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Growth-promoting activity of tissue inhibitor of metalloproteinases-1 (TIMP-1) for a wide range of cells

A possible new growth factor in serum

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Human tissue inhibitor of metalloproteinases-1 (TIMP-1), but not TIMP-2, has potent growth-promoting activity for a wide range of human and bovine cells. TIMP-1 seems to be a new cell-growth factor in serum and to stimulate the cells independently of its inhibitory activity.

Tissue inhibitor of metalloproteinase (TIMP-1 and TIMP-2); Cell-growth promoting activity; Serum growth factor; Human cell; Bovine cell

1. INTRODUCTION

The tissue inhibitor of metalloproteinases-1 (TIMP-1), a sialoglycoprotein with a molecular weight of around 30,000, is a specific inhibitor of matrix metalloproteinases (MMPs) such as interstitial collagenase, gelatinase/type IV collagenase (72 kDa and 92 kDa) and stromelysin, but not for metalloproteinases such as bacterial collagenase and thermolysin [1]. TIMP-1 is produced by various kinds of cells and found in every human body fluid examined [2,3], suggesting that TIMP-1 is a fundamental and ubiquitous protein in human beings. The amino acid sequence [4] of TIMP-1 deduced from its cDNA analysis is known to be identical to that of erythroid potentiating activity (EPA) [5], which stimulates the growth of erythroid precursors (BFU-E and CFU-E) [6] and the K-562 human erythroleukemia cell line [7]. We recently demonstrated that TIMP-1 produced by human bone marrow stromal cell line KH 102 stimulated the colony formation of BFU-E and CFU-E [8]. These findings prompted us to investigate the possibility that TIMP-1 possesses growth-factor activity for other cells besides erythroid precursor cells. Here we report that human TIMP-1 has potent growth-promoting activity for a wide range of cells and provide some facts that support the proposition that TIMP-1 is a new cell-growth factor in serum.

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2. MATERIALS AND METHODS

2.1. *Culture media*

Dulbecco's modified Eagle's minimal essential medium (D-MEM) was purchased from Gibco Laboratories, Grand Island, NY, USA. RPMI 1640 medium was from Flow Laboratories, Irvine, Scotland, UK. ASF-104, an albumin-free culture medium, was from Ajinomoto Ltd., Tokyo. Fetal calf serum (FCS) was from Bio Cell, Carson, CA, USA.

2.2. *Bovine and human cells*

The following cell lines were supplied by the Japanese Cancer Research Resources Bank (JCRB) and cultured in JCRB-recommended media: human lymphoblasts, non-secreting variant (WIL2-NS); human erythroleukemia cells (K-562); human myelogeneous leukemia cells (HL60); bovine pulmonary artery endothelial cells (CPA); Burkitt lymphoma cell lines Daudi and Ramos; human hepatoma cells (HLE). Human gingival fibroblasts (Gin-1), SV40-transformed human lung cells (WI-38VA13), and human skin epithelial cells (NCTC 2544) were purchased from the American Type Culture Collection, Rockville, MD, USA. Raji cells were generously provided by Dr. Shonen Yoshida (Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, Nagoya, Japan). Human aortic smooth muscle cells (HSM) were a kind gift of Dr. Yasuyuki Sasaguri (Department of Pathology, Kurume University School of Medicine, Kurume, Japan). Human breast adenocarcinoma cells (MCF7) were a generous gift of Dr. Shuji Yamamoto (Bio-Science Laboratory, Life Science Research Laboratories, Asahi Chemical Industry Co., Fuji-City, Shizuoka, Japan). Bovine articular chondrocytes (BAC) were a generous gift from Maruho Co., Osaka, Japan.

2.3. *Human TIMP-1 and TIMP-2 preparations*

Natural (n)TIMP-1 was purified in a homogeneous form from Gin-1 cell-conditioned medium as reported previously [9]. Recombinant (r)TIMP-1 that was expressed in a mouse fibroblast (C127) cell line and purified to more than 95% [10] was purchased from Celltech, Ltd., Slough, UK. nTIMP-2 was purified as described [11].

2.4. *Preparation of TIMP-1-free FCS*

TIMP-1-free FCS was prepared by the passage of FCS through an

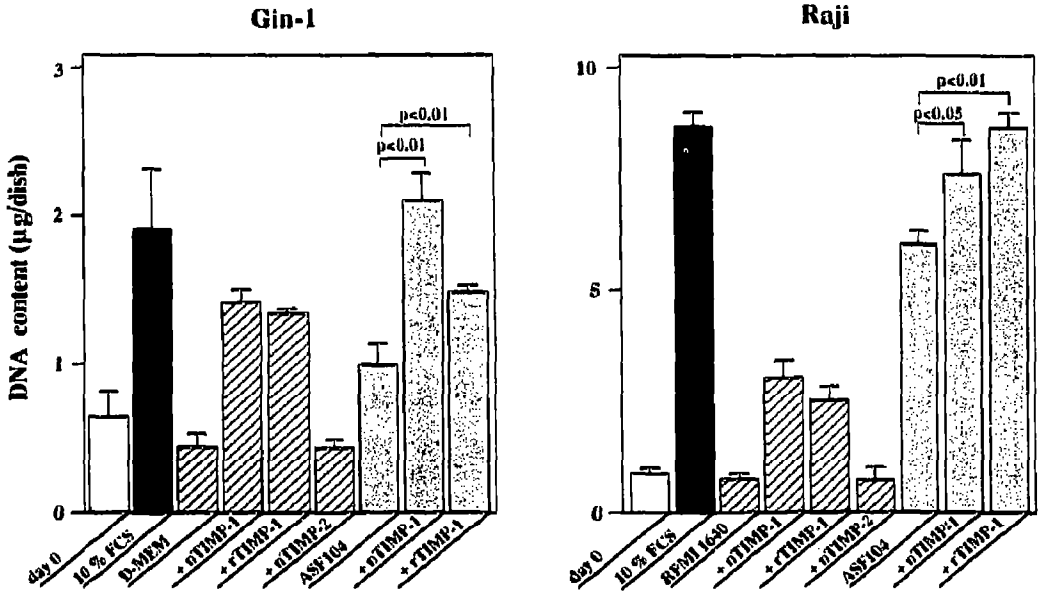

Fig. 1. Effect of TIMP addition on the growth of cells cultured in serum-free synthetic medium. The concentration of either human natural (n) TIMPs (TIMP-1 and TIMP-2) or human recombinant (r) TIMP-1 was 100 ng/ml (3.6 nM). DNA content at day 3 was determined with Burkitt lymphoma cell line Raji and at day 7 with human gingival fibroblasts (Gin-1). Results are the mean of three experiments, with standard deviation.

anti-TIMP-1 monoclonal antibody-Sepharose affinity column [9] to remove TIMP-1, and then sterilized by passage through a FALCON 7104 bottle stop filter (0.40 μm, Becton Dickinson, Lincoln Park, NJ, USA).

2.5. *Determination of TIMP-1 and DNA concentrations*

The TIMP-1 concentration was determined by a sandwich enzyme immunoassay [12]. DNA contents were determined by the method reported by Hinegardner [13].

3. RESULTS AND DISCUSSION

We first examined the effect of TIMP-1 addition on the growth of human gingival fibroblasts (Gin-1) and Burkitt lymphoma cell line Raji cultured in serum-free synthetic media (Fig. 1). Neither cell line was able to grow in basal media such as Dulbecco's modified Eagle's minimal essential medium (D-MEM) and RPMI 1640 medium. The cells, however, grew significantly in those basal media supplemented with 100 ng/ml (3.6 nM) of highly purified human natural (n)TIMP-1. Human recombinant (r)TIMP-1 had the same growth-promoting activity as nTIMP-1. This strongly supports our hypothesis that the growth-promoting activity can be ascribed to TIMP-1 alone. In enriched media such as ASF-104, both cells proliferated to a certain extent. However, both nTIMP-01 and rTIMP-1 significantly stimulated the cell growth up to the level close to that in 10% FCS. As Raji cells do not secrete MMPs, TIMP-1 seems to stimulate the cells independently of its inhibitory activity.

Recently, a new member of the metalloproteinase inhibitor family, TIMP-2, was reported [11,14–17]. TIMP-2 has a spectrum of antimetalloproteinase activities similar to that of TIMP-1 but differs from TIMP-1 in that it is unglycosylated and bound to the proenzyme form of 72-kDa gelatinase/type IV collagenase [16]. TIMP-2 is also clearly distinguished from TIMP-1 by not reacting with monospecific anti-TIMP-1 antibodies [14,17]. It has been recently reported that TIMP-2 regulates the autoactivation of interstitial collagenase [18] and 72-kDa gelatinase [19], and preferentially inhibits 72-kDa and 92-kDa gelatinases [20]. As shown in Fig. 1, TIMP-2 did not have any cell growth-promoting activity, thus demonstrating that there is a definite difference in the physiological role between TIMP-1 and TIMP-2. This result further supports the fact that the cell-proliferating activity of TIMP-1 is not merely related to its inhibitory activity. It is noteworthy

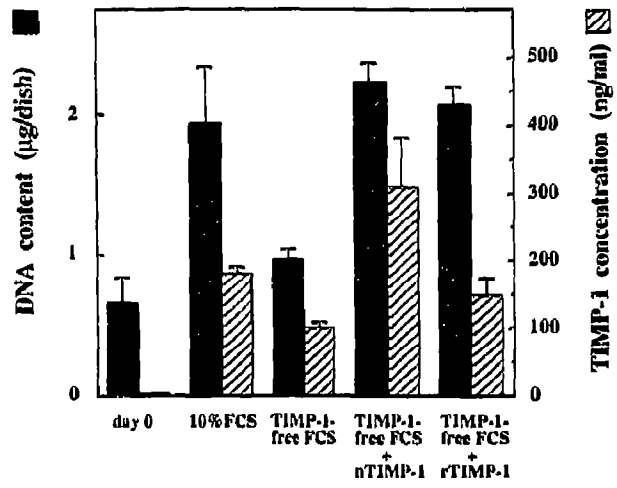

Fig. 2. TIMP-1-dependent proliferation of human gingival fibroblasts. The 10% FCS in the absence of cells contained 34.1 ng/ml TIMP-1. Results are the mean of three to six experiments, with standard deviation.

that TIMP-1 has a sequence homologous with human granulocyte-macrophage colony-stimulating factor (GM-CSF) in the N-terminal domain [21] but that TIMP-2 has no such sequence. This is consistent with the degrees of cell-growth promoting activity of both TIMPs mentioned above.

We have already reported that the TIMP-1 concentration in human serum is 183 ± 30 (mean ± S.D.) ng/ml [3]. TIMP-1 concentrations in 29 fetal calf serum (FCS) samples obtained from several different distributors ranged from 100 to 460 ng/ml (mean ± S.D., 213 ± 79). Although several chemically defined synthetic media have been developed, the requirement for serum still remains for many vertebrate cell lines, hinting that one or more further unknown serum factors may play key roles in regulating in vivo cell maintenance and proliferation. TIMP-1 may be such a factor.

When anti-TIMP-1 monoclonal antibodies (clone 7-6Cl) [9] were added to the culture medium of Gin-1 cells, almost complete suppression of cell growth was observed in the presence of 2 μg/ml antibody (data not shown). We next examined whether the cells might grow in TIMP-1-free FCS. As shown in Fig. 2, the cell proliferation was suppressed remarkably in TIMP-1-free FCS. The growth was, however, fully restored by the addition of nTIMP-1 or r TIMP-1 to the culture system. All the TIMP-1 (100.4 ± 7.8 ng/ml) detected in TIMP-1-free FCS culture medium was surely produced by the

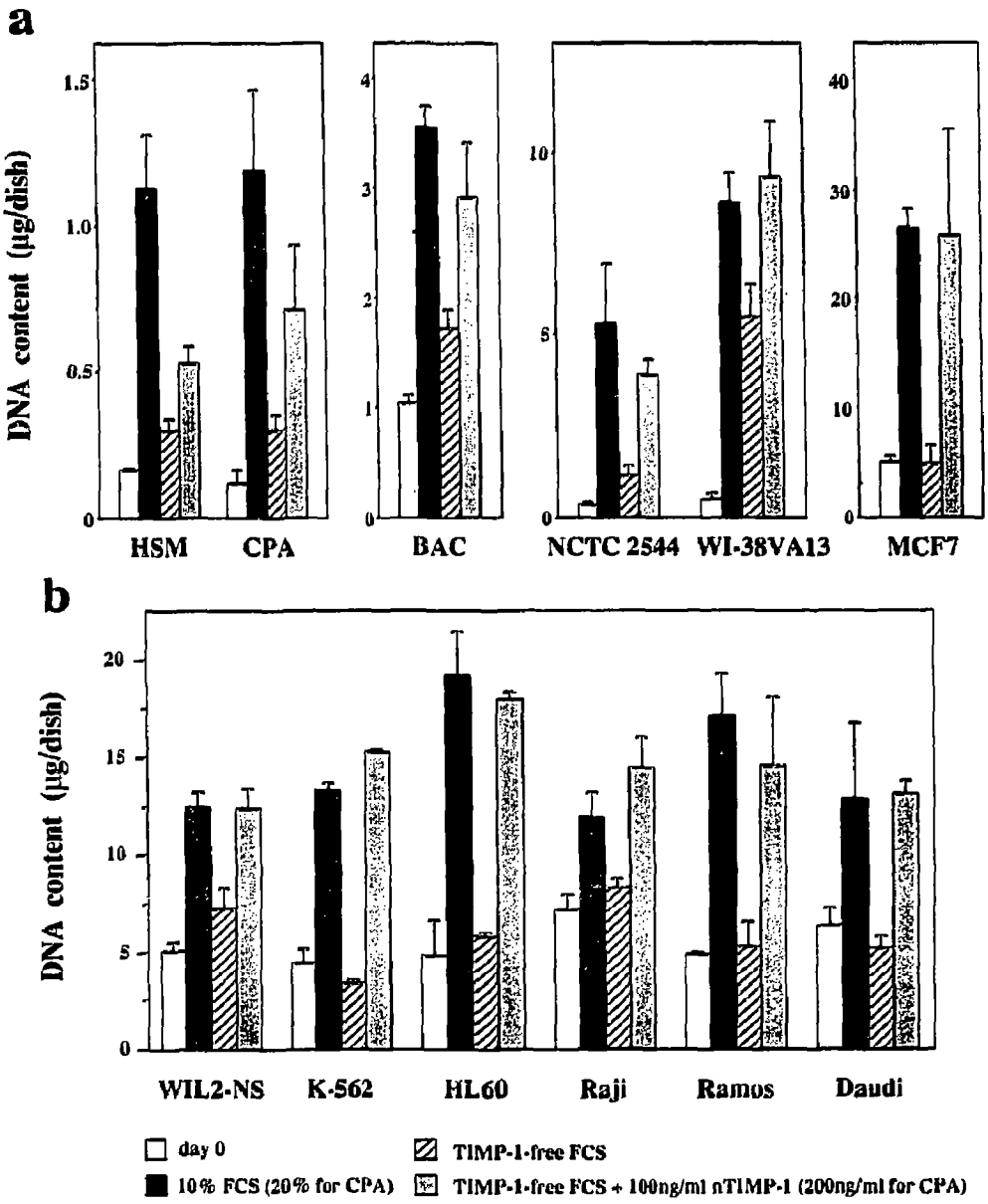

Fig. 3. TIMP-1-dependency of cell proliferation of various types of cells. DNA content at day 7 for adherent cells (a) and at day 2 for non-adherent cells (b) was determined. Results are the mean of three experiments, with standard deviation. HSM, human aortic smooth muscle cells; CPA, bovine pulmonary artery endothelial cells; BAC, bovine articular chondrocytes; NCTC 2544, human skin epithelial cells; WI-38VA13, SV40-transformed human lung cells; MCF7, human breast adenocarcinoma cells; WIL2-NS, human lymphoblasts; K-562, human erythroleukemia cells; HL60, human myelogeneous leukemia cells; Raji, Ramos, and Daudi, Burkitt lymphoma cell lines.

cells themselves. The cell proliferation was completely suppressed by a limited amount (54 ng/ml) of anti-TIMP-1 monoclonal antibody (clone 7-6Cl) added to TIMP-1-free FCS-containing cultures from the beginning to trap the newly synthesized TIMP-1 (data not shown). These findings strongly suggest that TIMP-1 produced by the cells themselves certainly stimulates their proliferation by either an autocrine or paracrine mechanism. As is evident from Fig. 2, the TIMP-1 content in the culture medium supplemented with rTIMP-1 was significantly low ($P<0.05$) compared with that in medium supplemented with the same amount of nTIMP-1. We found that some metalloproteinase(s) produced by Gin-1 cells during the 7-day culture, selectively acted on rTIMP-1 and made it insensitive to the sandwich enzyme immunoassay for TIMP-1 (data not shown).

The question then arose as to whether the growth-promoting activity of TIMP-1 was limited to some specific cells like Gin-1 cells. To answer this question, we examined the TIMP-1 dependency of cell proliferation with several human and bovine adherent and non-adherent cells. It appeared that the growth of all the cells examined was significantly dependent on TIMP-1 (Fig. 3). However, the growth of human aortic smooth muscle cells (HSM) was not fully restored by the addition of human TIMP-1. SV40-transformed human lung cells (WI-38VA13) grew quite well even in TIMP-1-free FCS. EPA, which has been revealed to stimulate specifically the proliferation of erythroid progenitor cells and K- 562 cells, can now be considered to be one aspect of the general cell growth-promoting activity of TIMP-1.

It is intriguing that TIMP-1 appears to be a single glycoprotein with both protease inhibitory activity and growth-promoting activity. In the case of adherent cells, for which the extracellular matrix is essential both structurally and functionally, we can easily understand that TIMP-1 has an important role, on the one hand, for the maintenance of extracellular matrix integrity and, on the other hand, for the proliferation of these cells in either autocrine or paracrine fashion. Even in the case of non-adherent cells, especially in the in vivo situation, the differentiation and proliferation of these cells might depend on their interaction with the surrounding environment. Actually, it is well recognized that the hematopoietic microenvironment plays an important part in the differentiation and proliferation of blood cells [22]. In view of the above, it is not illogical that TIMP-1 has both protease inhibitory activity and growth-promoting activity. Similar examples like endothelial cell growth factors 2a and 2b (ECGF-2a and -2b) have already been reported [23], which also have protease inhibitory activities. An investigation into the mechanism of the growth-promoting activity of TIMP-1, such as the search for a TIMP-1 receptor, is now underway in our laboratory.

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