SHORT REPORTS

Glycosaminoglycan Synthesis by Cultured Human Epidermal Cells

AKIRA KONOHANA, SHINGO TAJIMA and TAKEJI NISHIKAWA

Department of Dermatology, Keio University School of Medicine, Tokyo, Japan

Konohana A, Tajima S, Nishikawa T. Glycosaminoglycan synthesis by cultured human epidermal cells. Acta Derm Venereol (Stockh) 1986; 66: 56–58.

Human epidermal cells obtained from foreskin were grown without any feeder layers and glycosaminoglycan synthesis of the epidermal cells were investigated using ^{3}H-glucosamine as a tracer. At logarithmic and confluent phase of primary culture and confluent phase of 2nd passage culture glycosaminoglycans were found to be actively synthesized. The major component of synthesized glycosaminoglycans was hyaluronic acid. Our study indicates that cultured human epidermal cells constantly produce hyaluronic acid and suggests that glycosaminoglycans in the intercellular region of the epidermis seem to be synthesized by epidermal cells. *Key words: Epidermis; Mucopolysaccharides; Keratinocyte.* (Received April 10, 1985.)

A. Konohana, Department of Dermatology, Keio University School of Medicine, Shinanomachi 35, Shinjuku-ku, Tokyo 160, Japan.

Glycosaminoglycans (GAGs) in the dermis are an important component of dermal connective tissue and have been well studied. However, few data on GAGs in the epidermis have been reported. We proved that GAG content in human foreskin epidermis is about a half of the dermis and its major component is hyaluronic acid (unpublished data). Recently, the epidermis has been found to synthesize GAGs in vitro (1, 2). In the present work, we have cultured human epidermal cells without any feeder layers and have investigated the synthesis of GAGs under this condition.

MATERIALS AND METHODS

Epidermal cell culture

Foreskins were obtained during surgical circumcision, defatted, cut into strips and incubated with 0.25% trypsin in phosphate buffered saline (pH 7.4) at 4°C overnight. The epidermis was separated using forceps from the dermis and shaked in Dulbecco's Eagle medium (Gibco Laboratories, Ohio, USA) supplemented with 20% fetal calf serum (Gibco Laboratories, Ohio, USA) and 0.6 μg/ml hydrocortisone. Isolated epidermal cells were inoculated at the density 5×10^5 cells per 35 mm culture dish (Miles Laboratories, Illinois, USA) with 2 ml of the above-described medium and cultured at 37°C under 5% CO_2-95% air. The medium was changed twice a week. Two weeks after the inoculation epidermal cells became confluent and were subcultured according to the technique of Rheinwald et al. (3). In the 2nd passage culture 2×10^5 epidermal cells per dish were innoculated and became confluent within three weeks.

Labelling method of cultured epidermal cells

The cells were preincubated for 6 hours in the fresh medium supplemented with 20% fetal calf serum and 0.6 μg/ml hydrocortisone. Then, the cells were incubated in the fresh medium containing 100 μCi of D-[6-^{3}H(N)]-glucosamine hydrochloride (31.3 Ci/mmol, New England Nuclear) for 18 hours. The medium and cell fractions were harvested separately.

Isolation and identification of synthesized glycosaminoglycans

After digestion with pronase (Kakenseiyaku, Tokyo, Japan) at 50°C for 2 days, the sample was precipitated with 10% trichloroacetic acid at 4°C. The supernatant was dialysed against distilled water and lyophilized. Isolated GAGs were subjected to electrophoresis on cellulose acetate membranes with 0.1 M pyridine-0.47 M formate buffer, pH 3.0. Membranes were sliced into 2 mm strips, which were dissolved in 1.4-dioxane. Their radioactivity was counted in 10 ml of Aquasol-2

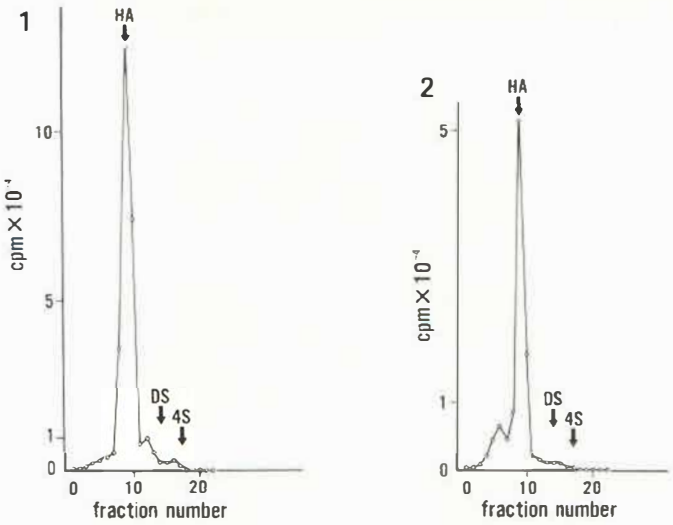

Fig. 1. Electrophoretic pattern of synthesized glycosaminoglycans isolated from the medium fraction of the 2nd passage culture. The 2nd passage culture of human epidermal cells was labelled with ^{3}H-glucosamine. Glycosaminoglycans isolated from the medium fraction were subjected to electrophoresis on cellulose acetate membranes. Membranes were sliced into 2 mm strips, dissolved in 1,4-dioxane and their radioactivity was measured. HA = hyaluronic acid; DS = dermatan sulfate; 4S = chondroitin 4-sulfate.

Fig. 2. Electrophoretic pattern of synthesized glycosaminoglycans isolated from the cell fraction of the 2nd passage culture. The 2nd passage culture of human epidermal cells was labelled with ^{3}H-glucosamine. Glycosaminoglycans isolated from the cell fraction were subjected to electrophoresis on cellulose acetate membranes. Membranes were sliced into 2 mm strips, dissolved in 1,4-dioxane and their radioactivity was measured. HA = hyaluronic acid; DS = dermatan sulfate; 4S = chondroitin 4-sulfate.

(New England Nuclear) by a liquid scintillation counter (Aloka, Japan). Aliquots of samples were treated with *Streptomyces* hyaluronidase (Seikagaku Kogyo, Tokyo, Japan) (4) and then subjected to electrophoresis.

RESULTS

In both medium and cell fractions from the primary cultures on the 7th and 14th days and from the 2nd passage culture on the 21st day, synthesis of GAGs were tested. Electrophoretic patterns of synthesized GAGs isolated from the medium and cell fractions of the 2nd passage culture (Figs. 1–2) showed a main single peak, which was susceptible to hyaluronidase (data not shown) and confirmed to be hyaluronic acid. Identical results were obtained in the primary culture on the 7th and 14th days (data not shown).

Distribution of synthesized GAGs between the medium and cell fractions was 29%:71%, 22%:78% and 32%:68% in the primary cultures on the 7th and 14th days and the 2nd passage culture, respectively.

DISCUSSION

Recently Brown et al. (5) reported that cultured normal human epidermal keratinocytes produced GAGs which consisted of hyaluronic acid (54%) and heparan sulfate (33%). The similar data were obtained by Roberts et al. (6). The present study proved that

cultured human epidermal cells can synthesize GAGs and the major component of synthesized GAGs is hyaluronic acid. However, our cells did not produce heparan sulfate as much as their cells. The difference of heparan sulfate synthesis may be explained by the difference of culture method. Their cells needed mouse fibroblasts as a feeder layer, but our cells were cultured without any feeder which might influence on GAG synthesis of epidermal cells. Epidermal cells cultured with a feeder layer grow rapidly (3) and are supposed to actively synthesize heparan sulfate, which is one of basement membrane components (7, 8), as SV-40 transformed keratinocytes were proved to produce mainly heparan sulfate (9).

Our results implying that epidermal cells mainly produce hyaluronic acid are compatible with our recent data showing that only hyaluronic acid is detected in analysis of GAGs in human foreskin epidermis (unpublished data). It may be suggested that alcian blue positive substance observed histologically in the intercellular region of the epidermis is hyaluronic acid and mainly synthesized by epidermal cells, not by fibroblasts in the dermis.

We studied epidermal cells at three different conditions, which gave identical results. The synthesis of GAGs by cultured cells in general is influenced by various cellular and extracellular factors (10, 11). Thus, our present study suggests that epidermal cells have an ability to synthesize hyaluronic acid constantly.

ACKNOWLEDGEMENT

The authors wish to thank Mrs Y. Fujii for her excellent technical assistance.

REFERENCES

1. King IA. Characterization of epidermal glycosaminoglycans synthesized in organ culture. Biochim Biophys Acta 1981; 674: 87–95.
2. Roberts GP, Marks R. The distribution of precursors of glycoconjugate synthesis in human epidermis. Arch Dermatol Res 1983; 275: 199–201.
3. Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. Cell 1975; 6: 331–344.
4. Hata R, Nagai Y. Distribution of acidic glycosaminoglycans in tadpole back skin. Biochim Biophys Acta 1973; 304: 408–412.
5. Brown KW, Parkinson EK. Glycoproteins and glycosaminoglycans of cultured normal human epidermal keratinocytes. J Cell Sci 1983; 61: 325–338.
6. Roberts GP, Jenner L. Glycoproteins and glycosaminoglycans synthesized by human keratinocytes in culture. Biochem J 1983; 212: 355–363.
7. Kanwar YS, Farquhar MG. Presence of heparan sulfate in the glomerular basement membrane. Proc Natl Acad Sci USA 1979; 76: 1303–1307.
8. Hassell JR, Robey PG, Barrach HJ, Wilczek J, Rennard SI, Martin GR. Isolation of a heparan sulfate-containing proteoglycan from basement membrane. Proc Natl Acad Sci USA 1980; 77: 4494–4498.
9. Brown KW, Parkinson EK. Extracellular matrix components produced by SV40-transformed human epidermal keratinocytes. Int J Cancer 1984; 33: 257–263.
10. Tomida M, Koyama H, Ono T. A serum factor capable of stimulating hyaluronic acid synthesis in cultured rat fibroblasts. J Cell Physiol 1977; 91: 323–328.
11. Hopwood JJ, Dorfman A. Glycosaminoglycan synthesis by cultured human skin fibroblasts after transformation with simian virus 40. J Biol Chem 1977; 252: 4777–4785.