

(19)
(12)(KR)
(A)(51) 。 Int. Cl.⁷
C07K 19/00(11)
(43)10-2004-0018598
2004 03 04(21) 10-2002-0049979
(22) 2002 08 23(71) ()
141-28

134

(72) 110 1404

(74)

:

(54) T a t - P T E N ,

NA PTEN Tat Tat-PTEN , D
 , , , .

11

1 Tat-PTEN DNA .
 , Tat PTEN Tat (protein transduction domain), PTEN
Xho I , Tat -Xpress
 .

2 Tat-PTEN .

3 pRSET A Tat-PTEN (pTat-PTEN) .

4 PTEN Tat-PTEN SDS-PAGE(sodium dodecyl sulfate-polyacrylamide gel electropho
 resis) .

5 Xpress (western blot)

6 Tat-PTEN , PTEN
Tat-PTEN

7 Tat-PTEN , Xpress
Tat-PTEN

8 (), TGF- β 1, Tat-PTEN TGF- β 1, Tat-PTEN

9 , TGF- β 1(10ng/ml), Tat-PTEN (0.25uM, 0.5uM, 1uM, 2uM) TGF- β 1(10ng/ml) , AKT
5uM, 1uM, 2uM) TGF- β 1(10ng/ml) , 1 2 TGF- β 1 10ng/ml , 3 6 Tat-PTEN 0.25uM, 0.5uM, 1uM, 2uM TGF- β 10ng/ml

10 Tat-PTEN 0.75uM () , 1 2 6 Tat-PTEN
0.75uM 30 , 5 , 24 , 48 , 72

11 () , PTEN, Tat-PTEN TGF- β 1, Tat-PTEN
, Tat-SOD , V Pi

12 () , TGF- β 1, Tat-PTEN TGF- β 1, Tat-PTEN , P

PTEN
Tat-PTEN , Tat
Tat-PTEN

PTEN(phosphatase and tensin homologue deleted from chromosome 10) MMAC1(mutated in multiple advanced cancers 1) TEP1(TGF β regulated and epithelial cell enriched phosphatase 1) (tumor suppressor gene) PTEN (mutation) (deletion) [Kong DA, Suzuki TT, Zou A, Sakurada LW, Kemp S, Wakatsuki T, Yokoyama H, Yamakawa T, Furukawa M, Sato, et al., 1997. PTEN1 is frequently mutated in primary endometrial carcinomas, Nat. Genet., 17: 143-144; Li DM amp; Sun H, 1997, TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor beta, Cancer Res., 57: 2124-2129; Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, et al., 1997, Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers, Nat. Genet., 15: 356-362]. PTEN 403 47,000 [Li J, Yen C, Liaw D, Podsypani na K, Bose S, Wang SI, Puc J, Miliarensis C, Rodgers L, McCombie R, et al., 1997, PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science. 275: 1943-1947; Li DM amp; Sun H, 1997, TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor beta. Cancer Res., 57: 2124-2129; Furnari FB, Lin H, Huang HJS, Cavenee WK, 1997, Growth suppression of glioma cells by PTEN requires a functional phosphatase

activity, Proc. Natl. Acad. Sci. USA, 94: 12479-12484]. PTEN / (serine/threonine phosphatase) (tyrosine phosphatase) (dual specificity) 가 , 3- (phosphoinositide 3-phosphatase) 3- (inositol phosphate 3-phosphatase) [Myers MP, Stolarov JP, Eng C, Li J, Wang SI, Wigler MH, Parsons R, Tonks NK, 1997, P-TEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase, Proc. Natl. Acad. Sci. USA, 94: 9052-9057; Myers MP, Pass I, Batty IH, Kaay JVD, Stolarov JP, Hemmings BA, Wigler MH, Downes CP, Tonks NK, 1998, The lipid phosphatase activity of PTEN is critical for its tumor suppressor function, Proc. Natl. Acad. Sci. USA, 95: 13513-13518]. (cell proliferation), (cell survival), (cell migration), (cell invasion) PTEN [Besson A, Robbins SM, Yong VW, 1990, PTEN/MMAC1/TEP1 in signal transduction and tumorigenesis. Eur. J. Biochem., 263: 605-611].

PTEN , WO 99/02704 PTEN , PTEN 6,020,199 PT EN , PTEN

1988 Green Frankel HIV Tat [Green M amp; Loewenstein PM, 1988, Autonomous functional domains of chemically synthesized human immunodeficiency virus Tat trans-activator protein, Cell, 55: 1179-1188; Frankel AD amp; Pabo CO, 1988, Cellular uptake of the Tat protein from human immunodeficiency virus, Cell, 55: 1189-1193], Fawell 1994 Tat 가 (crosslinking) [Fawell S, Seery J, Daikh Y, Moore C, Chen LL, Pepinsky B, Barsoum J, 1994, Tat-mediated delivery of heterologous proteins into cells, Proc. Natl. Acad. Sci. USA, 91: 664-668]. 가 Tat (denaturation) (transduction) 가 [Nagahara H, Vocero-Akbani, Adamina M, Synder EL, Ho A, Latham DG, Lissy NA, Becker-hapak M, Ezhevsky SA, and Dowdy SF, 1998. Transduction of full-length Tat fusion proteins into mammalian cells: Tat-p27kip1 induces cell migration. Nature Med. 4: 1449-1452]. Tat (protein transduction domain: PTD) 가 , (renaturation) , HSP90 (chaperone) [Schneider C, Sepp-Lorenzino L, Nimmesgern E, Ouerfelli O, Danishefsky S, Rosen N, Hartl FU, 1996, Pharmacologic shifting of a balance between protein refolding and degradation mediated by Hsp90, Proc. Natl. Acad. Sci. USA, 93: 14536-14541]. Tat

가 (filtration operation) (iridencleisis), (trephination) (trebeculectomy) (fibrous scar) (sclerectomy), 가 , DNA 가 , DNA- (dependent) RNA 가 C(Mitomycin C) 가 , 239-256]. 가 [, 1996, ,

TGF- β (Transforming growth factor- β) , TGF- β (wound healing) , TGF- (proliferative vitreoretinopathy) (conjunctival wound healing) [Cordeiro MF, 2002, Beyond mitomycin: TGF- β and wound healing. progress in retinal and eye research. 21: 75-89; Cordeiro MF, Gay JA, Khaw PT, 1999, human anti-transforming growth factor- β 2 antibody; a new glaucoma anti-scarring agent, IOVS, 40: 2225-2234].

가 , Tat-PTEN

가

Tat-PTEN

DNA

Tat-PTEN

(PTEN : 1) PTEN HIV-1 Tat
Tat-PTEN

Tat-PTEN DNA

Tat-PTEN

PTEN 가 Tat-PTEN Tat-PTEN Tat-PTEN Tat-

Tat-PTEN

TEN 5'- , Tat-PTEN DNA DNA P
HIV-1 Tat , DNA 가 , PCR DNA , DNA
, DNA

, PTEN Tat cDNA
, Tat PCR PTEN cDNA

Tat-PTEN BL-21
, Tat-PTEN

Tat-PTEN Tat-PTEN
(6 7). , Tat PTEN
(7).

Tat-PTEN TGF- (8), (9),
(11).

, 11 12 , Tat-PTEN Tat-PTEN , Tat PTE
N , Tat-PTEN , Tat-SOD , Tat PTEN
, Tat 가

Expression pRSET(Invitrogen, USA) , *BamH I* *Xho I* Xpress System Protein
(double stranded oligonucleotide) (ligation) , Automatic DNA sequencer(ABI 3100 DNA Analyzer system, Perkin-Elmer)

PTEN cDNA (Stratagene, La Jolla, CA, USA) (template)
TaqPlus Precision (Clontech, Palo Alto, CA, USA) , Cu/Zn SOD(Superoxide
dismutase) . PTEN SOD GenBank U93051(
: 5) X02317(: 6) . PTEN SOD (full length) cDNA
GenBank 가 . PTEN 5'-AAA-C
TC-GAG-ATG-ACA-GCC-ATC-ATC-AAA-GA-3'(: 7) 5'-AAA-AAG-CTT-TCA-GA
C-TTT-TGT-AAT-TTG-TGT-ATG-CTG-3'(: 8) , SOD 5'-AAA-CTC-GA
G-ATG-GCG-ACG-AAG-GCC-GTG-TGC-GTG-3(: 9) 5'-AAA-AAG-CTT-TTA-TT
G-GGC-GAT-CCC-AAT-TAC-ACC-3'(: 10) . *Xho I* 가 ,
Hind III . PCR 94 5 (pre-denaturation)
, 94 30 , 62 30 , 68 1 25 , 68 10 (last
-extension) . PCR 가 (agarose gel) TA- (Promega)
, BL21 (competent cell)(Invitrogen) (transformation)
. PTEN SOD cDNA 가 TA- *Xho I* *Hind III* , pRSET
(subcloning) Tat-PTEN (3), PTEN Tat-SOD
BL21(DE3)pLysS(F-, *omp T hsd SB* (rB-mB-) *gal dcm* (D
E3) pLys (CamR))(Invitrogen) 100ug/ml LB (pl
ate) . colony 100ug/ml LB (broth) 3ml 37
200rpm , 100ug/ml LB 400ml
37 OD600=0.6 , 1mM IPTG(isopropyl-1-thio- β -D-galactopyr
anoside) 가 8 , 10000 \times g 10 B
uffer A(20mM HEPES (pH 8.0), 100mM NaCl, 20mM) 20ml , (sonicator) 4
12500 \times g 30 , 8M 가 Buffer A
(inclusion body) Tat-PTEN PTEN Tat-
SOD 10000 \times g 10 8M Buffer A
, Tat-PTEN, PTEN, Tat-SOD 50%
His Bind resin (Novagen) 2.5ml 3 (binding buffer)(8M
, 5 1 \times (charge buffer)(50mM NiSO₄) 3 1 \times (gravity flow)
, 5mM , 0.5M NaCl, 20mM Tris-HCl, pH 7.9) 10 1 \times , 6 1 \times (6
0mM , 0.5M NaCl, 20mM Tris-HCl, pH 7.9) (100mM , 0.5M NaCl, 20mM Tris-
HCl, pH 7.9) 250mM 500mM 2ml
. Tat-PTEN PTEN 7.5% SDS-PAGE
(Bradford)

PTEN Tat-PTEN SDS-PAGE (coomassie staining) , PTEN 59k
Da, Tat-PTEN 60kDa 가 (4).
가 Tat-PTEN, PTEN Xpress (Invitrogen)
(5).

2:

(inferior conjunctiva) (Tenon capsule)
. 10% , 50ug/ml DMEM(Dulbecco's mod
ified Eagle's medium) 37 , 5% CO₂ 1 (confl
nt) , 0.05% , 5mM EDTA 100-mm , 3 5 (passage)

3: Tat-PTEN

TGF- β 1

Tat-PTEN PTEN 70% (confluency) ,
DMEM 2 37 , 5% CO₂ .

DMEM 3 , TGF- β 1(R amp; D Systems) DMEM
 10ng/ml 20 SDS-PAGE (nitrocellulose membrane)
 (transfer membrane) 0.1% Tween-20 5% (Nonfat milk powder) T
 BS(Tris-buffered saline) 1 (blocking) (blocking buffer) 1
 (primary antibody: Xpress antibody, Invitrogen, USA) 4 16
 1:1000 2 (secondary antibody: horseradish peroxidase-conjugated anti-mou
 se, anti-rabbit secondary antibody, Amersham) 1 ECL
 (Amersham) 1 (Amersham)

Tat-PTEN

Tat-PTEN 2 , PTEN P
 Tat-PTEN
 6
 TEN
 Tat-PTEN 2 , Xpress
 Tat-PTEN
 7
 Tat PTEN 가 , Tat

Tat-PTEN

TGF- β 1

8 TGF- β 1 P-AKT, P-ERK1/2, P-P38 가 가
 , Tat-PTEN TGF- β 1 TGF- β 1
 (extracellular matirx) (fibronectin) 가 , Tat-PTEN

Tat-PTEN

Tat-PTEN 0.25uM 2uM 가 10ng/ml
 TGF- β 1 , TGF- β 1 AKT 가
 (9).
 , Tat-PTEN 2 가, 가
 3 (10).

4: (Apoptosis Assay)

V-FITC (Annexin V-FITC assay)

Tat-PTEN , TACSTM annexin V-FITC
 Apoptosis Detection Kit (TEVIGEN) (early apoptosis) (late apop
 tosis) Tat-PTEN , Tat P
 TEN 2 , TGF- β 1 20 , 가 PBS V-FITC
 (1ug/ml V-FITC, 5ug/ml) (10mM H
 EPES, pH 7.4, 150mM NaCl, 5mM KCl, 1mM MgCl₂ , 1.8mM CaCl₂) , (mounting)
 (confocal microscope)

11 , Tat PTEN ,
 Tat-PTEN Tat-PTEN TGF- 1 Pi
 , TGF- 1 Tat-PTEN
 , (nuclear condensation) (apopto
 tic body) , TGF- 1 PTEN Tat
 . Tat-SOD 가 PTEN

FITC (permeabilization), 4%, PBS, 100ug/ml, PBS, TGF-β1 0.2%, X-100, V- (PI, Sigma) (Olympus Fluorescence microscope, BX60)

12, TGF-β1, Tat-PTEN (half-moon), (horse shoe) TGF-β1 Tat-PTEN

, PTEN DNA HIV-1 Tat Tat-PTEN, Tat-PTEN, TGF-가

(57)

1. PTEN : 1) PTEN Tat-PTEN HIV-1 Tat (

2. 1, : 12 Tat-PTEN

3. 1 Tat-PTEN DNA

4. 3, : 11 DNA

5. 3 4 Tat-PTEN

6. 5, 3 Tat-PTEN

7. 5 Tat-PTEN Tat-PTEN

8. 7,

9. 1 Tat-PTEN,

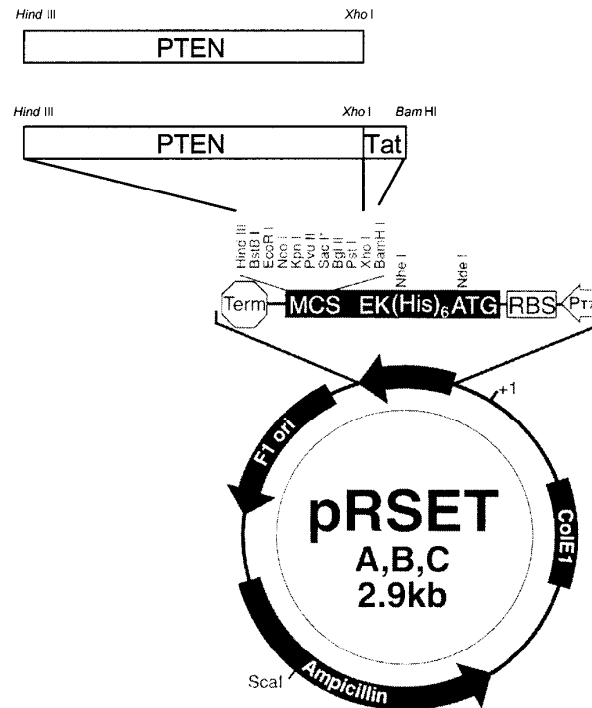
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GTCGTAAGAAACGTAGACAGCGCAGACGTGGT**CTCGAGATGACAGCCATCATCAA**
GAGATCGTTAGCAGAAACAAAAGGAGATATCAAGAGGATGGATTCGACTTAGACTTGAC
 CTATATTTATCCAAACATTATTGCTATGGGATTTCC**TGCAGAAAGACTTGAAGGCGTAT**
 ACAGGAACAATATTGATGATGTAGTAAGGTTTTTGGATTCAAAGCATAAAAAACCATTAC
 AAGATATACAATCTTTGTGCTGAAAGACATTATGACACCGCCAAATTAATTGCAGAGT
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 GTGAAGATCTTGACCAATGGCTAAGTGAAGATGACAATCATGTTGCAGCAATTCAGTGT
 AAAGCTGGAAAGGGACGAAC**TGGTGT**AATGATATGTGCATATTTATTACATCGGGGCAA
 ATTTTAAAGGCACAAGAGGCCCTAGATTCTATGGGGAAGTAAGGACCAGAGACAAAA
 AGGGAGTA**ACTAT**CC**AGTCAGAGGCGCTATGTGTATTATTATAGCTACCTGTTAAAG**
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 TCCAATGTTCAGTGGCGGA**ACTTGCAATCCTCAGTTTGTGGTCTGCCAGCTAAAGTGA**
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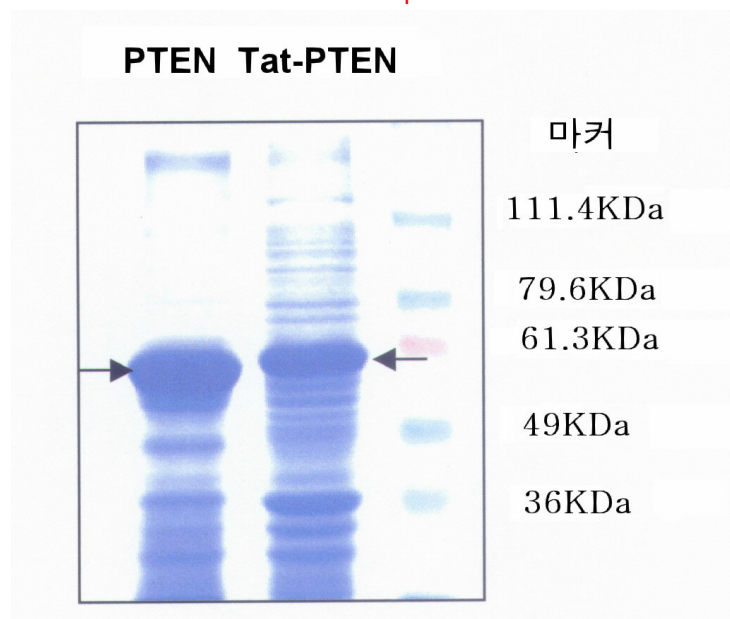
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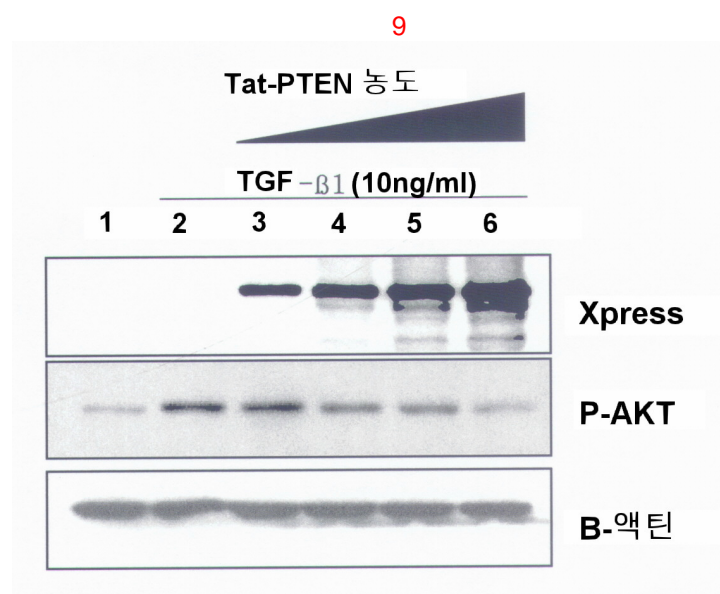
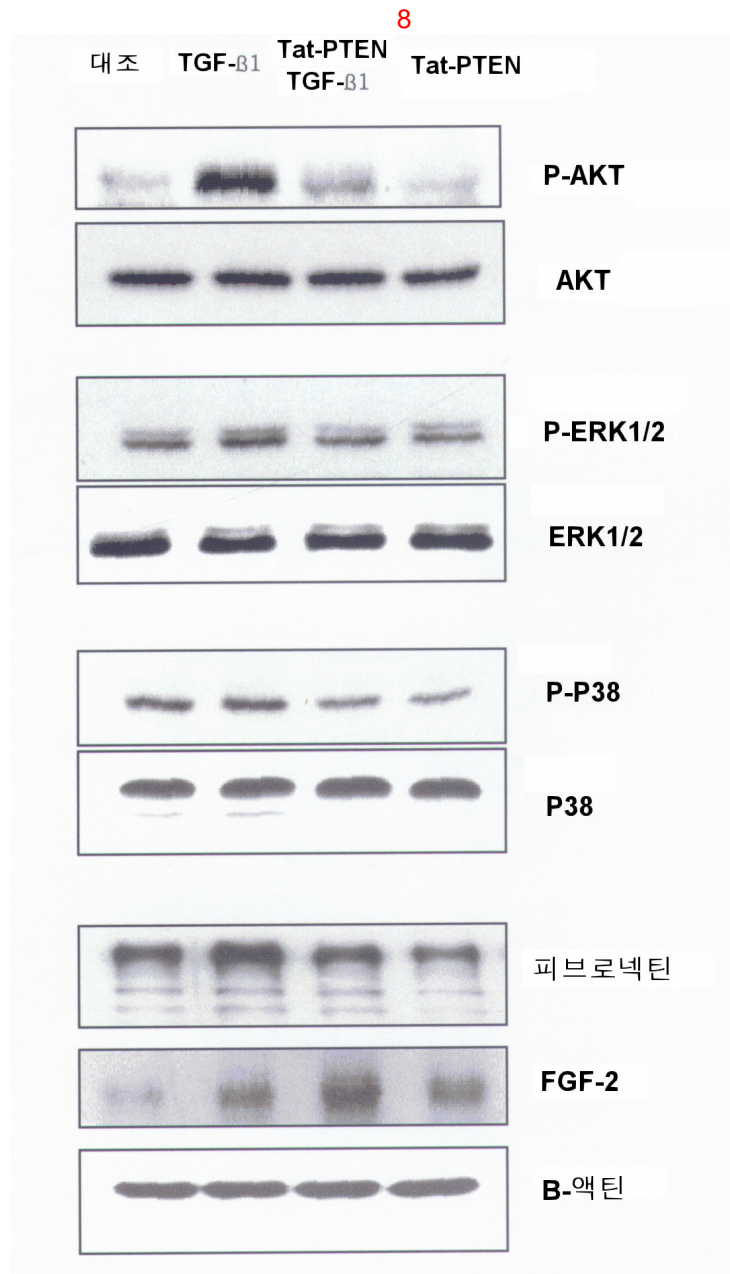
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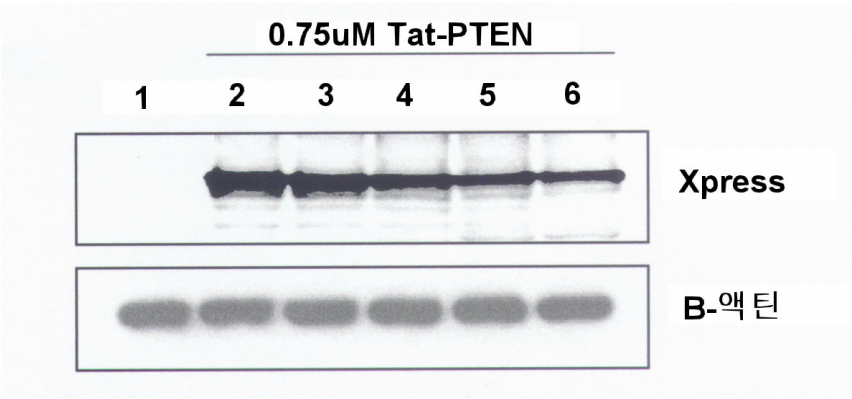
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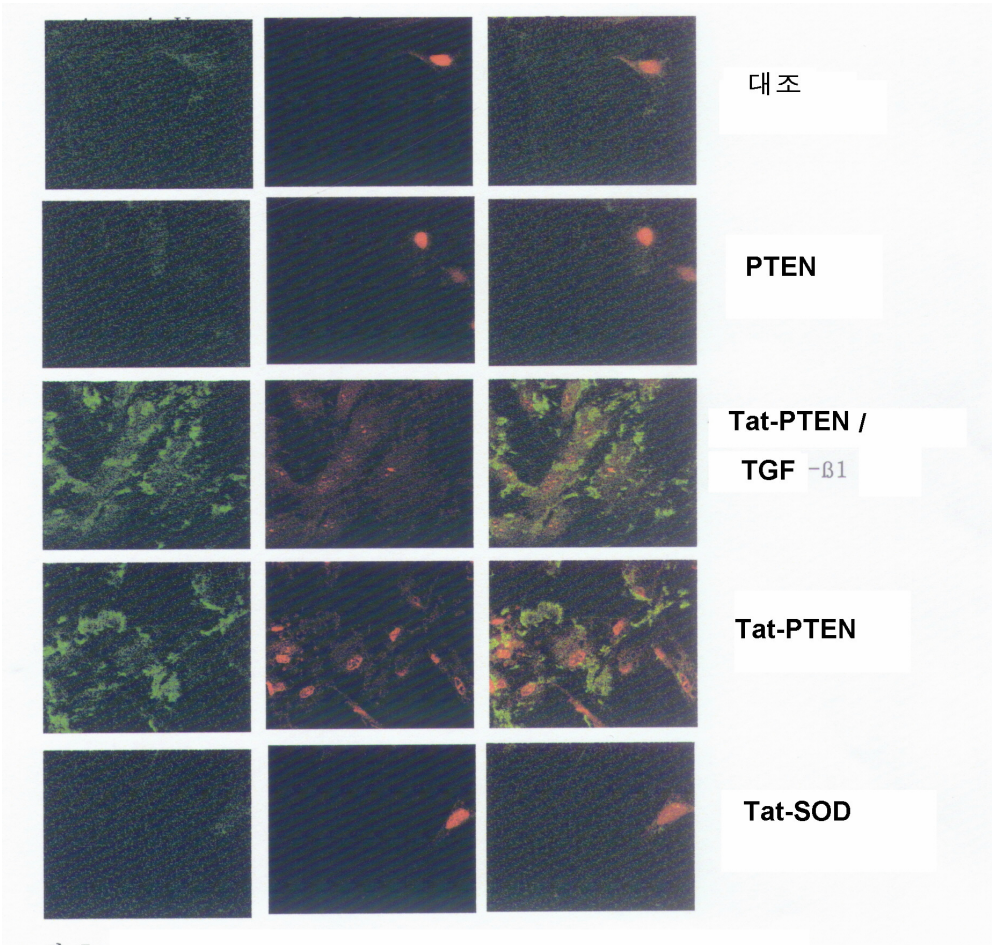




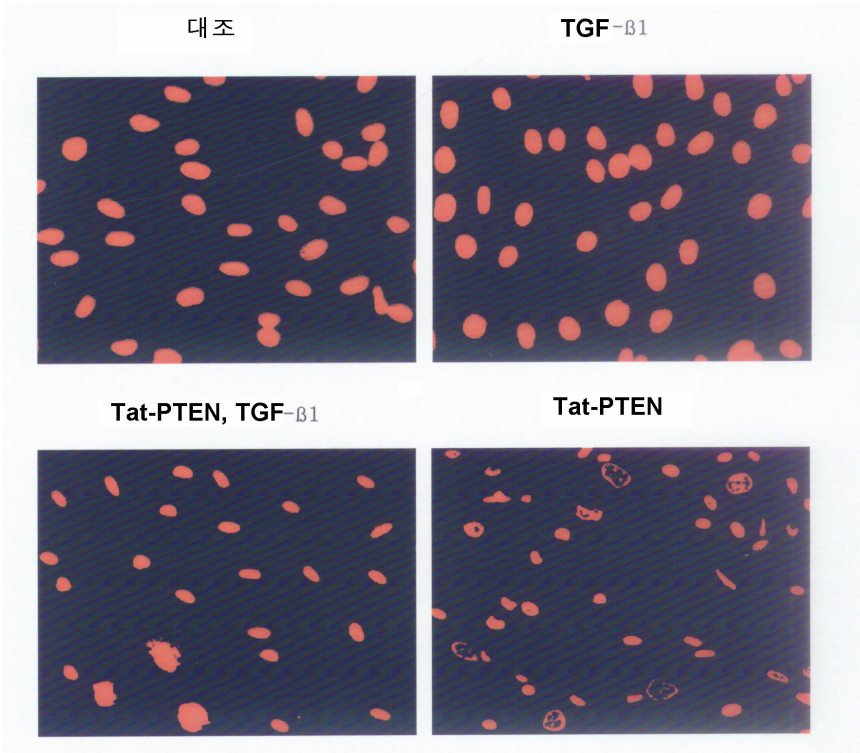
10



11



12



<110> Cowell Investment Capital
Yonsei University

<120> Tat-PTEN fusion protein, process for its preparation, and its use

<160> 14

<170> KopatentIn 1.71

<210> 1

<211> 13

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<213> Artificial Sequence

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1 5 10

<210> 2

<211> 11

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<213> Human immunodeficiency virus type 1

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1 5 10

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<211> 1212

<212> DNA

<213> Homo sapiens

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cataaaaacc attacaagat atacaatctt tgtgctgaaa gacattatga caccgcaaaa 240

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ccaggaccag aggaaacctc agaaaaagta gaaaatggaa gtctatgtga tcaagaaatc	900
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<213> Homo sapiens

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aactcagtta aaatgtctgt ttcaatgacc tgtattttgc cagacttaaa tcacagatgg	780
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29

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36

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33

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<223> Tat-PTEN fusion protein

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Met Arg Gly Ser His His His His His Gly Met Ala Ser Met Thr

1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp

20	25	30	
Arg Trp Gly Ser Gly Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg			
35	40	45	
Gly Leu Glu Met Thr Ala Ile Ile Lys Glu Ile Val Ser Arg Asn Lys			
50	55	60	
Arg Arg Tyr Gln Glu Asp Gly Phe Asp Leu Asp Leu Thr Tyr Ile Tyr			
65	70	75	80
Pro Asn Ile Ile Ala Met Gly Phe Pro Ala Glu Arg Leu Glu Gly Val			
85	90	95	
Tyr Arg Asn Asn Ile Asp Asp Val Val Arg Phe Leu Asp Ser Lys His			
100	105	110	
Lys Asn His Tyr Lys Ile Tyr Asn Leu Cys Ala Glu Arg His Tyr Asp			
115	120	125	
Thr Ala Lys Phe Asn Cys Arg Val Ala Gln Tyr Pro Phe Glu Asp His			
130	135	140	
Asn Pro Pro Gln Leu Glu Leu Ile Lys Pro Phe Cys Glu Asp Leu Asp			
145	150	155	160
Gln Trp Leu Ser Glu Asp Asp Asn His Val Ala Ala Ile His Cys Lys			
165	170	175	
Ala Gly Lys Gly Arg Thr Gly Val Met Ile Cys Ala Tyr Leu Leu His			
180	185	190	
Arg Gly Lys Phe Leu Lys Ala Gln Glu Ala Leu Asp Phe Tyr Gly Glu			
195	200	205	
Val Arg Thr Arg Asp Lys Lys Gly Val Thr Ile Pro Ser Gln Arg Arg			
210	215	220	
Tyr Val Tyr Tyr Tyr Ser Tyr Leu Leu Lys Asn His Leu Asp Tyr Arg			
225	230	235	240
Pro Val Ala Leu Leu Phe His Lys Met Met Phe Glu Thr Ile Pro Met			
245	250	255	
Phe Ser Gly Gly Thr Cys Asn Pro Gln Phe Val Val Cys Gln Leu Lys			
260	265	270	
Val Lys Ile Tyr Ser Ser Asn Ser Gly Pro Thr Arg Arg Glu Asp Lys			
275	280	285	
Phe Met Tyr Phe Glu Phe Pro Gln Pro Leu Pro Val Cys Gly Asp Ile			

290 295 300
 Lys Val Glu Phe Phe His Lys Gln Asn Lys Met Leu Lys Lys Asp Lys
 305 310 315 320
 Met Phe His Phe Trp Val Asn Thr Phe Phe Ile Pro Gly Pro Glu Glu
 325 330 335
 Thr Ser Glu Lys Val Glu Asn Gly Ser Leu Cys Asp Gln Glu Ile Asp
 340 345 350
 Ser Ile Cys Ser Ile Glu Arg Ala Asp Asn Asp Lys Glu Tyr Leu Val
 355 360 365
 Leu Thr Leu Thr Lys Asn Asp Leu Asp Lys Ala Asn Lys Asp Lys Ala
 370 375 380
 Asn Arg Tyr Phe Ser Pro Asn Phe Lys Val Lys Leu Tyr Phe Thr Lys
 385 390 395 400
 Thr Val Glu Glu Pro Ser Asn Pro Glu Ala Ser Ser Ser Thr Ser Val
 405 410 415
 Thr Pro Asp Val Ser Asp Asn Glu Pro Asp His Tyr Arg Tyr Ser Asp
 420 425 430
 Thr Thr Asp Ser Asp Pro Glu Asn Glu Pro Phe Asp Glu Asp Gln His
 435 440 445
 Thr Gln Ile Thr Lys Val
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<211> 618

<212> DNA

<213> Artificial Sequence

<220>

<223> Tat-SOD fusion protein

<400> 13

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aagaaacgta gacagcgcag acgtggtctc gagatggcga cgaaggccgt gtgcgtgctg	180
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gagtttggag ataatacagc aggctgtacc agtgcaggtc ctcaatttaa tcctctatcc	360

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<210> 14

<211> 205

<212> PRT

<213> Artificial Sequence

<220>

<223> Tat-SOD fusion protein

<400> 14

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr

1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp

20 25 30

Arg Trp Gly Ser Gly Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg

35 40 45

Gly Leu Glu Met Ala Thr Lys Ala Val Cys Val Leu Lys Gly Asp Gly

50 55 60

Pro Val Gln Gly Ile Ile Asn Phe Glu Gln Lys Glu Ser Asn Gly Pro

65 70 75 80

Val Lys Val Trp Gly Ser Ile Lys Gly Leu Thr Glu Gly Leu His Gly

85 90 95

Phe His Val His Glu Phe Gly Asp Asn Thr Ala Gly Cys Thr Ser Ala

100 105 110

Gly Pro His Phe Asn Pro Leu Ser Arg Lys His Gly Gly Pro Lys Asp

115 120 125

Glu Glu Arg His Val Gly Asp Leu Gly Asn Val Thr Ala Asp Lys Asp

130 135 140

Gly Val Ala Asp Val Ser Ile Glu Asp Ser Val Ile Ser Leu Ser Gly

145 150 155 160

Asp His Cys Ile Ile Gly Arg Thr Leu Val Val His Glu Lys Ala Asp

165 170 175

Asp Leu Gly Lys Gly Gly Asn Glu Glu Ser Thr Lys Thr Gly Asn Ala

180

185

190

Gly Ser Arg Leu Ala Cys Gly Val Ile Gly Ile Ala Gln

195

200

205