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DERIVATION OF LIFESPAN EXTENDED CELL LINE FROM SIMIAN VIRUS 40 INFECTED HUMAN UTERINE ENDOCERVICAL EPITHELIAL CELLS CULTURED ON COLLAGEN GEL

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Synopsis Primary culture of human uterine endocervical epithelial cells was performed. When cells were cultured on a plastic dish, these cells developed into a flat form, then lost their proliferative ability in a short time during continuous subculture. On the other hand, when cells were cultured on collagen gel and subcultured by explantation of this cell containing gel, the cells took on a roughly ovoid rather than a flat shape and active proliferation was attained which maintained confluency for as long as 8 weeks. Furthermore by adopting the Simian Virus 40 in the culture system for these cells, it was possible to extend the lifespan to approximately twice that of the control cell group.

Key words: Endocervical epithelial cell • Collagen gel culture • Simian Virus 40

Introduction

Recent improvement of cell culture techniques has made it possible to establish the culture systems of various types of normal human epithelial cells. In the field of gynecology, studies dealing with biological characteristics utilizing normal epithelial cell culture systems have also been reported. And many of these studies have concerned inevitably with experimental carcinogenetic problems in vitro. For the study of this purpose, it is requested essentially to keep stable culture systems that sustain active proliferation of the cells for a long time. Practically it is not so simple to establish such a satisfactory culture system compared with conventional primary culture yet[2]. The purpose of the present paper is to describe the result of endocervical epithelial cell growth cultured on collagen gel, and to evaluate the effect of Simian Virus 40 (SV40) on cultured cells for the extension of the lifespan.

Materials and Methods

Primary culture and collagen gel culture of endocervical epithelial cells.

Endocervical epithelial cell cultures were derived from cervical specimens obtained from hysterectomies performed for uterine fibromyoma. Endocervical tissue was selected from an area high in the cervical canal distant from the S-C junction. Most of the stromal tissue was removed, and explants of approximately $2\times2\times1mm^3$ were cut from the tissue, and were placed in 6cm culture dishes, then small amount of medium was added. The cultures were placed in a 37℃ humidified CO_2 incubator and fed twice a week with fresh medium. The culture medium consisted of TCM199 (HANDAI BIKEN) supplemented with 10% fetal calf serum (Gibco, NY, USA), 10μg/ml epidermal growth factor, 0.5μg/ml hydrocortisone, and 10^{-8}M estradiol (Sigma Chemical Co. St. Louis, MO, USA). Cells were subcultured with cell density 10^6/ml after 2 to 3 weeks in primary culture using 0.25% trypsin solution. On the other hand, type I collagen gel (Nitta Gelatin) was made in cell wells (24 wells, Corning). Subcultured cells were seeded on these collagen gel layers. When cultures on collagen gel became confluent, subcultured by explant subculture method. Namely collagen gel was removed from the well and cut into 2 to 4 pieces by scissors and each piece was put on newly prepared collagen gel, and small amount of medium was added to the well.

SV40 infection.

SV40 virus was prepared in CV-1 cells. Endocervical epithelial cells of 80% confluency cultured on collagen gel was incubated in 1ml of virus solution of plaque forming unit 10^8/ml. After 2 hours incubation at 37℃, virus containing medium was replaced with fresh medium. The SV40 infected and mock infected control cultures were maintained in

continuous growth by explant subculture method.

Immunofluorescent staining for SV40 T antigen and keratin 18.

Cells grown on chamber slide were washed with phosphate buffered saline (PBS) and fixed with ice-cold acetone. The fixed cells were incubated with the mouse monoclonal antibody against SV40 T antigen (Oncogene Science) for 30 minutes at 37℃ in a humidified chamber, washed with PBS, and subsequently incubated under the same condition with FITC-conjugated antimouse IgG antibody (VECTOR). Similarly keratin 18 was detected with the mouse monoclonal antibody against keratin 18 (SANBIO).

Results

Collagen gel culture of endocervical epithelial cells and SV40 infection.

The cells subcultured from the primary culture on the plastic dish exhibited flat polygonal appearance, typical of epithelial cells (Fig. 1). But these flat cells couldn't attain confluency after several subcultures with split ratio 1 : 4. On the other hand, subcultured on collagen gel, cells exhibited roughly ovoid shape and active proliferation (Fig. 2). When the culture became confluent, cells grown on gel were explant subcultured. New cell growth occured from the periphery of the subcultured gel, and attained confluency again. After repeated subculture at split ratio of 1 : 2 initially and 1 : 4 later for about 8 weeks, proliferation of the cells diminished and so-called senescence occured (Fig. 3). SV40 infected group exhibited senescence at the same time as mock infected group. How-

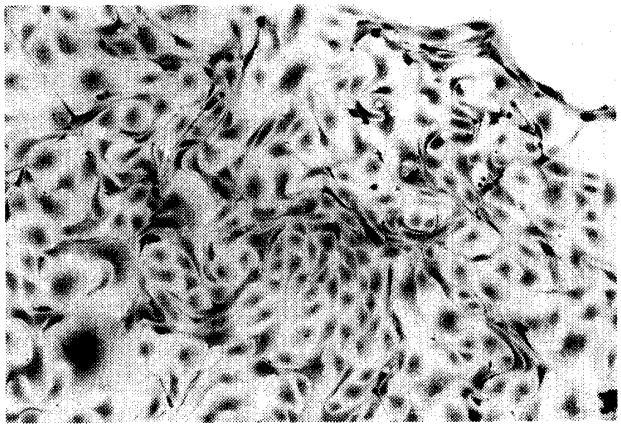

Fig. 1. Human uterine endocervical epithelial cells cultured on plastic dish (Giemsa stain, Original magnification ×100).

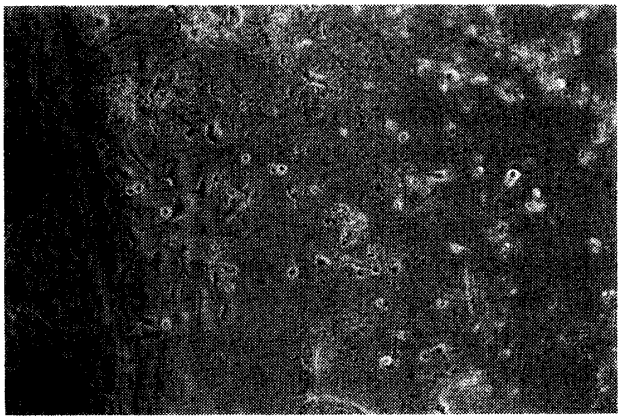

Fig. 3. Senescing cells cultured on collagen gel (Phase-contrast microscopy, Original magnification ×100).

Fig. 2. Human uterine endocervical epithelial cells cultured on collagen gel (Phase-contrast microscopy, Original magnification ×100).

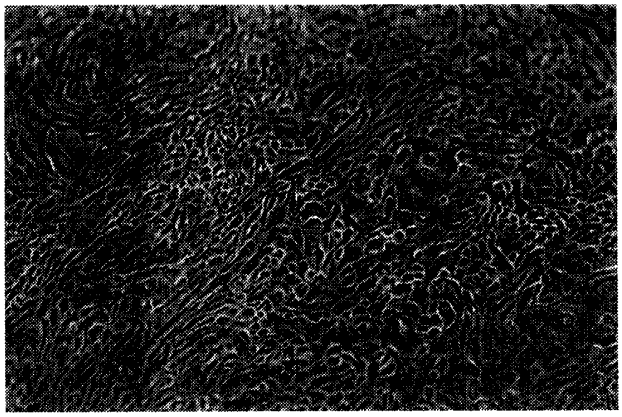

Fig. 4. Lifespan extended cell line cultured on collagen gel (Phase-contrast microscopy, Original magnification ×100).

ever, in the SV40 infected group, even from the senescing cells, actively proliferating cells appeared and these cells attained confluency (Fig. 4). Although these closely packed cells exhibited to be small in size initially, during subculture they took quite similar appearance to that of parent cells. These cells maintained proliferative ability as long as 8 weeks after the senescence of parent cells. But after this rapid growth period, proliferation diminished gradually and confluency couldn't be attained. This phenomenon seemed to be the occurrence of crisis.

Immunofluorescent study.

Immunofluorescent staining for SV40 T antigen was detected within the nuclei of lifespan extended cell line (Fig. 5). And also these cells stained positively for cytoplasmic keratin 18 (Fig. 6).

Discussion

Generally, it is not so easy to establish the stable culture system of normal human epithelial cells compared with that of fibroblastic cells. When cultured on plastic dish, epithelial cells gradually become flat form and proliferative ability diminishes with subculture. And confluency can't be attained even in rather early stages. Uterine endocervical epithelial cells have also followed a similar process. However such a culture system is not appropriate for the in vitro transformation study. On the other hand, cultured on collagen gel, endocervical epithelial cells maintained the proliferative ability without flattening for 8 weeks.

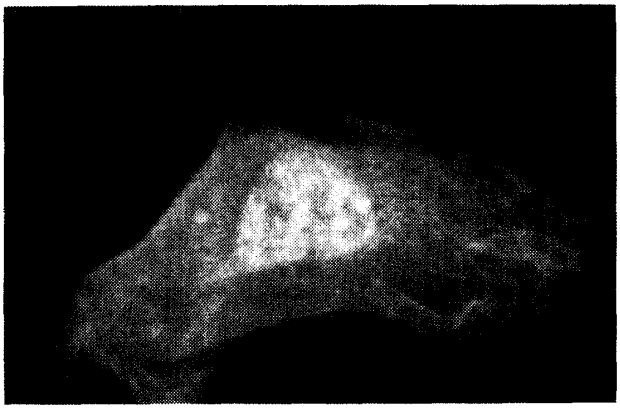

Fig. 5. Immunofluorescent staining for SV40 T antigen (Original magnification ×400).

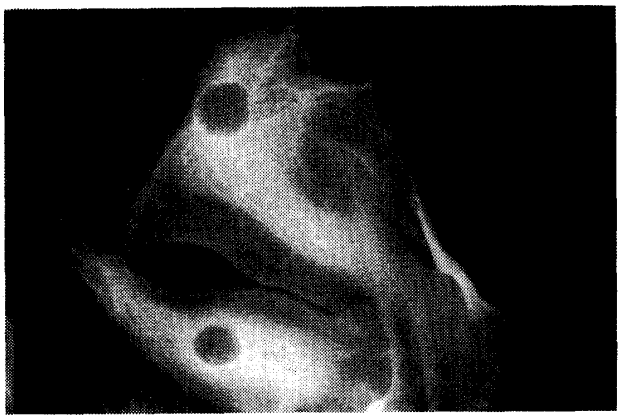

Fig. 6. Immunofluorescent staining for keratin 18 (Original magnification ×200).

Explant subculture method, that utilizes collagen gel as a divisible substrate, seemed to be useful for in vitro transformation experiment. Because cell loss during this subculture procedure is less than that of dispersion subculture method using enzymes such as trypsin.

Immunofluorescent staining of keratin subtype was utilized for identification of cultured cells in this study. Recently expression pattern of keratin subtype in the human uterine cervix has been clarified, and keratin 18 is known to be expressed only in endocervical epithelial cells not only in vivo but also in vitro[6)8)]. Immortalization is thought to be the first step of in vitro neoplastic transformation, and SV40 has often been applied for immortalization experiments of human cells[1)3)4)]. In the field of gynecology, only a few studies that used SV40 has thus far been reported. In this study, infection of this virus caused extension of lifespan 2-fold of mock infected control cells, and after the extended lifespan, these cells ceased proliferation. This phenomenon seemed to be the so-called crisis. It is well known that transformation by SV40 is caused by T antigen of the virus[7)]. Recently new interest on the T antigen has evolved from the viewpoint of its interaction with antioncogene products. Thus it has been clarified that Rb protein, product of Rb tumor suppressor gene, interacts with SV40 T antigen[5)]. Because defect of Rb gene is known to be associated with human cancer, SV40 is expected to contribute not only to animal cell experiments but also to the in vitro human carcinogenesis study.

In summary, collagen gel culture and explant subculture method seemed to be useful for establishment of stable epithelial cell culture system with active proliferation. And using this culture method, lifespan extension experiment of uterine

endocervical epithelial cells by infection of SV40 was performed.

References

1. *Chang, S.E.:* In vitro transformation of human epithelial cells. Biochim. Biophys. Acta, 823: 161, 1986.
2. *Harris, C.C.:* Human tissues and cells in carcinogenesis research. Cancer Res., 47: 1, 1987.
3. *Reddel, R.R., Ke, Y., Gerwin, B.I., McMenamin, M. G., Lechner, J.F., Su, R.T., Brash, D.E., Park, J.B., Rhim, J.S. and Harris, C.C.:* Transformation of human bronchial epithelial cells by infection with SV40 or Adeno-12 SV40 hybrid virus, or transfection via strontium phosphate coprecipitation with a plasmid containing SV40 early region genes. Cancer Res., 48: 1904, 1988.
4. *Sach, G.H.:* Human cell transformation by Simian Virus 40—A review. In Vitro, 17: 1, 1981.
5. *Schreier, A.A. and Gruber, J.:* Viral T antigen interaction with cellular proto-oncogene and anti-oncogene products. J. Natl. Cancer Inst., 82: 354, 1990.
6. *Smedts, F., Ramaekers, F., Robbin, H., Pruszczynski, M., Muijen, G.V., Lane, B., Leigh, I. and Vooijs, P.:* Changing patterns of keratin expression during progression of cervical intraepithelial neoplasia. Am. J. Pathol., 136: 657, 1990.
7. *Topp, W.C. and Pollack, R.:* Transformation by SV40 and polyoma Virus. In DNA Tumor Viruses (ed. J. Tooze), 205. Cold Spring Harbor Laboratory, Cold Spring Harbor, 1980.
8. *Turyk, M.E., Golub, T.R., Word, N.B., Hankins, J. L. and Willanks, G.D.:* Growth and characterization of epithelial cells from normal human uterine ectocervix and endocervix. In vitro cell. Dev. Biol., 25: 544, 1989.

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概要 ヒト子宮頸管上皮細胞の初代培養を行つた．移植片培養法によつて得た細胞はプラスチックディッシュ上では扁平な上皮様形態を示し継代とともにその増殖能は急速に減衰した．しかしながら，タイプⅠコラーゲンゲル上では丸みを帯びた，より立体的な形態を取り，細胞付着ゲル片の移植継代法によつて，8週間にわたりconfluencyを維持できる増殖能を示した．さらに，この培養系にSimian Virus 40を感染させることにより寿命の2倍に延長した細胞を発生せしめることができた．