cells. *Develop Growth Differ* 37: 711–716
- Kubohara Y, Saito Y, Tatemoto K (1995b) Differentiation-inducing factor of *D. discoideum* raises intracellular calcium concentration and suppresses cell growth in rat pancreatic AR42J cells. *FEBS Lett* 359: 119–122
- Morris HR, Taylor GW, Masento MS, Jermyn KA, Kay RR (1987) Chemical structure of the morphogen differentiation inducing factor from *Dictyostelium discoideum*. *Nature* 328: 811–814
- Ohe Y, Ishikawa K, Itoh Z, Tatemoto K (1996) Cultured leptomenigeal cells secrete cerebrospinal fluid proteins. *J Neurochem* 67: 964–971
- Okada A, Kinoshita Y, Maekawa T, Hassan MS, Kawanami C, Asahara M, Matsushima Y, Kishi K, Nakata H, Narabayashi Y, Chiba T (1996) Erythropoietin stimulates proliferation of rat-cultured gastric mucosal cells. *Digestion* 57: 328–332
- Schaap P, Nebl T, Fisher PR (1996) A slow sustained increase in cytosolic  $Ca^{2+}$  levels mediates stalk gene induction by differentiation inducing factor in *Dictyostelium*. *EMBO J* 15: 5177–5183
- Smith GM, Shine HD (1992) Immunofluorescent labeling of tight junctions in the rat brain and spinal cord. *Int J Dev Neurosci* 10: 387–392
- Tanno H, Nockels RP, Pitts LH, Noble LJ (1993) Immunolocalization of heat shock protein after fluid percussive brain injury and relationship to breakdown of the blood-brain barrier. *J Cereb Blood Flow Metab* 13: 116–124

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