Biotechnol. Bioprocess Eng **2000**, 5: 92-98

Immortalization of Rat Kidney Glomerular Mesangial Cell and Its Coculture with Glomerular Epithelial Cell

Toshinobu Kida, Sachi Fujishima, Masatoshi Matsumura, and Pi-Chao Wang*

Institute of Applied Biochemistry, Tsukuba University, 1-1-1 Tennodai, Tsukuba City 305-8572, Japan

Abstract Mesangial cell has several key roles in the control of glomerular function: it participates in the regulation of glomerular filtration rate, macromolecular clearance, and as both a source and target of numerous hormones and autocrines. Many of these insights into mesangial cell function have been obtained by studying mesangial cells in culture. However, no suitable cell lines have been established yet. We here reported the immortalization of rat kidney glomerular mesangial cell by transfection of E6 and E7 genes of human papillomavirus type 16 (HPV-16) via electroporation and lipofection. The results showed that only electroporation could transfect the genes to mesangial cells and the transfected cells maintained the viability for longer than 6 months. Fluorescence microscopic observation showed that cellular contractility and phagocytosis, which are the two main phenotypes of mesangial cells, are well maintained after transfection. The coculture of transfected mesangial cells with rat glomerular epithelial cells showed that the growth of mesangial cells was suppressed by epithelial cell, but the growth of epithelial cells was enhanced by mesangial cells. Moreover, an enhancing effect on the phagocytosis of mesangial cell was also observed in coculture. Such results may imply that the glomerular cell-cell interaction plays an important role in the regulation of cell proliferation and differentiation

Keywords. immortalization, glomerular mesangial cell, glomerular epithelial cell, E6 and E7 genes, cellular contractility, phagocytosis

INTRODUCTION

The kidney glomerulus contains three different cell types: endothelial cells and mesangial cells are closely associated at the inner surface of the glomerular basement membrane, whereas visceral epithelial cells cover its outer shell [1]. The mesangial cell is multipotential in its functions and capacities and has been presumed critical to the regulation of glomerular hemodynamics [2,3] Because the glomerular mesangial cells contract *in vitro* in response to a variety of hormones, it has been proposed that this cell modifies the glomerular ultrafiltration coefficient via alternations in glomerular capillary filtration surface area [4]. Moreover, as a specialized pericyte, it also participates in the regulation of macromolecular trafficking and clearance, and as both a source and target of numerous hormones and autocrines [5]. Many of these insights into mesangial cell function have been obtained by studying mesangial cells in culture [6,7]. The accumulation of extracellular matrix (ECM) in the mesangium or the over-proliferation of mesangial cells causes the irreversible progression of glomerular sclerosis and chronic renal failure, as was observed in the course of glomerularnephritis and diabetic nephropathy [8,9] Communication circuits between cells or between cells and matrices are found to be important for maintenance of normal tissue physiology and for the initiation and persistence of pathophysiologic abnormalities of glomerular inflammation which may lead to glomerulor scleorsis. Recently, using cell culture technique and a coculture system, the interactions between monocyte/macrophage and mesangial cells [10,11], and between glomerular endothelial cells and mesangial cells [12] were investigated, and the results showed that the cell-cell interaction might induce and/or inhibit mesangial cell growth which has close relationship to the glomerularnephritic disease.

Mesangial cell culture is considered a useful system for carrying out all of these experiments. However, normal mesangial cell culture can only be obtained from primary culture of isolated glomeruli, and its viability can not be maintained over 30 doublings. Therefore, it is difficult to observe the long-term response of mesangial cell to various stimuli in primary culture. In order to clarify the long-term response of mesangial cell to various stimuli, an established mesangial cell is necessary. Mackay *et al.* [13] reported to establish a mesangial cell line in 1988 However, it was derived from transgenic mice. Recently, the E6 and E7 genes of human papillomavirus type 16 (HPV-16) have been reported to be detectable for immortalization of human keratinocytes because E6 and E7 genes can cooperate in the transformation of epidermal keratinocytes *in vitro* [14]. Using *in vitro* transcription/translation system, the E7 gene product of HPV-16 has been shown to be

* Corresponding author
Tel: +81-298-53-6581 Fax: +81-298-53-4605
e-mail cogitate@sakura.cc.tsukuba.ac.jp

able to bind to and coprecipitate with the cellular P105-Rb protein (reinoblastoma susceptibility gene product), which is responsible for the regulation of cell growth [15], and the E6 protein has been shown to bind the cellular p53 protein [16], which is responsible for the regulation of cell cycle. We have tried to immortalize mesangial cell by using *in vitro* transfection system and here reported the results including the observation of main phenotypes and the cellular contractility and phagocytosis of transfected mesangial cells. Moreover, we also reported the coculture effect of epithelial cell on the growth of mesangial cell; such kind of cell-cell interaction between these two cells has been poorly understood.

MATERIALS AND METHODS

Isolation and Culture of Mesangial Cell

Glomeruli were isolated from male Wistar rat at the age of 4-6 weeks, by the method of Kreisberg *et al.* [17]. Briefly, thin (2-4mm) strips of decapsulated kidney cortex were minced in culture medium. Small fragments of tissue were pushed through 212 μm-mesh stainless-steel screens with a glass pestle. The resulting mixture containing glomeruli was passed over a graded series of screens, and single unencapsulated glomeruli were finally retained on a fine mesh screen, which were then washed and sedimented in Hanks medium (Nissui). These steps resulted in a preparation of gromeruli virtually free of non glomerular contaminants. For isolation of mesangial cell, isolated glomeruli were incubated with 0.2% trypsin (Gibco BRL) in phosphate buffered saline (PBS) at 37°C in a humidified 5% CO_2 incubator for the removal of extracellular matrices. After centrifugation at 250 g for 5 min, the recovered glomeruli were washed with Hanks medium supplemented with 10% FBS (Gibco BRL). After that, fresh medium of DMEM: HamF12 (2:1) supplemented with 10% FBS was added to cell culture to replace the old medium every 2-3 days, and mesangial cells were used for further experiments after 3-4 passages.

Epithelial Cell Line, Culture Medium, and Plasmids

Rat glomerular epithelial cell line SGE1 was gifted by Dr. T. Okigaki [18], and the cells were cultured in DHSF medium (per liter: DMEM, 5 g; Ham's F12, 5.3 g; $NaHCO_3$, 1.9 g; ITS, 6.25 mg; EGF, 1 μg) supplemented with 10% FBS. Plasmids pDE6 and pDE7 containing E6 and E7 genes, respectively, were kindly gifted by Dr. Yasumoto [19], and were transformed to *E coli* JM109 for the amplification of DNA. All of the DNA manipulation and purification of plasmids were carried out using standard methods, described elsewhere [20].

Electroporation

25 μg each of pDE6 and pDE7 DNA was linearized by digestion with restriction enzyme, *Hind*III. After purification, pDE6 and pDE7 were dissolved in 1 mL PBS buffer containing 11 mM glucose, and cotransfected to mesangial cells (5×10^6 cells) by electroporation at 950 μF, 260 volt twice by using a Gene Pulser (Bio Rad). The transfected cells were cultured in a selected medium (DMEM:HamF12 (2:1) supplemented with 10% FBS and 0.7 mg/mL neomycin) for one week. The viable trasfected cells were then cloned using a limiting dilution technique. Positive clones were selected from single cells and cultured to confluency for further analysis of the cell morphology.

Lipofection

DOSPER liposomal transfection kit (Boehringer Mannheim) was used for transfection. 3×10^5 cells in 2 mL culture medium were incubated in a 6 well plate at 37°C, 5% CO_2 until cells reached 50-80% confluent. On the day of transfection, cell medium was replaced by DOSPER/DNA mixture, and 1 μg DNA with 6 μL DOSPER (1,3-di-oleoyloxy-2-(6-carboxy-spermyl)-propylamide) in 1 mL Hepes-bufferd saline (HBS;100 μL) was added to cell culture, drop by drop. Culture medium was then replaced by fresh medium after 6 hr incubation at 37°C. The viable transfected cells were then cloned using a limiting dilution technique, and positive clones were selected from neomycin selective medium.

Coculture of Mesangial Cells with Epithelial Cells

To examine the coculture effect on the cell growth of immortalized mesangial cells with epithelial cells, mesangial cells and epithelial cells were cultured in 24-well Labtek slides (Nunc), respectively. When the cell growth of mesangial and epithelial cells reached 30% and 80%, respectively, a silicon ring with the same diameter of 24-well Labtek slide was mounted on the top of the slide which was attached with 80% confluent of epithelial cells, and then the slide attached with 30% confluent of mesangial cells was mounted on the silicon ring (Fig. 1). The surfaces of slides each attached with mesangial or epithelial cells were faced to each other and medium was supplied sufficiently up to the edge of top slide. At least 14 wells were prepared in each culture so that cell count could be performed in duplicate for one week. The time course of cell growth was then estimated on the basis of the counted cell numbers.

Same experiment was also conducted to examine the coculture effect on the cell growth of epithelial cell with immortalized mesangial cell. All manipulations are the same as described above, except that the cell numbers of mesangial and epithelial cells are reversed as 80% and 30%, respectively.

Phagocytosis

For evaluation of one of the main properties, phagocytosis, of mesangial cell, the immortalized cells were cultured in 24-well Labtek slides and treated with 1 μL/mL of 0.1 μm fluorescent plain microspheres (Polyscience Inc.) for 3 h. After that, cells were washed with PBS, fixed in 10% buffered formaldehyde on ice for 20 min, then mounted in 90% glycerol/PBS, and finally observed via fluorescence microscopy.

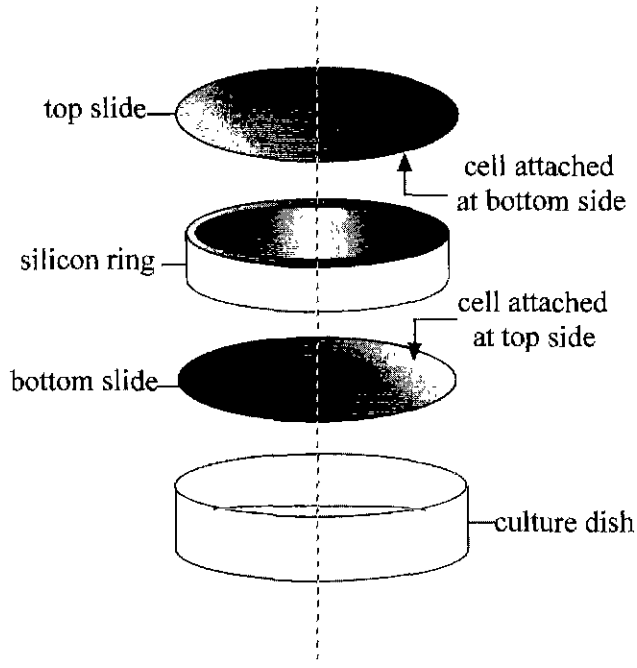

Fig. 1. Experimental set up for coculture

Contractility

For evaluation of another main property, contractility, of mesangial cells, the immortalized cells were cultured in 24-well Labtek slides and treated with 2.5 μL/mL anti-α-actin (ICN Biomedicals) for 3 h. Then the secondary antibody FITC-labeled IgG was added for two more hours of incubation. After that, cells were washed with PBS, fixed in formaldehyde, then mounted in glycerol/PBS as described above, and finally observed via fluorescence microscopy.

CR1 Expression

For evaluation of the specific property of glomerular epithelial cell line SGE1, the expression of complement receptor type 1 (CR1) was investigated via fluorescence microscopy. Manipulation of cell was described as above, except that cells were pre-treated with 0.1% BSA for 30 min for blocking the non-specific reaction and then treated with FITC-labeled anti-CD35 (CR1) antibody.

RESULTS AND DISCUSSION

Isolation of Glomeruli and Primary Culture of Mesangial Cell

Fig. 2(a) shows that epithelial cells appeared around glomeruli soon after the isolation of glomeruli in the first week. After the change of fresh DMEM:Ham's F12 (2:1) medium supplemented with 10% FBS every 2-3 days, epithelial cells disappeared gradually while smooth muscle-like mesangial cells appeared approximately after two weeks (Fig. 2(b)). Four weeks later, epithelial cells disappeared totally and mesangial cells reached confluent (Fig. 2(c)). Such phenomena have been observed in most cases for the outgrowth of mesangial cells from isolated glomeruli [21-23].

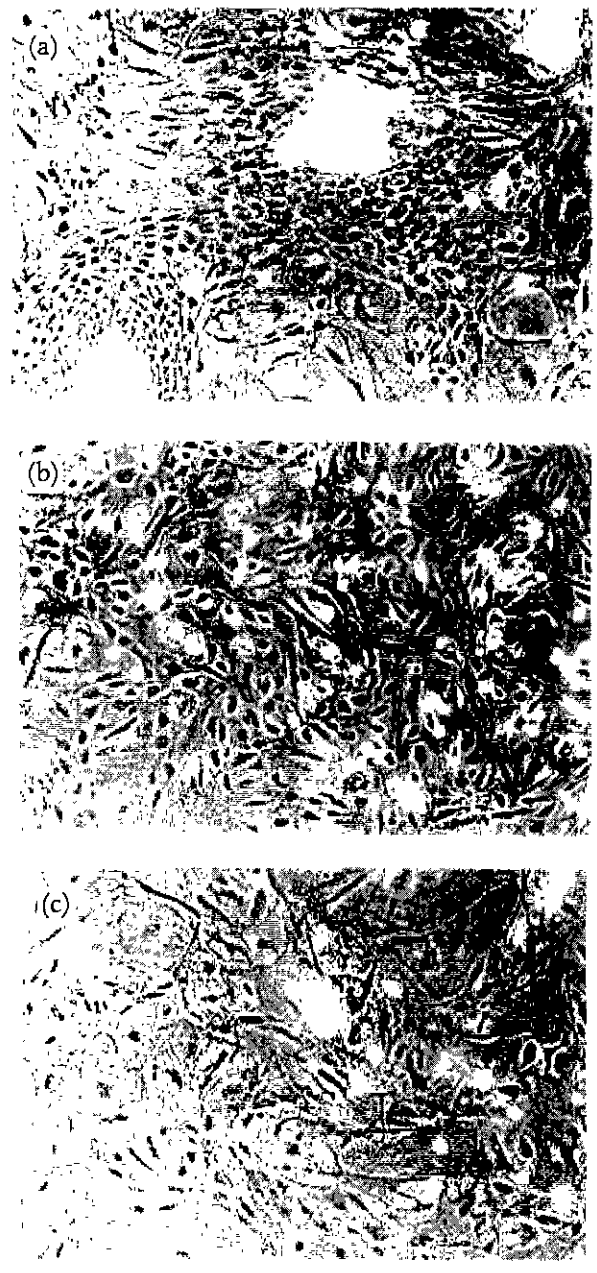

Fig. 2. Phase contrast micrograph of mesangial cells outgrown from isolated glomeruli. (a) Cobblestone-like epithelial cells appeared around glomeruli in 1 week after glomeruli isolation; (b) Epithelial cells disappeared gradually and smooth muscle-like mesangial cells appeared after 2 weeks, (c) Mesangial cells replaced epithelial cells after 4 weeks

Immortalization of Glomerular Mesangial Cells

The mesangial cells in 3-4 passages were used for the

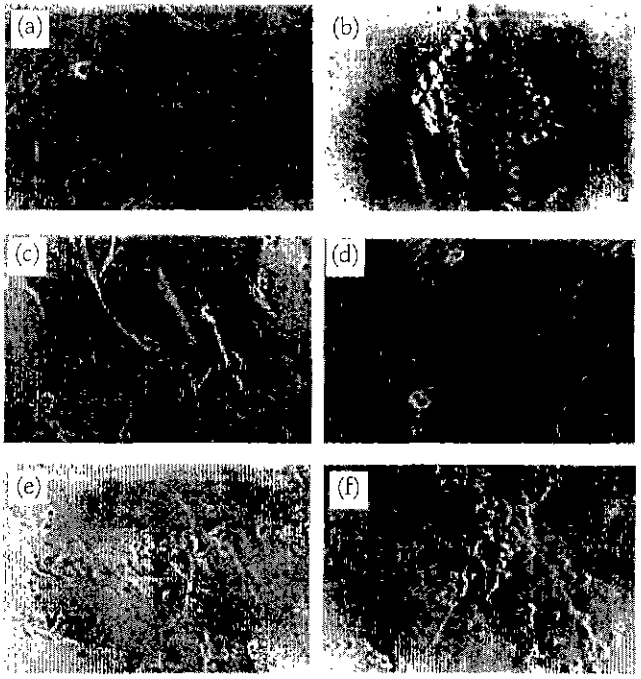

Fig. 3. Phase contrast micrograph of mesangial cells after transfection with E6 and E7 genes. (a) Electroporated cells showed no morphological change soon after transfection; (b) Electroporated cells aggregated after the addition of selective medium; (c) Electroporated cells recovered in one month after the addition of selective medium; (d) Lipofected cells showed no morphological change soon after transfection; (e) Lipofected cells floated in medium after the addition of selective medium, (f) Lipofected cells become inviable in one month after the addition of selective medium

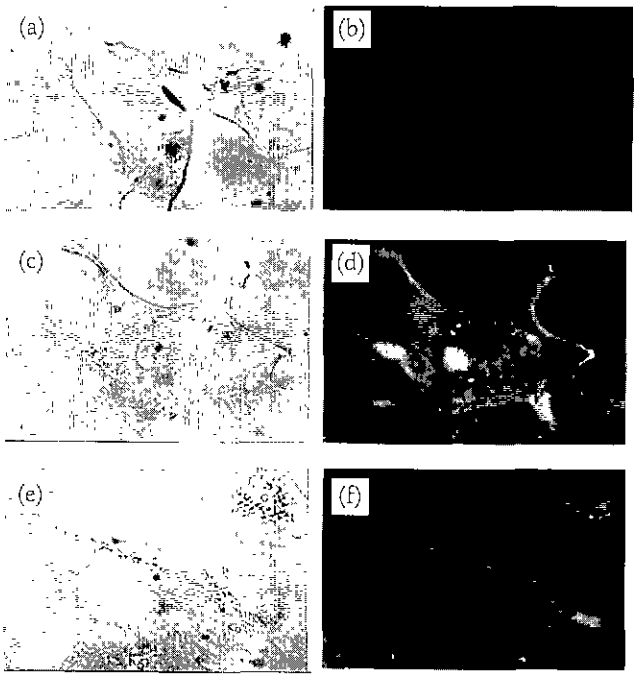

Fig. 4. Phase contrast and fluorescent micrographs of mesangial cells cultured in 6 months after transfection. (a) Phase contrast micrograph of control cells; (b) Fluorescent micrographs of control cells, (c) Phase contrast micrographs of cells treated with fluorescent plain microspheres; (d) Fluorescent micrographs of cells treated with fluorescent plain microspheres, (e) Phase contrast micrographs of cells stained with anti-α-actin antibody, (f) Fluorescent micrographs of cells stained with anti-α-actin antibody.

immortlization experiment by transfection of E6 and E7 genes. Fig. 3 shows the cell mophological changes after electroporation and lipofection, respectively. The results of electroporated cells were shown in Fig. 3(a), (b), and (c), and lipofected cells, in Fig. 3(d), (e), and (f). It is interesting to find that cell morphology did not change soon after gene transfection either by electroporation or by lipofection method (Figs. 3(a) and (d)), while addition of selective medium immediately caused the electroporated cells to aggregate (Fig. 3(b)) and the lipofected cells to float (Fig 3(e)) However, after one month of medium change every 2-3 days, the electroporated cells recovered and grew well in the selective medium (Fig. 3(c)), whereas the lipofected cells became nonviable (Fig. 3(f)) Other experiments comparing the method of electroporation with lipofection by transfecting Green Fluorescent protein (GFP) DNA, also showed the same results (data not shown). Such results may suggest that electroporation is more suitable for the transfection of genes to mesangial cells The higher possibility of trasfection via electroporation is probably due to the high quantity of DNA and large number of cells as compared with those used in lipofection

The electroporation caused one half of the 5×10^6 cells inviable, and the subsequent selective medium excluded the non-transfected cells. Finally, 30 positive clones were screened by limiting dilution and cultured in medium for more than 6 months without losing its properties of contractility and phagocytosis. Moreover, the gene expression of E6 and E7 in mesangial cells has been confirmed by RT-PCR (data not shown). Therefore, the prolonged life span of transfected cells is due to E6 and E7 rather than other factors such as spontaneous mutation.

Although HPV-16 E6 and E7 genes were reported to be able to immortalize human epidermal keratinocytes without producing malignancy [24,25], not all cells carrying E6 or E7, or both, are immortalized [26]. In this study, we demonstrated that the co-transfection of E6 and E7 succeeded in prolonging the viability of mesangial cell for more than 6 months.

Cell Morphology and Property

In order to examine whether the mesangial cell maintained its morphology and property after transfetion, cell contractility and phagocytosis were investigated using phase contrast microscopy and fluorescence microscopy, respectively. Fig 4(a) and (b) show the phase contrast micrograph and fluorescent micrograph of control cells without any treatment with related antibodies Cells treated with fluorescent plain microspheres revealed that many small microspheres were phagocytosized through fluorescence microscopic observation (Fig. 4(d)), which can hardly be detected using phase con-

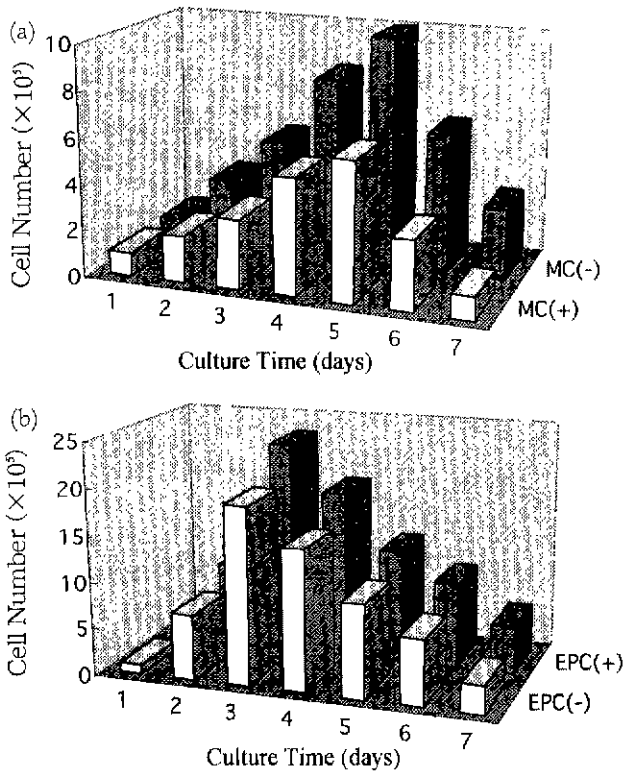

Fig. 5 Time course of cell growth in coculture: (a) Coculture effect on mesangial cell growth by confluent epithelial cell, MC(–) mesangial cell in single culture MC(+): mesangial cell in coculture with epithelial cells; (b) Coculture effect on epithelial cell growth by confluent mesangial cell; EPC(–) epithelial cell in single culture; EPC(+): epithelial cell in coculture with mesangial cells

trast microscopy (Fig 4(c)). Fig. 4(f) shows the fluorescent micrograph of mesangial cells treated with anti-α-actin antibody It displays prominent cytoskeletal staining for actin with parallel fibrils throughout the cytoplasm, but it is not observable using phase contrast microscopy (Fig 4(e)) All these results may suggest that mesangial cells maintain its morphology and property after gene transfection, although whether other features, such as secretion of hormones and extracellular matrices, are maintained or not still need to be investigated.

Cell Proliferation in Coculture

30% confluent immortalized mesangial cells were cocultured with 80% confluent epithelial cell in DMEM· Ham's F12 (2:1) medium for one week Cells were obtained by trypsinization and centrifugation, and viable cell number was determined by trypan blue exclusion using a hemocytometer. Fig 5(a) shows the time course of mesangial cells during one week, grown in coculture with epithelial cells. As compared to the single cultured mesangial cells MC(–), the cocultured cells MC(+) revealed significant suppression in cell growth by epithelial cells, although both MC(+) and MC(–) showed

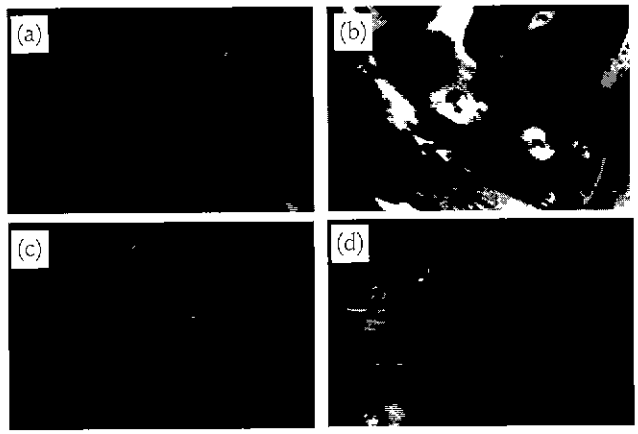

Fig. 6. Fluorescent micrograph of mesangial cell property in single culture [MC(–)] and coculture with epithelial cell [MC(+)]. (a) MC(–) treated with fluorescent plain microspheres, (b) MC(+) treated with fluorescent plain microspheres; (c) MC(–) stained with anti-α-actin antibody; (d) MC(+) stained with anti-α-actin antibody.

same tendency to reach the growth peak on day 5 and then declined rapidly on day 6.

On the contrary, when 30% confluent epithelial cells were cocultured with 80% confluent immortalized mesangial cells in DHSF medium, the cocultured epithelial cells, EPC(+), displayed a higher cell growth as compared to single-cultured cells, EPC(–), although both EPC(+) and EPC(–) showed the same tendency to reach growth peak on day 3 and then declined gradually. These results may suggest that the cell-cell interaction occurred in the coculture of mesangial and epithelial cells and triggered secretion of the autocrine and paracrine cytokines or extracellular matrices, which resulted in the induction and/or suppression effect on cell growth. In this study, it is obvious that coculture of mesangial cells with epithelial cells showed a suppression effect on cell growth of mesangial cells, while an enhancing effect was observed in the epithelial cell cocultured with mesangial cell

Change of Cell Morphology and Property in Coculture

In order to examine whether the coculture resulted in any change on the cell morphology and property, cell contractility and phagocytosis for mesangial cell, and phagocytosis and CR1 expression, a marker for epithelial cell SGE1 [27,28], were investigated. The results were shown in Figs. 6 and 7 For mesangial cells, a remarkable increment of phagocytosis in MC(+) was observed as compared to that of MC(–) (Figs. 6(a) and (b)), but no significant changes in cellular skeleton between MC(+) and MC(–) were detectable (Figs. 6(c) and (d)). For epithelial cells, neither the CR1 expression nor the phagosytosis was found changed in both EPC(+) and EPC(–) Although many reports have mentioned that the cell-cell interaction between monocyte/macro-phage and mesangial cell had enhanced the cell growth of mesangial [10,11,27], all of these investi-

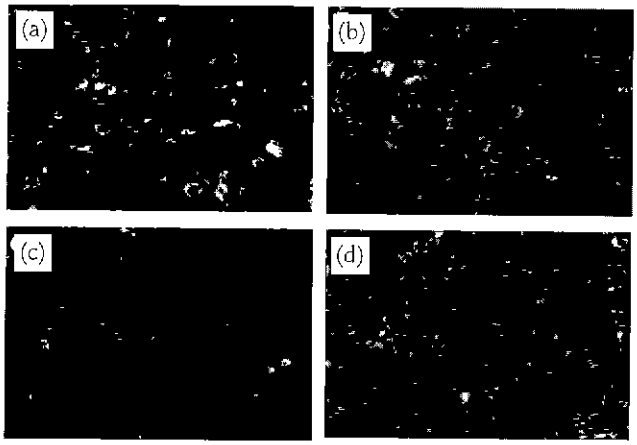

Fig. 7. Fluorescent micrograph of epithelial cell property in single culture [EPC(–)] and coculture with mesangial cell [EPC(+)] (a) EPC(–) treated with anti-complement receptor type 1 (CR1) antibody, (b) EPC(+) treated with anti-complement receptor type1(CR1) antibody, (c) EPC(–) treated with fluorescent plain microspheres, (d) EPC(+) treated with fluorescent plain microspheres.

gations were focused on the mechanisms of glomerularnephritis induced by the proliferation of mesangial cells and over-production of extracellular matrices. Recently, Saeki *et al.* [12] pointed out the coculture of glomerular endothelial cell suppresses the cell growth of mesangial cell, which may imply that the interaction of glomerular cells can regulate cell proliferation to avoid unfavorable glomerularnephritis caused by the proliferation of mesangial cells Although coculture of endothelial cell and mesangial cell has been reported, coculture of glomerular epithelial cell and mesangial cell has not been disclosed yet, probably due to the separated locations of these two cells at the opposite sides of basement membrane However, it should be noted that basement membrane comprises a porous structure and cell-cell interaction between epithelial cells and mesangial cells such as autocrine or paracrine cytokines can pass through porous structure of basement membrane and influence each other either in cell proliferation or differentiation. We firstly demonstrated that mesangial cell could be regulated by epithelial cell both in cell growth and cell property. The fact that mesangial cell growth was suppressed while phagocytosis ability enhanced in the coculture with epithelial cells supports our hypothesis that cell-cell interaction exists between epithelial and mesangial cells This fact suggests that the glomerular cells *in vivo* may regulate each other via cell-cell interaction to approach an optimum condition between them.

In conclusion, we have succeeded in immortalizing rat kidney glomerular mesangial cell by transfecting E6 and E7 genes via electroporation. The transfected cell maintained its viability for more than 6 months without losing its main properties Moreover, an enhancing effect on the phagocytosis as well as a suppression effect on cell growth of mesengial cells cocultured with epithelial cells may suggest the cell-cell interaction exists between these two cells.

Acknowledgments This study was partly supported by a Grant-In-Aid for Scientific Research C-2 (10650777) from the Ministry of Education, Science and Culture of Japan.

REFERENCES

[1] Michael, A F, W F Keane, L. Raij, R L Vernier, and S. M Mauer (1980) The glomerular mesangium *Kidney Int.* 17: 141-154

[2] Blantz, R C , K. S. Konnen, and B J. Tuchker (1976) Angiotensin II effects upon the glomerular micorcirculation and ultrafiltration coefficient of the rat. *J Clin. Invest* 57: 419-434

[3] Schlondorff, D (1987) The glomerular mesangial cell an expanding role for a specialized pericyte. *FASEB J.* 1 272-281

[4] Narita, I., T. Morioka, K. Yoshida, F Shimizu, and M Arakawa (1989) Monocyte secretes factors that regulate glycosaminoglycan (GAG) synthesis by mesangial cells (MCs) *in vitro Kidney Int.* 35 357

[5] Ruef, C., K. Budde, J Lacy, W Northemann, M Baumann, R. B Sterzel, and D L Colema (1990) Interleukin 6 is an autocrine growth factor for mesangial cells *Kidney Int.* 38 249-257

[6] Ausiello, D , J. J. Kreisberg, C Roy, and M J. Karnovsky (1987) Construction of cultured rat glomerular cells of apparent mesangial origin after stimulation with angiotensin II and arginine vasopressin *J. Clin Invest* 65: 754- 761

[7] Mene, P, M S Simonson, and M. J. Dunn (1989) Physiology of the mesangial cell. *Physiol Review* 69 1347-1352

[8] Cohen, A. H and W. A. Border (1982) Mesangial proliferative glomerulonephritis. *Semin Nephro.* 2: 228-240

[9] Kashgarian, M (1985) Mesangium and glomerular disease *Lab. Invest* 52 569-571

[10] Lovett, D. H , J L Ryan, and R B Sterzel (1983) Stimulation of rat mesangial cell proliferation by macrophage Interleukin 1 *J Immunol* 131 2830-2836.

[11] Morioka, T., I Narita, F. Shimizu, and Y. Oite (1990) The production, by cultured human monocytes, of mesangial cell proliferation factors differeing from Interleukin 1 and Interleukin 6. *Clin Exp Immunol* 83 182-186.

[12] Saeki, T., T. Morioka, M. Arakawa, F. Shimizu, and J Oite, (1991) Molulation of mesangial cell profiferation by endothelial cells in coculture. *Am. J. Pathol* 139 949-957

[13] MacKay, K , L. J Strikeer, S Elliot, C A Pinkert, R L Brinster and G E. Striker (1988) Glomerular epithelial, mesangial, and endothelial cell lines from transgenic mice *Kidney Int* 33. 677-684

[14] Hawley-Nelson, P., K H Vousden, N. L Hubbert, D. R Lowy, and J T Schiller (1989) HPV16 E6 and E7 proteins cooperate to immortalize human skin keratinocytes *EMBO J.* 8 3905-3910

[15] Dyson, N , P. M. Howley, K. Munger, and E. Harlow (1989) The human papillomavirus-16 E7 oncoprotein is able to bind the retinoblastoma gene product *Science* 248 934-936.

[16] Werness, B A , A J Levine, and P. M. Howley (1990) Association of human papillomavirus type 16 and 18 E6 proteins with p53 *Science* 248 76-79.

[17] Kreisberg, J. I., M A Venkatachalan, and P. Y Patel (1984)

Cyclic AMP-associated shape change in mesangial cells and its reversal by prostaglandin E2. *Kidney Int.* 25: 874-879.

[18] Yamada, M., M. Kawaguchi, H. Takamiya, H. Wada, and T. Okigaki (1988) Establishment and characterization of an epithelial cell line SGE1, from isolated rat renal glomeruli. *Cell Struct. Funct* 13: 495-513.

[19] Hashida, T. and S. Yamamoto (1991) Induction of chromosome abnormalities in mouse and human epidermal keratinocytes by the human papillomavirus type 16 E7 oncogene. *J. Gen. Virol.* 2: 1569-1577.

[20] Sambrook, J., E. Fritsch, and T. Maniatis (1989) Molecular Cloning a Laboratory Manual (Cold spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

[21] Foidart, J. B., C. A. Dechenne, P. R. Mahieu, C. E. Creutz, and J. Demeg (1979) Tissue culture of normal rat glomeruli: Isolation and morphological characterization of two homogeneous cell lines. *Investi. Cell Pathol* 2. 15-26.

[22] Singhal, P. C., N. Gibbons, and M. Abramovici (1992) Long-term effects of morphine on mesangial cell proliferation and matrix synthesis. *Kidney Int* 41: 1560-1570.

[23] Ooi, Y. M., M. A. Weiss, A. Hsu, and B. S. Ooi (1983) Mechanism of suppression of mouse mesangial cell proliferation by macrophage supernatants. *J. Immunol.* 130: 1790-1796.

[24] Yasumoto, S., J. Donigen, and J. A. Dipaolo (1987) Differential early viral gene expression on in two stages of human papillomavirus type 16 DNA-induced malignanant transformation. *Mol. Cell Biol. 7: 2165-2172.*

[25] Noda, T., H. Yajima, and Y. Ito (1988) Progression of the phenotype of transformedcells after growth stimulation of cells by a human papillomavirus type 16 gene function. *J. Virol* 62: 313-324.

[26] Munger, K., W. C. Phelps, V. Bubb, P. M. Howley, and K. Schlegel (1989) The E6 and E7 genes of the human papillomavirus type 16 together are necessary and sufficient for transformation of primary human keratinocytes. *J. Virol.* 63: 4417-4421.

[27] Wang, P. C., S. Kimura, K. Todokoro, T. Okigaki, and Y. Kao, Matsumura, M. (1996) Stable expression of human compliment receptor CR1 gene on rat renal glomerular epithelial cell SGE1. *J. Cell. Eng* 1: 179-182.

[28] Wang, P. C., K. Horisawa, and M. Matsumura (1999) Construction of glomerular epithelial cells in vitro for removal of immune complex. *Arti. Organs* 2. 170-175.

[29] Veis, J. H., W. Yamashita, Y. J. Liu, and B. S. Ooi (1990) The Biology of mesangial cells in glomerulonephritis biology of mesangial cells. *Soc. Exp. Biol. Med.* 160-167.

[30] Cosio, F. G., D. D. Sedemak, and S. Jr. Wahman (1990) Cellular receptors for matrix proteins in normal human kidney and human mesangial cells. *Kidney Int.* 38: 886-895.

[Received October 11, 1999, accepted February 5, 2000]