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(11)
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(43) 2001 09 06

(73) 134

(72) 1 870 542 - 1104
721 - 2 102 101
103 606
87 303 1105

(74)
:

(54)

(MMP)

4

1a MT1 - MMP proMMP - 2가 MMP - 2

1b APMA proMMP - 2가 MMp - 2

2 PMA proMMP - 2가 MMP - 2

3a proMMP - 2 MMP - 2

3b 3a MMP - 2

4 MT1 - MMP MMP - 2

5a FLAG FLAG

5b FLAG MMP - 2

s) (endostatin) (matrix metalloproteinases, MMP

가 (embryonic development),
 (tumor metastasis),
 (: Cawston, Br. Med. Bull., 51:385 - 401, 1995).
 MMP - 1(matrix metalloproteinase - 1) MMP - 8(matrix metalloproteinase - 8)
 (collagenase) , MMP - 2 MMP - 9 (gelatinase) , MMP - 3 MMP - 10
 (stromelysin) , MMP - 14(1 , MT1 - MMP)
 MMP(membrane type MMP) MMP
 MMP N - (pro - domain) MMP
 , N - MMP
 (: Stetler - Stevenson et al., J. Biol. Chem., 265:13933 - 13938, 1990). MMP
 MMP - 2 (basement membrane) type (type collagen)
 , MMP - 2 proMMP - 2 MMP - 2 MMP(soluble MM
 P) 1 (membrane type -
 1 MMP, MT1 - MMP) (: Sato et al., Nature, 370:61 - 65, 1994)

20kDa 가 (murine hemangioendothelioma) 가 (xenograft)
 (: O'Reilly M.S. et al., Cell, 86:353 - 364, 1994; Boehm T. et al., Nature, 390:404 - 407, 1997),
 (: Boehm T, et al., Nature, 390:404 - 407, 1997).
 (vascular endothelial growth factor, VEGF) bFGF(basic fibroblast growth factor) (apoptosis)

(in vitro) (in vivo)

MMP - 2 proMMP - 2(promatrix metalloproteinase - 2) MMP - 2 MT1 - MMP
 P MMP - 2

merase chain reaction, PCR) cDNA (poly)
 PCR pFLAG - CMV - 1
 , FLAG
 20kDa PAGE ,
 , MMP - 2
 sMT1 - MMP FLAG 가
 2 proMMP - 2 MT1 - MMP Sf9 proMMP - 2 , MMP -
 , MMP - 2

te 13 - acetate) proMMP - 2 , PMA(phorbol 12 - myristate 13 - acetate) proMMP - 2 MMP

- 2

(immuno - depleted) , proMMP - 2 MMP (anti - FLAG antibody bead)

pro - MMP - 2 , MT1 - MMP

proMMP - 2 (pro - domain) proMMP - 2 MMP - 2 , APMA(p - aminop

henylmercuric acetate) proMMP - 2 MMP - 2 가 ,

MT1 - MMP APMA proMMP - 2 ,

MT1 - MMP APMA proMMP - 2 ,

MMP - 2 MMP - 2 , MT1 - MMP

proMMP - 2 MMP - 2 ,

sMT1 - MMP proMMP - 2 MMP - 2 ,

proMMP - 2 MMP - 2 , MT1 - MMP

MT1 - MMP proMMP - 2 MMP

MMP - 2

가

1:

(human umbilical vein endothelial cells, HUVEC)

20%(w/v) FBS, 100units/Mℓ , 100μg/Mℓ , 3ng/Mℓ bFGF(

basic fibroblast growth factor, Upstate Biotechnology, U.S.A.) 5units/Mℓ M19 (Life

Technologies, U.S.A.)가 75cm² , 37 5% CO₂ ,

(human fibrosarcoma cell) HT1080 (ATCC CC

L121) (human embryokidney cell) HEK293 () 10%

FBS, 100units/Mℓ , 100μg/Mℓ (DMEM, Life Tec

hnologies, U.S.A.)

2:

C 183 cDNA 5' GGAAGCTTCATACTCATCAGGACTT

PCR : 1) , 3' GGGGTCGACCTATTTGGAGAAAGAGGTCATG(: 2)

PCR PCR Hind /Sa1I N

FLAG(DYKDDDDK) (epitope) (linker) pFLAG - CMV - 1

(KODAK, U.S.A.) pFLAG - CMV - 1 - endostatin 1

HEK293 pFLAG - CMV - endostatin pcDNA3.1(Clontech, U.S.A.) , G

418(0.6mg/Mℓ)

(: Sasaki T. et al., EMBO J., 17:4249 - 4256(1998)).

, 0.1μg/Mℓ 200μℓ FLAG (KODAK, U.S.A.)가 100μℓ

FLAG 가 A (Upstate Biotechnology, U.S.A.) 100μℓ 4 24

, 4000rpm 2 10μℓ 15% SDS - PAGE

FLAG DYKDDDDK

HT1080(ATCC CCL121) , MMP - 2

3:

MMP - 2, MT1 - MMP가 proMMP - 2 (pro - domain)
 (: Sato et al., J. Biochem., 119:209 - 215, 1996), proMMP - 2
 (transmembrane - deleted) sMT1 - MMP(soluble membrane type - 1 MMP)
 Sf9(ATCC CRL1711) (: Jo et al., J Biochem.
 Mol. Biol., 32:60 - 66, 1999).

4: proMMP - 2 MMP - 2

proMMP - 2 MMP - 2, 20mM Tris - HCl(pH 7.5), 150mM NaCl, 5mM CaCl₂, 100 μ M ZnCl₂ 0.025% Brij 35 MMP 40μℓ 0, 0.31, 0.63, 1.3, 2.5, 5.0, 10 20μg/ℓ 3 10ng proMMP - 2 12ng sMT1 - MMP 가 37 3 . , APMA proMMP - 2 proMMP - 2 가 (: Okada et al., Eur. J. Biochem., 194:721 - 730, 1990), proMMP - 2 , 12ng sMT1 - MMP 1mM APMA(Sigma Chemical Co., U.S.A.) 가 37 30 . 0.1% 9% SDS - PAGE , 2.5% Triton X - 100 SDS 50mM Tris - HCl(pH 7.5), 150mM NaCl 10mM CaCl₂ 24 , 0.25% R250 .

1a MT1 - MMP proMMP - 2가 MMP - 2
 . 1a , 1 proMMP - 2(68kDa) ,
 2 9 12ng sMT1 - MMP가 proMMP - 2
 . 1a 2 , proMMP - 2
 (68kDa) MT1 - MMP , MMP - 2가 64kDa (intermediate form)
 , 3 9 (64kDa)

1b APMA proMMP - 2 MMP - 2
 . 1b , 1 proMMP - 2(68kDa) , 2
 9 1mM APMA가 proMMP - 2
 . 1b 2 , proMMP - 2 APMA M
 MP - 2(62kDa) , 3 9 MMP - 2
 - 2(62kDa) .
 , sMT1 - MMP APMA proMMP - 2 MMP - 2 ,

5:

4 MT1 - MMP APMA proMMP - 2 MMP - 2
 , proMMP - 2 MMP - 2
 .
 1 37 24 60ng/Mℓ PMA 0.1, 1, 3, 5 10mg/Mℓ
 , 10μℓ
 (immuno - depleted) 60ng/Mℓ PMA(Alexis, U.S.A.)
 37 24 , 10μℓ ,
 (immunoblotting)
 FLAG , MMP - 2 pr
 oMMP - 2 MMP - 2

2 PMA(phorbol 12 - myristate 13 - acetate) proMMP - 2가 MMP - 2
 . 2 , 1 , 2
 PMA , 3 8 2
 , 4 , proMMP - 2(68kDa) PMA MMP - 2(62kDa)
 MMP - 2(62kDa)

3a proMMP - 2 MMP - 2
 , 3b 3a proMMP - 2 MMP - 2
 MMP - 2 , 2 PMA , 3 PMA
 , 4 , 5 4
 A , PMA가
 1, 3 5 proMMP - 2(68kDa)가 MMP - 2(62kDa)
 2 4 proMMP - 2가 MMP - 2

proMMP - 2 MMP - 2가 , 가 , proMMP - 2 MMP - 2
 , PMA proMMP - 2가 MMP - 2

6: MMP - 2 MT1 - MMP
 4 5 , proMMP - 2가 MMP - 2
 , proMMP - 2 MMP - 2 가 MT1 - MMP , MMP - 2
 MT1 - MMP

3 proMMP - 2 1mM APMA 37 30 MMP - 2
 Mca - Pro - Leu - Gly - Leu - Dpa - Ala - Arg - NH₂ (Bachem, U.S.A.)
 (: Yamamoto et al., J. Med. Chem., 41:1209 - 1217, 1998).
 , 20ng MMP - 2 20ng MT1 - MMP 0, 0.31, 0.63, 1.3, 2.5, 5, 10 20μg
 1 μ M 가 40Mℓ MMP
 , 37 30 0.1M (pH 4.0) 가 , KO
 NTRON SFM 25 328nm (excitation) , 393nm

4 MT1 - MMP MMP - 2
 MMP - 2() MT1 - MMP() (%)
 가
 . MMP - 2
 , proMMP - 2 MMP - 2 sMT1 - MMP
 , sMT1 - MMP MMP - 2 50% 2.0μg/Mℓ
 0.82μg/Mℓ

4 5 , sMT1 - MMP가 proMMP - 2 가 proMMP -
 2 MMP - 2 , proMMP - 2 MT1 - MMP
 MMP - 2

7: proMMP - 2

, MT1 - MMP APMA proMMP - 2 MMP - 2 ,
 proMMP - 2 (coimmunoprecipitation) , proMMP - 2가
 proMMP - 2

1μg/Mℓ proMMP - 2 HT1080 0, 0.1, 1 10μg
 4 2 , proMMP - 2 , 25μℓ
 FLAG (KODAK, U.S.A.)가 가 1 가 , 4000rpm
 30 , 0.5% NP - 40(Nonidet P - 40, Sigma, U.S.A.) 500μℓ
 PBS 5 , MMP - 2 (Santa Cruz Biotechnology, U.S.A.)
 FLAG (western blotting)

5a FLAG FLAG ,
 5b MMP - 2 5a , 1
 가 HT1080 , 2 4
 0.1, 1 10μg 가 HT1080 , 5b , H HT1080
 30μℓ , 1 4 5a 1 4
 MMP - 2 . 5a 5b ,
 (20kDa) 가 , proMMP2(68kDa)가
 가

MP - 2 , APMA proMMP - 2 proMMP - 2 MMP - 2 MT1 - MMP proMMP - 2 M .

MT1 - MMP
MMP - 2

MMP - 2

(proMMP - 2)

(57)

1.

(MMP)

2.

1 ,

1

(MT1 - MMP)

- 2

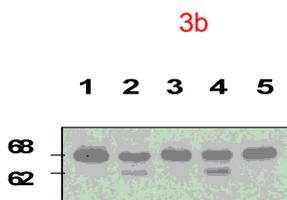
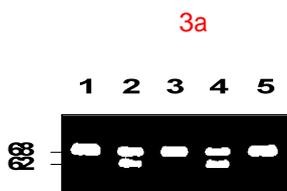
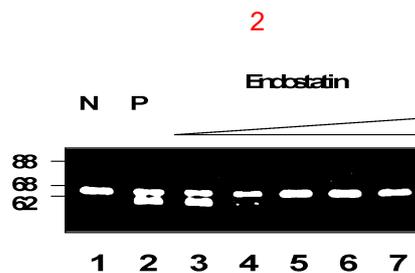
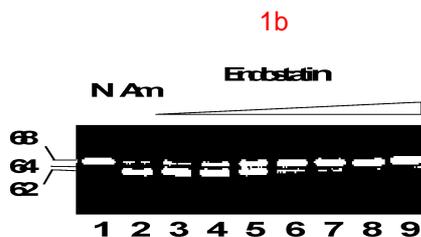
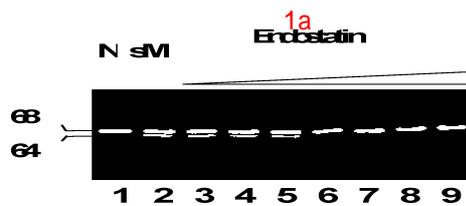
(proMMP - 2)가

- 2(MMP - 2)

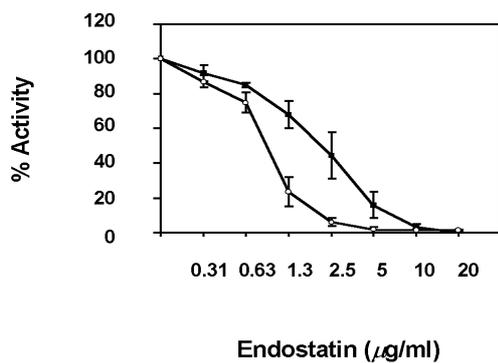
3.

1 ,

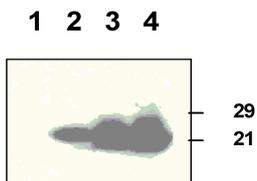
- 2(MMP - 2)



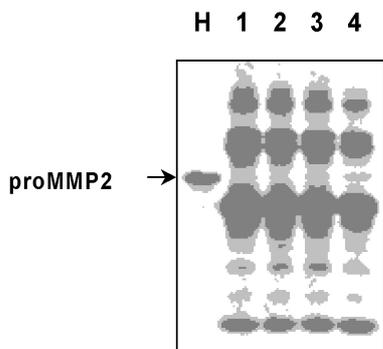
4



5a



5b



<110> KWON, Young-Guen <120> A Method for Inhibiting Degradation of Extracellular Matrix by Endostatin through Inhibition of the Catalytic Activity of Matrix Metalloproteinases
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 DNA <212> Artificial Sequence <220> <223> 5' primer <400>
 gt; 1 gggaagcttc atactcatca ggactttcag c
 <210> 2 <211>

31 <212> DNA <213> Artificial Sequence <220> <223> 3' primer <400&
gt; 2 ggggtcgacc tatttggaga aagaggtcat g

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