題目：Structural and application of recombinant rhodostomin --- 形態發生之機制 1/3
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MDCK cells.

Taken together, the property of fibronectin matrix may play a role in microvilli formation of MDCK cells. MDCK cells cultured on collagen IV dish, was present on the apical portion of the epithelium cultured on dish or collagen IV dish. Finally, using immunocytochemical methods we observed that fibronectin matrix deposition distinguishes rhodopsin in cells cultured on collagen IV dish. Furthermore, inhibition of fibronectin assembly by fibronectin matrix was not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or 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not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not well or not wel
Results

Total content of fibronectin was increased in MDCK cells cultured on collagen

The level of assembled fibronectin matrix was increased in MDCK cells cultured

on collagen fibrils.

What is the meaning of the increased fibronectin in MDCK cells cultured on

collagen fibrils? Many previous studies have proved that cells could secret and

assemble fibronectin into a fibrillar form that accumulates around their surface; they

also indicated that assembled fibronectin matrix played important roles in controlling
cell proliferation as well as differentiation. So we wonder whether collagen influence

cell proliferation as well as differentiation? We found that the amount of fibronectin

matrix was increased in MDCK cells cultured in normal dish or collagen gel-coated

dish (Figure 1). Comparing the total content of fibronectin in media containing

collagen fibrils to incorporate exogenous fibronectin, MDCK cells cultured on collagen fibrils was caused by stimulating the ability of different concentrations of FBS. We found that increased level of fibronectin in

dish (Figure 2, especially, fibril formation was prolonged by simulating the ability of

collagen fibrils). For different time points, total content of fibronectin was obviously increased in MDCK cells cultured on collagen gel-coated dish or on 0.3% collagen gel-coated dish in normal culture media containing normal

concentrations of fetal calf serum (FBS).
formation of fibronecin matrix (Figure 3), and also induced the loss of microroughness. Microroughness were detected at previous. As the result showed, fibronecin inhibited the
sequence for another 24 hours. The level of fibronecin matrix and the formation of
hours and then added with 200 µl of fibronecin with containing RGD or RGF
microroughness in MDCK cells. We cultured MDCK cells in serum-free medium for 24 hours between the cytoplasmic and fibronecin, and to detect the effect in the formation of
contaminating adhesive-βγ-catenin aspartic acid (RGD) sequence to disrupt the association
formation of microroughness in MDCK cells. We used fibronecin-a-disintegrin protein
epithelial cell, we wondered whether the fibronecin matrix was essential of the
1994). Because loss of microroughness was an indicator of dedifferentiation of
1987). Otherwise, fibronecin matrix assembly was loss in several types of
required for differentiation of murine erythropoietic progenitor cells. In cell proliferation as well as differentiation, for example, fibronecin matrix was
Recent studies indicated that assembled fibronecin matrix played important roles
really induced the disappearance of microroughness.

RGD inhibited the formation of fibronecin matrix of MDCK cells, but

cultured on collagen fibrils.

increased fibronecin matrix in the disappearance of microroughness of MDCK cells

of MDCK cells to form the fibronecin matrix. However, what is the meaning of
collagen-simulated MDCK cells to incorporate exogenous fibronecin and the ablility
aboundantin molecular weight of 120 KD, 70 KD and 40 KD. Here we proved that
MDCK cells cultured on collagen gel-coated dish, these fragments were especially
otherwise, we also detected obvious expression pattern of fragments of fibronecin in
significantly increased in MDCK cells cultured on collagen gel-coated dish.
were also detected in MDCK cells cultured on collagen gel.

Interestingly, the thromboxane A2 measurements of 120KDa, 70KDa, and 40 KDa protein in MDCK cells cultured on collagen gel was most sensitive to collagenase digestion. However, cells cultured in normal dish and on collagen gel-coated dish. This result indicated that both of the cells could produce the basic need of internal thromboxane. Although cells cultured on normal dish reduced to the same level in MDCK when MDCK cells were cultured with collagen (figure 5). When treated with immunoblotting detection of thromboxane. Total content of thromboxane was increased were treated with 0.2% collagenase for different time and collected the cell lysate. The cells collagenase in the detection of thromboxane. We cultured the MDCK cells on dish, on such as on collagen gel-coated dish, on collagen gel and in collagen gel. Because we used the collagenase to collect the cells, so firstly we had to determine the effect of thromboxane matrix would be affected when cells cultured on different conditions. Formed cysteine structure and exhibited epithelial polarity. So we wondered whether lab had shown that when MDCK cells were cultured in 0.3% collagen gel, they by collagen and within affect the polarity of the cells. However, previous data in our previous data we had known that thromboxane matrix of MDCK cells was affected. 

Thromboxane depended on collagen gel were sensitive to collagenase digestion.

The formation of cell polarity, ex. the formation of microvilli in MDCK cells. The phenomenon showed in thromboxane matrix really had an important role in dish. The phenomenon showed in thromboxane matrix really had an important role in round-up phenotype and reduced the adhesion of MDCK cells to adherence to culture.
Ibuprofen fragments and the disappearance of microvilli of MDCK cells. Could
with collagen fibril, we wondered if there was any interaction between these
significant expression of fibronectin fragments were detected in MDCK cells cultured
with matrix metalloproteinase (MMP) and may affect the normal functions. In our data,
metastatic melanoma (M638) and many affect the normal functions in our data.
Many studies indicated that fragments of ibuprofen induced cells to express
Fragments of ibuprofen induced the disappearance of microvilli in MDCK cells.

Protective effect to disrupt the collagen signals of dedifferentiation,
ibuprofen deposited around the MDCK cells in collagen gel might offer the
microvilli still exhibited in cells cultured in collagen gel. The thick layer of
dish. Combining with previous data in our laboratory, this may explain why the
cultured in collagen gel was more than that on cells cultured on collagen gel-coated
same pattern of the fragments of ibuprofen. Second, the intact ibuprofen of cells
two important things: first, all of the cells cultured with collagen were expressed the
with collagen fibril (in collagen gel-coated dish, on gel and in collagen gel), we found
(9). However, when we compare the expression pattern of ibuprofen in cells cultured
Fibronectin of cells cultured on gel was more sensitive to collagenase digestion (figure
the cell lysate. The total content of fibronectin were detected by immunoblotting.
for different time. The cells were pretreated with 0.2% collagenase before collecting
cells on normal dish, on collagen gel-coated dish, on collagen gel or in collagen gel.
MDCK cells would be changed by different conditions of collagen. We cultured the
different conditions of collagen, I plan to see whether the total content of fibronectin in
Here we compared the total content of fibronectin in MDCK cells cultured on
digestion.
The ibuprofen in cells cultured on collagen gel was most sensitive to collagenase
Previous studies indicated that collagen might change the distribution of extracellular matrix of tubular cells. In order to see whether collagen would affect the distribution of tubular cells cultured on collagen was not affected.

Deposition of tubule collagen in MDCK cells cultured on collagen was not affected.

activity of MMP-2 and MMP-9 to digest tubule collagen might induce by the stimulation of Wnt-3, Wnt-7, Wnt-9 were found. However, the collagen in these experiments, A and others, not including collagen. However, the level of collagen that on normal dish. Previous data pointed out that the quantities of MMP-2 and MMP-9 were higher. In 24 hours, the activity of MMP-3 in cells cultured on collagen until was higher. MMP-9 has been expressed the MMPs (MMP-3 and MMP-9) that could digest the tubule collagen. The results of human plasma (Richland O. Huc) for the determination of MMP activity by immunoblotting showed that the immunoreactive bands obtained from the supernatants of the cells were induced by collagen. We purified tubule collagen from the supernatants, we wonder whether collagen would induce the cells to express specific collagenase. Cells cultured on collagen might express significantly amount of collagenase.

MMP-2 and MMP-9 were activated both in MDCK cells cultured on collagen.

MMP-2 and MMP-9 were activated both in MDCK cells cultured on collagen.
even in cells treated with rhocostomin-RGD,
distribution of fibronectin was not changed in cells cultured on collagen fibril (figure).

MDCK cells cultured on collagen fibril by confocal microscope. However, the
Collagen gel coated dish.

and then extracted by sodium deoxycholate. Dish: normal culture dish, GC: fibroblast. We cultured 1x10^6 cells on the dish for 24 hours. The cells were scraped.

Figure 1. Total content of fibronectin increased in cells cultured on collagen gel coated dish.

Treatement hours

- 0
- 4
- 8
- 12
- 24
The cells were treated with 2% sodium deoxycholate to separate the fibronectin being collagen fibrils. We cultured MDCK cells in normal media for the indicated time points.

Figure 2. The level of assembled fibronectin matrix increased in cells cultured on GC dish.

- &-actin
IB:

- Fibronectin
IB:
collagen coated dish, RGD: Rho-RGD (50 μg/ml), RGE: Rho-RGE (50 μg/ml).

Indication for the fibronecin in the cytoplasm. Dish: normal culture dish, GC:
assembled into the ECM. The soluble fraction of 3% Triton X-100 was an
cells was treated with 2% sodium deoxycholate to separate the fibronecin being
sequence (Rho-RGD) or RGE sequence (Rho-RGE) (50 μg/ml) for 24 hours. The
cultured MDCK cells in serum free media with rhodostomin containing RGD

Figure 3. Rhodostomin inhibited fibronecin assembly of MDCK cells. We
SEM.

Figure 4. Rhodostomin-induced disappearance of microvilli in MDCK cells

**Rho-GEF** **Rho-RGD** **Dish**
For collection, Fibronectin was detected by immunoblotting. Dish: normal culture. The cells were collected for different time, the cells was scraped and on collagen gel for 24 hours. Treated with collagenase for different time, the cells was scraped and on collagen gel coated dish and on collagen coated gel.

Figure 5. Fibronectin deposited on collagen gel was sensitive to collagenase digestion. P-actin: IB. Fibronectin: IB.
The cells were treated with 0.2% collagenase for collecting the cells. The cells were cultured with different conditions sensitive to collagenase digestion. MDCK cells were cultured on collagen gel was most

Figure 6: The fibronectin matrix in cells cultured on collagen gel was most

p-actin

IB:

Fibronectin

IB:

Dish: GC  OC  IC
Another 24 hours. The microvilli were detected by SEM.

Figure D, E and F: 40 KD4, figure G, H, and I: ALL were treated in 20 ng/ml for 70 KD4, and then cultured with fibronectin fragments: 120 KD4, figure A, B and C: 70 KD4.

MDCK cells. The MDCK cells were pretreated with serum starvation for 24 hours, Figure 7. Fragments of fibronectin induced the disappearance of microvilli in
Distribution of fibronectin was detected by confocal microscopy. The cells were fixed with 3.7% paraformaldehyde and the rhodostomin containing RGD sequence (Rho-RGD) or RGD sequence (Rho-RGE) coated on the dish. We cultured the cells with the fibronectin in MDCK cells. We cultured the cells with fibronectin.