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(KR)  
(B1)

(51) 。 Int. Cl. <sup>7</sup>  
A61K 38/31

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(11)  
(24)

2003 02 17  
10 - 0372516  
2003 02 04

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10 - 2000 - 0006175  
2000 02 10

(65)  
(43)

2001 - 0083966  
2001 09 06

(73)

134

(72)

1 870

542 - 1104

721 - 2

102 101

103 606

87 303 1105

(74)

:

(54)

(MMP)

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)

, (murine hemangioendothelioma) 가  
 20kDa 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)  
 last growth factor) , (apoptosis)

, (in vitro) (in vivo)

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

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

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

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 75cm<sup>2</sup> , 37 5% CO<sub>2</sub> ,

(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

PCR . cDNA 5' GGGGAAGCTTCATACTCATCAGGACTT

T - CAGC( : 1) , 3' GGGGTCGACCTATTTGGAGAAAGAGGTCATG( : 2)

PCR , PCR Hind /SaII , 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 CaCl<sub>2</sub>, 100 μ M ZnCl<sub>2</sub> 0.025% Brij 35 MMP 40μl 0, 0.31, 0.63, 1.3, 2.5, 5.0, 10 20μg/ml 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 CaCl<sub>2</sub> 24 , 0.25% R250 .

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

1b APMA proMMP - 2 MMP - 2 proMMP - 2(68kDa) , 2 9 1mM APMA가 proMMP - 2 proMMP - 2(62kDa) , 3 9 . 1b 2 , 3 9 . MP - 2(62kDa) , 3 9 . MP - 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  
 , 60ng/Mℓ PMA(Alexis, U.S.A.) 37 24 , 10μℓ ,  
 (immuno - depleted) (immunoblotting) . , MMP - 2 pr  
 FLAG  
 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 , 3a 3b , 1  
 , 2 PMA , 3 PMA  
 , 4 A PMA 5 4  
 . PMA가  
 1, 3 5 proMMP - 2(68kDa)가 MMP - 2(62kDa)  
 .

2 4 proMMP - 2가 MMP - 2 .

proMMP - 2 MMP - 2가 , PMA proMMP - 2가 MMP - 2  
 , 가 , proMMP - 2 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 - NH<sub>2</sub> (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  
2 MMP - 2 , MT1 - MMP proMMP -  
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

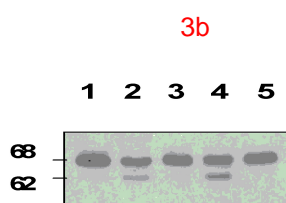
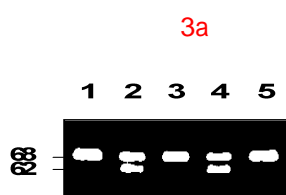
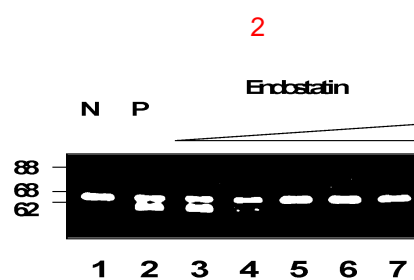
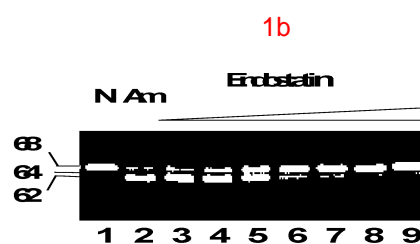
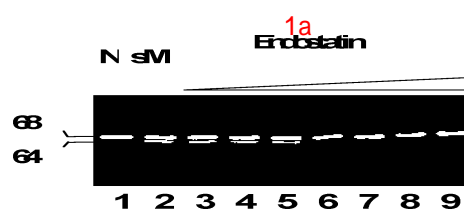
- 2(MMP - 2)

3.

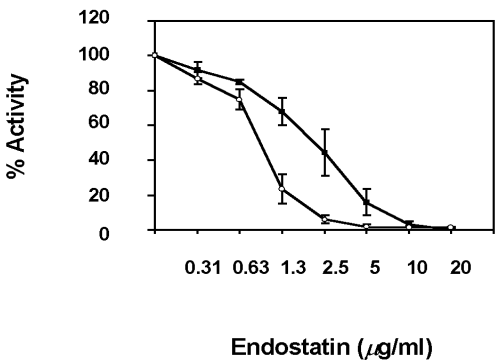
1 ,

- 2(MMP - 2)

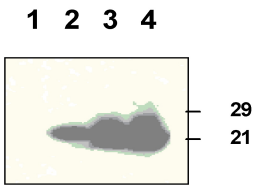




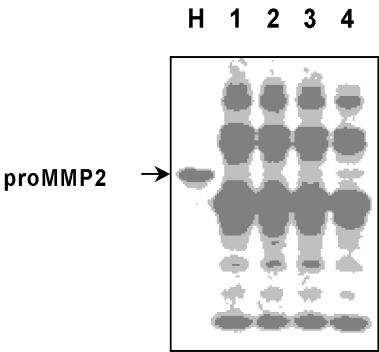
4



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  
es <130> DP90906 <160> 2 <170> KOPATIN 1.5 <210> 1 <211>  
31 <212> DNA <213> 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

31