

Animals

Cell lines and culture methods

C

, DPM E αKO() , (3),

- DMEM 10% - (BG)

95% 5 % CO2 37 C. MC 7 10

.

F MC , 96-

2500 / DMEM 10% BG . F ,

DMEM 3% BG 5 / PDGF (L ,

G I , N), 100 /L 17β- (E2, -A , .

L , MO) E OH . I , (50 /L)

15 E2 , PDGF (5 /)

30 E2 . MC

C -G L C A

(P , M , I) .

DPM ,

.

F , MC (2×10^5) 6 DMEM

10% BG . ,

DMEM 0.5 % BG , 18 ,

200 . C PB - ,

DMEM 0.5% BG E2, E OH , PDGF

. A 24 , - 4

,

. E 4 .

E2 , MC (2×10^5)

6 DMEM 10% BG .

, DMEM BG , 6 ,

, 5-100 /L E2 PDGF .

C PB 15 PDGF

-80 .

F , MC (2×10^4) DMEM 10%

BG 6 -

. , DMEM

0.5% BG , 16 , E2 E OH

PDGF (5 /) BG (3%). A 24 ,

4% NEL

(I C D D K ; , B ,)
 . DAPI .

qRT-PCR

E2 NA P C
 N A (PCNA) MC, 6 DMEM
 10% BG 24 . 0.5%
 BG 18 , 100 /L E2 E OH 30
 5 / PDGF, 100 /L 0.1% B A , 3% BG
 24 NA N (Q , , CA).
 DNA ILO (L), -PC
 Q B G (Q) : *PCNA*:
 C AGCCA GGGCG GAAC,
 GAA AC AG GC AAGG G C GCA , *GAPDH*:
 CAC GAAGGGCA C GG, CA G CA ACCAGGAAA GAG.

Gene transfer

E α MC E2- - ,
 E α (GFP) (-GFP-E α)
 MC E α KO . A GFP
 (-GFP) . -GFP-E α -GFP
 (4). MC (2×10^5) 6 DMEM
 10% BG 24 , -GFP-E α -

GFP. , DMEM 10% BG
8 . -
96 MC .
GFP .
A 60-80 % GFP 24 -GFP-E α -
GFP ().
E2 ,

Western blotting assay

(4). MC

IPA IP (, L , CO)

(EMD, L G , IL). L

D -PAGE P DF ().

, -A , -E K,

-G K3 α / β , PTEN (C , D , MA), α - (EMD), E K (L

), GAPDH, E α (MC20), A , PP2A , (C B ,

C , CA), MKP-1, (BD , J , CA). ECL-

(GE , P , PA) .

PP2A activity assay

PP2A PP2A

(B , L P , N). C M-PE

() . P

. E (150 μ)

PP2A . B PP2A

.

96- ,

. P 15

650 . A

0-2000 /L.

Statistical analysis

A EM. C

- - . M ANO A

$P<0.05$

1. M JB, G , N M, G H, K, L Q, A MJ,
N H, B E, H , I LK, K H.

Circulation. 2012; 126: 1993-2004.

2. D , K A, G A, D A, C P, M M. E
(E) (E)

. Development. 2000; 127:4277 4291.

3. K H, E M, L JP, , K M, A M, B E,
C O, O M B , M ME. A

. J. Clin. Invest.. 2001; 108:611 618.

4. L Q, HK, E H, B E, B D, P DC, K H.

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2A. *J. Biol. Chem.* 2003; 278:4639 4645.