Volume 255, number 2, 423-426 FEB 07647 September 1989

Differentiation of intestinal epithelial cell line (IEC-18) by an acid extract of rat small intestine

Takafumi Shintani, Tohru Fushiki, Shin-ichi Fukuoka, Hiromi Takahashi-Iwanaga* and Etsuro Sugimoto

Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto 606 and **Department of Anatomy, School of Medicine, Niigata University, Niigata 951, Japan*

Received 17 July 1989; revised version received 14 August 1989

A factor which may induce differentiation of intestinal epithelial cell lines in vitro was found in an acid extract of adult rat small intestine. The addition of a partially purified acetic acid extract of rat small intestine to IEC-18 cell culture dishes increased sucrase activity within 48 h. Thymidine incorporation markedly decreased within 24 h. Significant development of microvilli-like structures was observed on the acid extract-treated IEC-18 cells, compared with controls. This activity of rat acid extract was heat-stable and the apparent molecular weight of the factor was 400–800. These findings suggested that the factor may be related to the epithelial differentiation of rat small intestinal crypt cells.

Differentiation; Culture cell; Small intestine; Sucrase

1. INTRODUCTION

The small intestine is a functionally differentiated system consisting of crypt stem cells and highly differentiated function cells. The majority of crypt stem cells give rise to epithelial absorptive cells, which die within 2 or 3 days on extrusion from the tips of the villi into the intestinal lumen. However, little is known about the epithelial differentiation phenomenon.

Quaroni et al. [1] established intestinal epithelial cell (IEC) lines. According to their results [1,2], they may be undifferentiated epithelial crypt cells, although it has not been possible to induce their complete differentiation in vitro. Recently, Kedinger et al. [3] succeeded in inducing their complete differentiation by sandwiching IEC-17 cells, which are similar to IEC-18 cells, between two homotopic fetal rat intestine mesenchymes, followed by grafting under the kidney capsule of an adult rat. This led us to speculate that the fetal rat gut mesenchyme releases a factor which enables the morphogenesis and cytodifferentiation of crypt cells.

In this study, we found a factor which can at least partially induce IEC-18 cell differentiation in vitro, and partially purified it from an acid extract of adult rat small intestine.

Correspondence address: T. Shintani, Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan

2. MATERIALS AND METHODS

2.1. *Purification of epithelial cell differentiation factor*

Two hundred Wistar rats, weighing 200–250 g, were starved for 16 h and then killed by decapitation. The duodenum and jejunum were taken out immediately and the lumen was washed with ice-cold saline. The small intestine was minced and frozen until the purification. The frozen pool of minced small intestine was melted in 0.5 N acetic acid, followed by homogenization. The homogenate was boiled for 5 min in a water bath, sonicated for 10 min at 4°C, and then stirred for 48 h at 4°C. The supernatant was filtered through a glass filter and then lyophilized. The lyophilized extract was dissolved in 150 ml of water. Unless otherwise stated, 0.2 ml of the resultant solution was added to 1 ml of cell culture medium throughout the present experiments. Further purification was carried out by Sephadex G-10 gel filtration and high-performance liquid chromatography (HPLC), on

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

a 4R503 reverse-phase column (Synchrome Inc.; 1.5 × 5 cm) and a silica column (Sorbax SIL., DuPont Instrument; 4.6 × 150 mm), respectively.

2.2. *Cell culture conditions*

The epithelial cell line (IEC-18), which was established by Quaroni and Isselbacher of duodenal fragments of rats was purchased by Dainippon Seiyaku Co. (Japan). We cultured these cells at 37°C under an atmosphere of 5% CO_2 in air. The medium used comprised of DMEM containing 10% fetal calf serum, and penicillin, 5 IU/ml, and streptomycin, 5 mg/ml, were added as antibiotics. Other conditions were the same as previously reported [1,2].

2.3. *Differentiation assay*

IEC-18 cell differentiation was followed by measuring the increase in sucrase activity, this being well established indicators of differentiation in the jejunum [4,5]. The medium with the acid extract of the small intestine was given to 50% confluent cell cultures in 24-well multiplates. Sucrase activity was measured every 12 h after starting cell culture with the medium containing rat small intestine acid extract. Cell proliferation was measured as the incorporation of [^{3}H]thymidine [6].

2.4. *Preparation of samples for observation by scanning electron microscopy*

The medium with the small intestinal extracts was given to the 50% confluent cultures. After 2 days, the cells were fixed with a 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, at 4°C overnight, postfixed with 1.0% OsO_4, and then circles of 10 mm in diameter were punched out of the bottoms of the dishes. Subsequently, the specimens were dehydrated through a graded series of ethanol, critical-point-dried using CO_2, evaporation-coated with gold-palladium, and examined in a scanning electron microscopy (Hitachi S-450 LB) at an accelerating voltage of 10 kV.

3. RESULTS AND DISCUSSION

The fetal rat gut mesenchyme was reported to enable the morphogenesis and cytodifferentiation of crypt cells. Though the mechanism underlying the differentiation of crypt cells has been investigated [7,8], the material responsible for the cell differentiation remains unclear. In this experiment, we tried to identify the material which triggers the differentiation of nondifferentiated crypt cells into epithelial function cells.

The effect of the acid extract of rat small intestine on IEC-18 cell proliferation and differentiation was investigated. As shown in table 1, in IEC-18 cells the incorporation of [^{3}H]thymidine stopped within 1 day after the addition of the acid extract, but cell viability remained at 70% throughout the experiment, as judged with the trypan blue staining method. The activity of sucrase which has been established as a mature epithelial cell marker enzyme, was markedly increased 2 days after the addition (fig.1). These findings suggested that addition of the acid extract of rat small intestine caused cessation of cell proliferation and differentiation into mature intestinal epithelial cells at the same time.

Table 1

Effect of the acid extract of rat small intestine on thymidine incorporation in IEC-18 cells

Addition	[^{3}H]thymidine incorporation (× 10^2 dpm/well)			
	0 h	12 h	24 h	36 h
Acid extract	5.50 ± 1.3	2.50 ± 0.4	0.38 ± 0.04	0.51 ± 0.06
Saline	5.35 ± 1.1	11.1 ± 2.3	22.0 ± 1.5	45.6 ± 4.2

The medium with the acid extract (see section 2) was added to the 96-well dishes (about 50% confluent, 3 × 10^4 cells/well at first). Thymidine incorporation was examined by a previously described method [6]

Fig.2 shows a typical image obtained on scan-

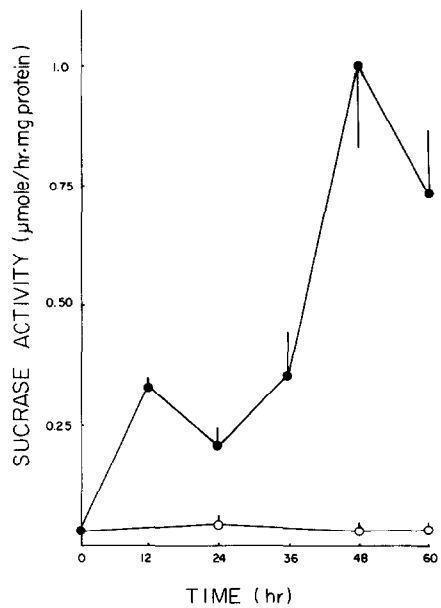

Fig.1. Effects of the acid extract of rat small intestine on sucrase activity. The medium with the acid extract of rat small intestine (see section 2) was given to cultures in 24-well plates (about 50% confluent on the first day), and then after incubation for certain periods, the enzyme substrate solution was added to the cultures after washing with Hank's solution. Sucrase activity during incubation in the presence (●) and absence (○) of the acid extract was determined as described [4,5].

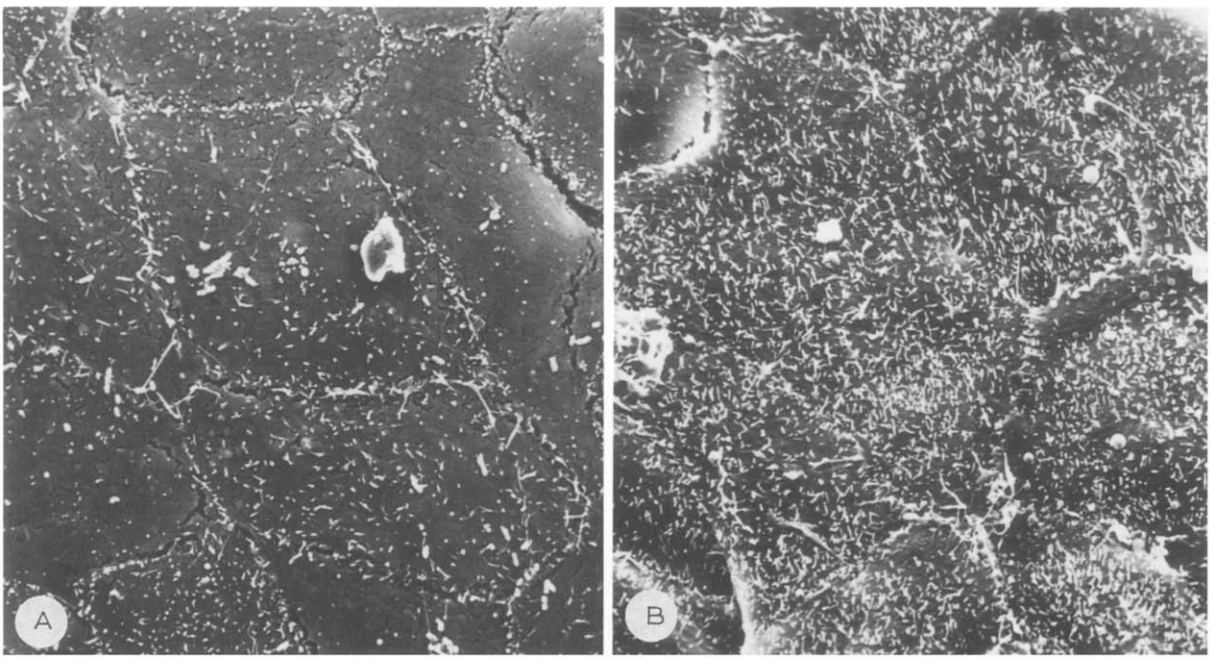

Fig.2. Observation by means of scanning electron microscopy (SEM). Cultures at 2 days after the addition of a medium with the small intestinal extract were fixed with a 5% glutaraldehyde solution at 4°C overnight, and then circles of 10 mm in diameter were punched out of the bottoms of the dishes for observation by SEM (× 2600). (A) Control. (B) IEC-18, with acid extract added.

ning electron microscopy observation. Development of microvilli-like structures was observed on the experimental cell surface (fig.2B). In the control cells (fig.2A), about 50% of the cells only had short microvilli-like structures, 0.1–0.2 μm in length on average. In the experimental cells, to which the acid extract had been added 2 days before observation, dense microvilli-like structures of 0.5 μm in length on average were observed on the surface of 70–80% of the cells. These findings are very consistent with the changes in enzyme activity during the culture of IEC-18 cells with the acid extract of the small intestine mentioned above. Some of the cells in the experimental group had many microvilli of more than 1 μm in length. On the contrary, in the control dishes, such differentiated cells were not found at all. Therefore, the acid extract of rat small intestine may contain a regulatory factor for epithelial cell differentiation. This is characteristic of the small intestine, since acid extracts of rat liver, kidney and spleen did not exhibit such an activity. Then we carried out further purification of the factor, using its apparent cell differentiation activity as an index. About 1 liter of acetic acid extracts was obtained from the small intestines of 200 Wistar male rats. The extracts were passed through a 4R503 column (Syn-Chrom Inc.; 1.5 × 5 cm) at neutral pH and then lyophilized. The concentrate was subjected to Sephadex G-10 gel filtration and then HPLC on a silica column (Sorbax SIL; 4.6 × 150 mm), with elution with 10% ethanol at 1.8 ml/min. As shown in fig.3, apparent cell differentiation activity was detected for a peak of absorbance at 220 nm, which is indicated by the arrow. Gel filtration on Sephadex G-10 and HPLC on a Water I-125 column showed that the apparent molecular weight of the active fraction was between 400 and 800.

Kurokawa et al. [9] suggested that TGFβ1 may promote the development of intestinal epithelial cells. Recently, it was found that TGFβ1 can stop the proliferation of IEC-6 epithelial cells but that it has no effect on the expression of microvilli-marker enzymes [10]. The present active fraction is obviously distinguishable from these growth factors in its molecular weight and mode of action. After 5 min boiling of the acid extract of small intestine at 100°C, differentiation activity remained.

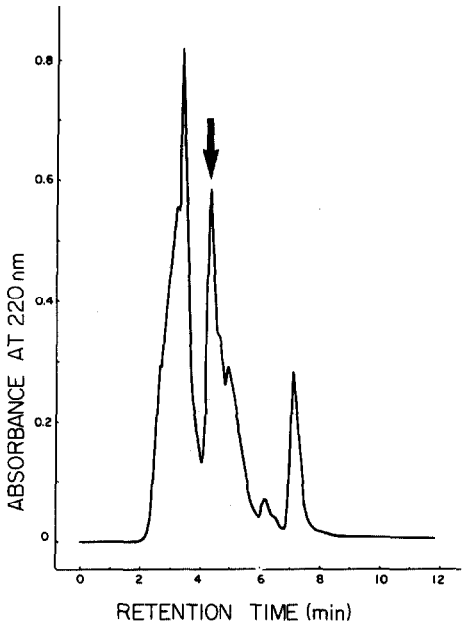

Fig.3. HPLC of the crude preparation from the acid extract of rat small intestine. The acetic acid extract of rat small intestine was passed through a 4R503 reverse-phase column (1.5 × 5 cm) at neutral pH and then concentrated by lyophilization. The concentrate was subjected to Sephadex G-10 gel filtration and then an analytical HPLC column (Dupont, Sorbax SIL; 4.6 × 150 mm) in a system equipped with a model 660 programmer and a model 510 pump (Waters). The biological activity toward IEC-18 was assayed. The active fraction peak is indicated by the arrow.

But 20 min autoclaving at 120°C abolished about 50% of the activity.

In this experiment, we added the acid extract to a medium (0.2 ml into 1 ml) and gave the medium to 50% confluent cells. This may not be enough to induce the complete differentiation of every cell, since when we added more acid extract to the dishes, higher sucrase activity was observed. However, these microvilli-like structures induced by the acid extract may not be completely developed microvilli, like those that can be seen in the rat small intestine, since their length was not uniform and their density was much lower than that in the rat lumen. We cannot rule out the possibility of other co-operative factors that induce complete differentiation.

Overall, IEC-18 cells have been reported to have poor microvilli structures and it has not been possible to induce their complete differentiation in vitro, so our present findings must be a clue as to epithelial development at the level of materials in vitro.

Acknowledgement: This work was supported by a Grant-in-Aid for Scientific Research 63636003, 01636006, and 62470126 from the Ministry of Education, Science and Culture of Japan.

REFERENCES

[1] Quaroni, A., Wands, J., Trelstad, R.L. and Isselbacher, K.J. (1979) J. Cell. Biol. 80, 248–265.
[2] Quaroni, A. and May, R.J. (1980) Methods in Cell Biology, vol. 218, pp. 403–427, Academic Press, New York.
[3] Kedinger, M., Simmon-Assmann, P.M., Lacroix, B., Marxer, A., Hauri, H.P. and Haffen, K. (1986) Dev. Biol. 113, 474–483.
[4] Fortin-Magana, R., Hurwitz, R., Herbert, J.J. and Kretchmer, N. (1969) Science 167, 1627–1628.
[5] Dahlquist, A. (1964) Anal. Biochem. 7, 18–25.
[6] Aratani, Y., Sugimoto, E. and Kitagawa, Y. (1987) FEBS Lett. 218, 47–51.
[7] Gordon, J.I. (1989) J. Cell. Biol. 108, 1187–1194.
[8] Lipkin, M. (1987) in: Physiology of the Gastrointestinal Tract (Johnson, L.R., ed.) pp. 255–300, Raven, New York.
[9] Kurokawa, M., Lynch, K. and Podolsky, D.K. (1987) Biochem. Biophys. Res. Commun. 142, 775–782.
[10] Barnard, J.A., Beauchamp, R.D., Coffey, R.J. and Moses, H.L. (1989) Proc. Natl. Acad. Sci. USA 86, 1578–1582.