

mRNA 가 mRNA 2 가

CMAS- AS- 5' 3' mRNA AS- *c-myb, c-myc* CMAS-

k-ras mRNA AS- 가 AS- 가 AS-

RiAS- AS- mRNA RiAS- mRNA AS-

2 *c-myb, c-myc* *k-ras* (RiAS)- RiAS- mRNA AS-

AS- CMAS- RiAS-

CMAS- RiAS-

mRNA 가 mRNA 2 가

c-myb, c-myc *k-ras* (protooncogene) CMAS(covale
ntly-closed multiple antisense)- CMAS-

RiAS- *c-myb, c-myc* *k-ras* RiAS(ribbon-type antisense)-
RiAS- (cohesive end) 가

(aberrant gene expression) AS-

(antisense oligodeoxynucleotides, " " "AS-

(Tompson, C. B., et al., *Nature* , 314, 363-366, 1985). , (Chavany, C., et al., *Mol Pharm.*, 48,
738-746, 1995).

AS- (Watson-Crick base pairing) mRNA (specificity) (dupl
(10-30) AS- ()DNA-()mRNA (transl
affinity) . AS- mRNA () mRNA (translation)
ex) RNaseH mRNA가 mRNA () (Dolnick, B. J., C
(ribosomal complex) DNA (triple-helix)
ancer Inv. , 9, 185-194, 1991). , AS-

(Young, S. L., et al., *PNAS*, 88, 10023-10026, 1991).

AS-phosphorothioate, "PS" DNA가) AS- 10 B (Offensenger, W. B., et al., *EMBO J.*, 12, 1257-1262, 1993). AS- (Tomita, N., et al., *Hyper-tension*, 26, 131-136, 1995). (nude mice) A Ri_a (subunit) (Nesterova, M., et al., *Nat. Med.*, 1, 528-533, 1995). AS- (Crohn's disease) 가 AS- (Roush, W., *Science*, 276, 1192-1193, 1997).

AS- honate, "MP") AS- PS(phosphorothioate) AS- (methylphosphonate, RNaseH 가 AS- mRNA 가

mRNA mRNA 2 / 3 (Gryaznov, S., et al., *Nucleic Acids Res.*, 24, 1508-1514, 1996). mRNA (mRNA 2 가

AS- 가 () AS- 가

c-myc AS- (protooncogene) Myb 가 (cell cycle) G1/S (proliferation) (differentiation) *c-myc* (hematopoietic cell) (Melani, C., et al., *Cancer Res.*, 51, 2897-2901, 1991). (leukemic cells) *c-myc* (overexpression)

AS- *c-myc* (chronic myelogenous leukemia) K562 (promyelocytic cancer) HL-60 (Kimura, S., et al., *Cancer Res.*, 55, 1379-1384, 1995). *c-myc* AS- (phosphodiester, "PO")

AS- PS AS- (Anfossi, G., et al., *Proc. Natl. Acad. Sci. USA*, 86, 3379-3383, 1989) PO AS- 가 AS-

c-myc mRNA (malignancies) AS- (molecular therapeutics) 가 AS- *c-myc*

, *c-myc* mRNA 2 8 AS- *c-myc* (covalently-closed multiple antisense (ribbon-type antisense, "RiA S")- 가

【발명의 요약】 mRNA 가 mRNA 2 2 가 mRNA 2 *c-myc*, *c-myc* *k-ras* mRNA

가 mRNA (multiple antisense)- mRNA) CMAS-

2 *c-myc* mRNA (multiple antisense)- mRNA) CMAS-

CMAS(covalently-closed multiple antisense)-

c-myb mRNA (mRNA) RiAS- (cohesive end) AS-
 , 2
 , *c-myc* mRNA *k-ras* mRNA RiAS-
 (aberrant gene expression) AS-

1 *c-myb* (closed multiple antisense oligo, CMAS-)

2 CMAS-
 A: 5% (Metaphor) 가
 1; 2; 14
 3; 60 4; CMAS-
 B: (denaturing polyacrylamide)

1 3;
 2 4;
 3 *c-myb* (ribbon-type AS oligo, RiAS-)
 4 RiAS-

A: 15% 가
 1; 58 MIJ-78 2; 116 RIAS-
 B: MIJ-78 RiAS-
 1 3;
 2 4;
 5 (serum) CMAS- (degradation pattern)

A: AS-
 1; (), 2; 50%
 3; FBS 4; (CS)
 B: CMAS-
 1; (), 2; 50%
 3; FBS 4; (CS)
 6 RiAS- (degradation pattern)

A. MIJ-78
 1; (), 2; 50%
 3; FBS 4; (CS)
 B: RiAS-
 1; (), 2; 50%
 3; FBS 4; (CS)

7 HL-60 *c-myb* *c-myb* CMAS-
 A: (total) RNA *c-myb* RT-PCR
 1; 60 CMAS- 0.3 µg + (lipofectin) 1 µg,
 2; 60 CMAS- 1 µg + 1 µg,
 3; (scrambled) AS- 1 µg + 1 µg.
 B: RNA *c-myb* RT-PCR
 : *c-myb* mRNA RT-PCR
 : - mRNA RT-PCR

8 HL-60 *c-myb* mRNA *c-myb* RiAS-
 A: (total) RNA *c-myb* RT-PCR
 1; RiAS- 0.1 µg + (lipofectin) 0.8 µg,
 2; RiAS- 0.1 µg + 0.8 µg,
 3; SC- 0.2 µg + 0.8 µg.
 B: RNA *c-myb* RT-PCR
 : *c-myb* mRNA RT-PCR

: - mRNA RT-PCR
 9 HL-60 60 CMAS AS-
 - - : CMAS- (1), - - : CMAS- (2), - - : AS-
 - - : S-MIJ-7, :
 (1) (2) : AS-
 10 HL-60 *c-myb* RiAS-
 A: *c-myb* RiAS- MTT
 B: *c-myb* RiAS- [³H]- (thymidine)
 11 *c-myb* RiAS- HL-60
 A: *c-myb* RiAS- , B: SC- , C:
 12 *c-myb* RiAS- HT-29
 A: *c-myb* RiAS- , B: SC- , C:
 13 *c-myc* RiAS- H7-29
 A: *c-myc* RiAS- , B: SC- , C:
 14 *k-ras* RiAS- HT-29
 A: *k-ras* RiAS- , B: SC- , C:

mRNA 가 mRNA 2 2 가
 mRNA 가 mRNA 2 2 가
 mRNA ; ii) (i) 가 mRNA 2 2 가
 AS- ; iii) (ii) AS- , 가
 AS- 가 AS-
 AS- 8 8 *c-myb*
 AS- 8 8 4 (MIJ-1, MIJ-2, MIJ-3 MIJ-4)
 -4) CMAS- , 3 (MIJ-3, MIJ-4 MIJ-17) Ri
 AS- mRNA 2
 (1) 가
 (phosphodiester backbone) AS-
 . PS AS- MP AS-
 AS- 가 , 가
 oration) 가 DNA (repair) (misincorp
 가 (cationic liposome) -
 가 가 AS-
 가 가 AS-
 (covalently-closed multiple antisense, CMAS)-
 CMAS- CMAS- , AS-
 5' 3'
 AS- 4 가
 AS- 4 AS- 1, 2, 3
 multiple antisense)- (MIJ-1, MIJ-2, MIJ-3 MIJ-4) CMAS(covalently-closed
 10% (1) . CMAS- 15% PAGE
 (electrophoretic mobility pattern) (2 A) . CM
 AS- , PAGE
 - 60 , 120 180 3 - . CMAS-
 2 (2 B) .
 RiAS- (RiAS)- RiAS-
 AS- RiAS- AS-

가 , AS- 2 AS- 가

CMAS- (ligation) (cohesive end) 가

AS- RiAS- (3

RiAS- 3, 4 5 3 (MIJ-3, MIJ-4 MIJ- 17) 가 RiAS- RiAS- 가 2 (6 mRNA P AGE (MIJ-78) 2 RiAS- (4 A). RiAS PAGE 116 (major band) RiAS- (4 B). 가 , CMAS- RiAS- 가 , CMAS- 60 (CMAS- , 5 A) 58 (RiAS- , 6 RiAS- 24 (calf serum) 24 , FBS CMAS- RiAS- 가 가 (5 B 6 B). , CMAS- 가 mRNA (5 , CMAS- (Lipofectin) . MIJ-5, *c-myb* CMAS- MIJ-5 가 mRNA SC- mRNA RiAS- CMAS- RiAS- SC- (scrambled- RiAS-) . Ri A가 (8). RiAS- 30% *c-myb* mRNA mRNA PCR CMAS- RiAS- 90% (7 B). CMAS- RT-PCR *c-myb* MIJ-5 가 *c-myb* 가 CMAS- 가 *c-myb* (8 B). (aberrant gene expression) AS- (CMAS- RiAS-), *c-myb* CMAS- *c-myb* RiAS- 가 MTT , [³H] , *c-myb* CMAS- (incorporation) (soft) 가 3가 가 가 MTT , MIJ-5 *c-myb* CMAS- 가 가 가 (9). , 60 AS- MIJ-5A HL-60 80% 가 (sha m control) 가 *c-myb* CMAS- 가 , RiAS- 91% (9 A). , SC- *c-myb* RiAS- 가 , MIJ-5 가 90% (2). MIJ -5A 11% 32% 70% 가 , MIJ-5 가 , SC-

c-myb RiAS- 92%
 (3). , SC- 7.9% 7.1%
 , *c-myb* RiAS- [³ H] , RiAS-
 HL-60 93% , SC- 16.8%
 15.4% (10 B). , *c-myb* RiAS- HL-6
 0 SC- (11). (proto
 oncogene) *c-myc* *k-ras* RiAS- , *c-myc* RiAS- *k-ras* RiAS- 가
 , HT-29 SC- RiAS-
 , *c-myc* RiAS- , *c-myc* RiAS- *k-ras* RiAS- (12, 13
 14).
 , RiAS- (nuclease) 가 , RiAS-

< 1 > AS- mRNA
 AS- (arbitrary)
c-myb mRNA
 DNAsis (Hitach Software,) *c-myb*
 100 가
 30 5' 60 가
 3가 가 2
 , 3가 2 가 8 *c-myb* mRNA (

[1]

c-myb mRNA 서열로부터 선별된 앤티센스 올리고에 대한 8개의 목표 서열

이름	상보적 위치	타입	사이즈(mer)	서열
MIJ-1	253-267	앤티센스	15	서열번호 1
MIJ-2	401-415	앤티센스	15	서열번호 2
MIJ-3	613-627	앤티센스	15	서열번호 3
MIJ-4	1545-1559	앤티센스	15	서열번호 4
MIJ-6	253-267	앤티센스	15	서열번호 9
MIJ-16	585-602	앤티센스	18	서열번호 10
MIJ-17	961-978	앤티센스	18	서열번호 5
MIJ-19	97-114	센스	8	서열번호 11

1 8 (antisense) 2
 , 8 (MIJ-1, MIJ-2, MIJ-3 MIJ-4)가
 CMAS- , 3 (MIJ-3, MIJ-4 MIJ-17)가 RiAS-
 < 2 > CMAS(covalently-closed multiple antisense)-
 AS- 1 4 AS-
 ((MIJ-1, MIJ-2, MIJ-3 MIJ-4) CMAS-
 , AS- 4 CMAS- (1)
 . 4 60 AS- 6
 AS- 60 AS- 7
 (ligation) AS- 85 2 가
 . T4 1 가 16 16 -
 . CMAS- 5% 가 (Metaphor agarose, FMC, USA) 12% PA
 GE , 60 (Exonuclease III)

가 , AS- 2 CMAS- III AS- 90
 (duplex formation) mRNA
 nse) . CMAS- 15% PAGE AS- CMAS(covalently - closed multiple antise
 (2 A) (60), 2 (120) 3 (180) (2
 PAGE B). CMAS- AS- AS- RiAS-
 < 3 > AS(RiAS)- AS- RiAS-
 , RiAS- 3, 4 5 3 (loop) (stem)
 mRNA 가 mRNA 2 (MIJ-3, MIJ-4 MIJ-17)
 가 3 7 MIJ-78 5' (MIJ-78) 5' 2
 . 58 MIJ-78 MIJ-78 MIJ-78 AS- MIJ-78
 5'(p) GATC-3' 2 MIJ78 5' (cohesive) 85
 MIJ-78 (ligation template) MIJ-78 가 16 24
 2 가 T4 DNA 1 가 3) RiAS- 15%
 (diad)- (3). RiAS- RiAS- (116)
 3 RiAS- 가 2 (6)가 RiAS- RiAS-
 3 mRNA RiAS- 가 (duplex) RiAS- (tors
 ional stress) RiAS- PAGE (MIJ-78)
 (4 A). RiAS- (4 B). RiAS- , PAGE
 (116)가 (4 B). RiAS- , MIJ-78(AS-)
 < 2 > CMAS- RiAS- CMAS- RiAS-
 < 4 > CMAS- RiAS- , CMAS-
 RiAS- (phosphodiester) (60) CMAS- 1 µg 가
 , FBS (가 ; HyClone, Logan, Utah, USA)
 가 . 100 µl 15% AS- 가 37 24
 . AS- 15% PAGE 가 37 24 . 160 U/µg
 (Takara, Japan) CMAS- 가 37 24 . RiAS-
 CMAS- 60 가 , FBS 24 ()
 5 A). , CMAS- 가 , FBS 24
 RiAS- 58 가 24 (5 B).
 (6 A). , RiAS- 가 24
 < 5 > CMAS- RiAS- *c-myb* mRNA AS- 가
 CMAS- RiAS- mRNA
 < 5-1 > HL-60(promyelocytic leukemia cell line) K562 (chronic myelogenous leukemia cell line)
 RPMI 1640(Gibco BRL, USA) 10
 % FBS(Gibco BRL, USA) 1% / (Gibco BRL, USA) 가 37 , 5% CO₂
 (stock)
 5 AS-
 < 5-2 > CMAS- RiAS-
 0.3 µg CMAS- 0.8 µg (Gibco BRL, USA) 0.2 µg RiAS- 0.8 µg
 20 µl OPTI-MEM 40 (RPMI 1640 + 10% FBS)가
 가 AS- 가 (RPMI 1640 + 10% FBS)가
 OPTI-MEM 5X10⁵ /ml 48-

(Falcon, USA) 100 $\mu\ell$ 40 $\mu\ell$ - 0 1
 2 가 AS- 37 , 5% CO₂ 4 10% FBS가
 OPTI-MEM 100 $\mu\ell$ 가 100 $\mu\ell$ 가
 OPTI-MEM 20 $\mu\ell$ 4 가 100 $\mu\ell$ 가 가
 37

<5-3> RNA RT-PCR
 Tripure™ (Boehringer Manhein, Germany) RNA
 RNA Access™ RT-PCR (promega, USA) 가 10 μg 80 $\mu\ell$ 가
 RT-PCR AMV (5 U/ $\mu\ell$), Tf1 DNA (5 U/ $\mu\ell$), dNTP(10 mM, 1 $\mu\ell$) MgSO₄ (25
 mM, 2.5 $\mu\ell$) 가 cDNA 48 45 DNA (termal cycler, Hyb
 aid, USA) PCR 25 PCR
 1% 가 (gel documentation program, Bio-Rad, USA)

<5-4> RT-PCR
 RT-PCR 1% 가 DNA (New England Biolab, USA) 0.4 M Na
 OH 4 ECL 3' 30 (Amersham Lif
 e Science, England) 30 9 5X SSC,
 0.02% SDS가 6 M 62 , 60 1% SDS가 5X SSC
 0.1% SDS가 1X SSC 15
 HRP(horseradish peroxidase) - 30
 CMAS- mRNA
 , CMAS-
 , 0.3 μg MIJ-5 , *c-myb* CMAS- HL-60
 1 μg MIJ-5 MIJ-5 SC- *c-myb* mRNA 37%
 RNA 95% (7 A). CMAS- 가 MIJ-5A *c-myb* mRNA mRNA

RiAS- mRNA
 HL-60 , RiAS- SC- RiAS-
 μg 0.2 μg) 0.8 μg (8 A). , SC- RiAS- (0.1
 A mRNA (40 nM) 0.2 μg *c-myb* mRN
 mRNA RiAS- 30% *c-myb*
 , HL-60 CMAS- RiAS- 가 mRNA
 , 90% 가 MIJ-5 RT-PCR *c-myb* (message) DNA
 panel) (internal) (30) MIJ-5 RT-PCR *c-myb* (7 B). (bottom
 , 0.2 μg *c-myb* RiAS- (8 B).

< 6 > *c-myb* CMAS- *c-myb* RiAS-
c-myb 가 *c-myb* AS- *c-myb* CMA
 S- *c-myb* RiAS- 가
 (incorporation) *c-myb* CMAS- *c-myb* RiAS- MTT , [³ H]
 가 (soft agarose) 가

<6-1> MTT
 MTT(3, -4[4,5-Dimethylthiazol-2-yl]2,5-diphenyl-tetrazolium bromide, "MTT")
 HL-60 OPTI-MEM , 96- 50 $\mu\ell$ (CMA
 S- 0.01 - 1 $\mu\ell$ /15 $\mu\ell$, RiAS- 0.2 $\mu\ell$ /15 $\mu\ell$) (0.2 μg /15 $\mu\ell$)
 5 100 $\mu\ell$, PBS
 MTT (5 mg/M ℓ in PBS; Sigma, USA) 20 $\mu\ell$ (100 μg) 가 37 4 . 0.1 N
 HCl 100 $\mu\ell$ 가 (ambient) 1
 , CMAS- ELISA 570 nm 가 M
 IJ-5 가 . 0.13 μg (0.24 μg)

CMAS-AS- , MIJ-5A 80% HL-60 가 (9). , 60
c-myb CMAS-
 RiAS- , RiAS- 91% (10 A).
 , SC- , *c-myb* RiAS-
<6-2> 가
c-myb CMAS- *c-myb* RiAS-
 가 6 K562
 37 , 5% CO₂ 24 . 0.8% 가 20% FBS
 2x RPMI-1640 가 6-
 . 6- 4 5 15 . 20
 , CMAS- MIJ-5 90% (2). MIJ-5A 70%
 , SC- 11% 32%

[2]

K562 세포의 콜로니 형성에 대한 *c-myb* 올리고의 효과

올리고			콜로니의 수	형성된 콜로니의 %
구조	크기(mer)	유형(type)		
선형	15	AS-MIJ-1	55	44.4
선형	15	S-MIJ-3	110	88.7
선형	15	SC-MIJ-1	84	67.7
선형	60	AS-MIJ-5A	29	23.4
CMAS	60	AS-MIJ-5	9	7.2
리포펙틴 단독			109	88.0
처리되지 않은 대조군			124	100.0

, *c-myb* RiAS- 92%
 (3). , SC- 7.9% 7.1%

[3]

K562 세포의 콜로니 형성에 대한 *c-myb* 올리고의 효과

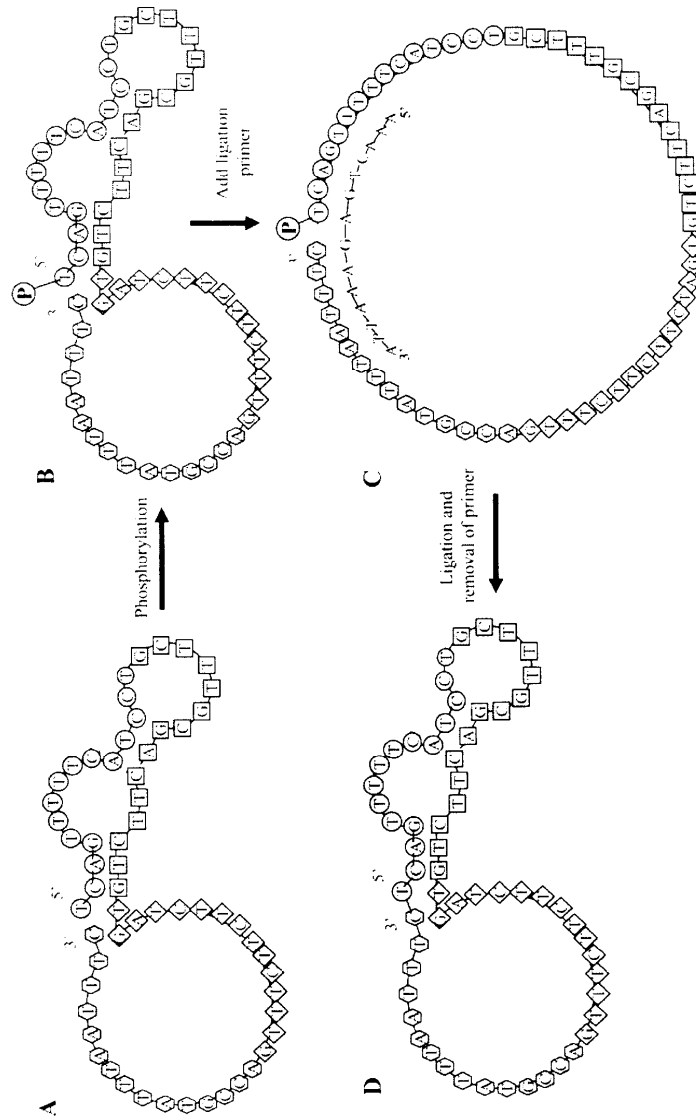
올리고	크기(mer)	콜로니의 수	형성된 콜로니의 %
RiAS-올리고	116	7.6 - 1.53	7.8
혼합 올리고	116	0.5 - 2.12	92.1
리포펙틴 단독		91.3 - 4.16	92.9
처리되지 않은 대조군		98.3 - 4.04	100.0

<6-3> [³H]

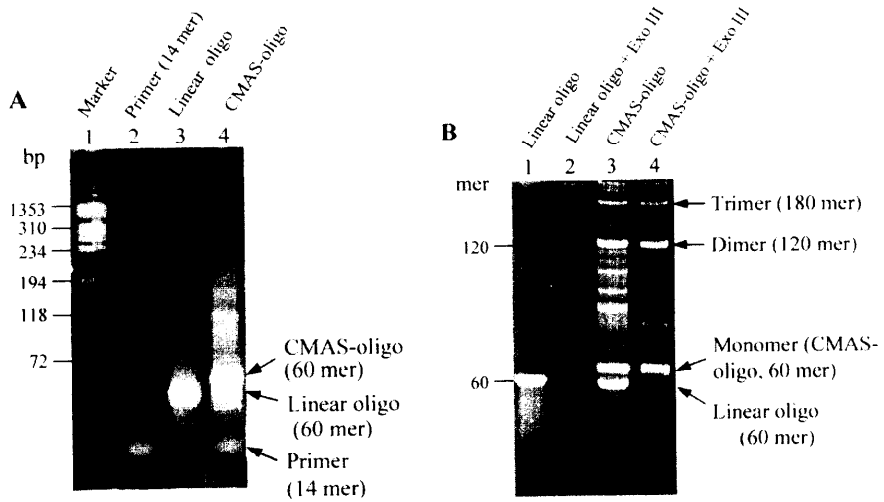
c-myb RiAS- [³H]
 . [³H] , HL-60 AS- 0
 .5 μCi [³H] (2.0 Ci/mmol; Amersham, England) 가 16 3 (triplicate)
 , (glass microfiber) , PBS, 5% TCA (absolute
) . [³H] , X-100, PPO POPOP
 (liquid scintillation counter)
 , RiAS- (0.2 μg) HL-60 93% (10 B). , SC-
 16.8% 15.4% 가 (mild) , *c-m*
yb RiAS- (scrambled) HL-6
 0 (11).
< 8 > *c-myc* RiAS- *k-ras* RiAS-
c-myb RiAS- (proton
 cogene) *c-myc* *k-ras* RiAS- 3 , *c-my*
c RiAS- *k-ras* RiAS- 가 ,

- 4 , T4 (T4 ligase) .
- 7.
- 4 CMAS- .
8. *c-myb* () 가 *c-myb* mRNA 2 1, 2, 3 CMAS- 4 , 4
9. 8 , 6 , 4 CMAS
10. 1 , (3) 가 AS- AS- 가 AS- (2) AS- 가 AS- , AS- 2 (ribbon-type AS- ; RiAS-) , AS-
- 11.
- 10 T4 .
12. 10 가 RiAS- .
13. *c-myb* () 가 *c-myb* mRNA 2 3, 4 () 5 RiAS- .
14. AS- 2 , 10 RiAS- . AS- 13 , 7 AS- 2 , 10 RiAS- .
15. 13 14 , AS- 5'-(p)GA TC-3' RiAS- .
16. 4 CMAS- 10 RiAS- AS- (liposome) .
17. 16 (cationic) AS- .
18. 4 CMAS- 10 RiAS- , , , .
- 19.
- 20.
- 21.
- 22.
- 23.

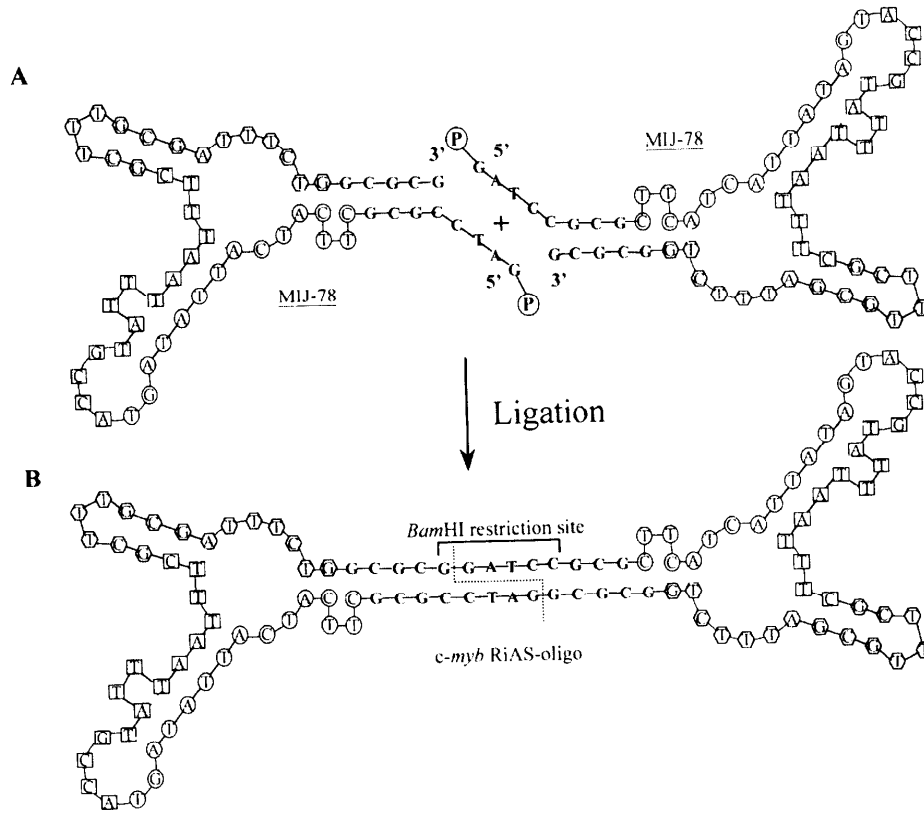
1



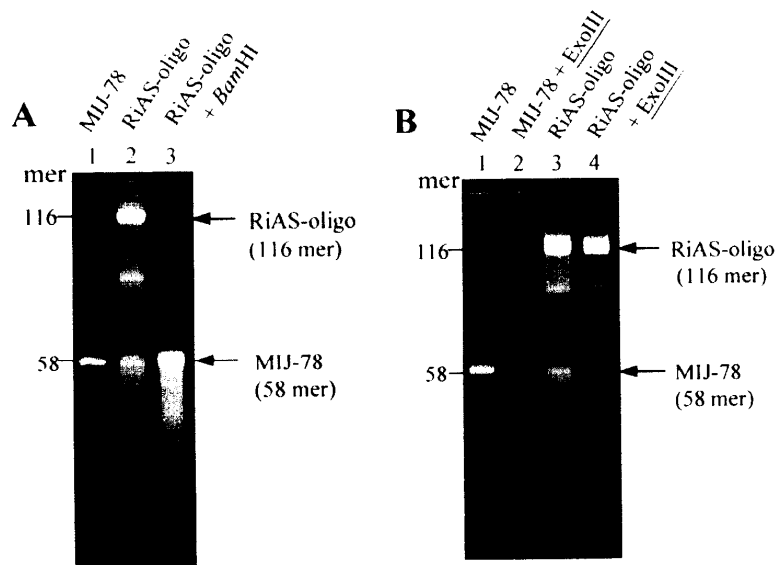
2

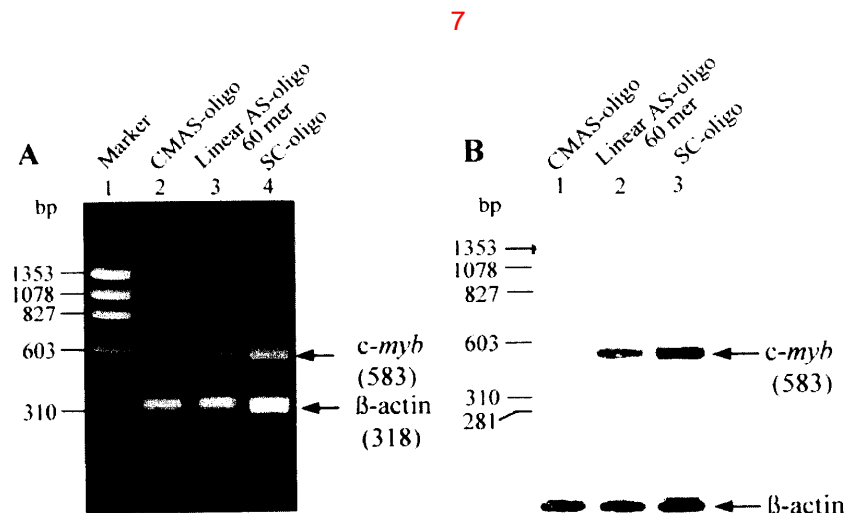
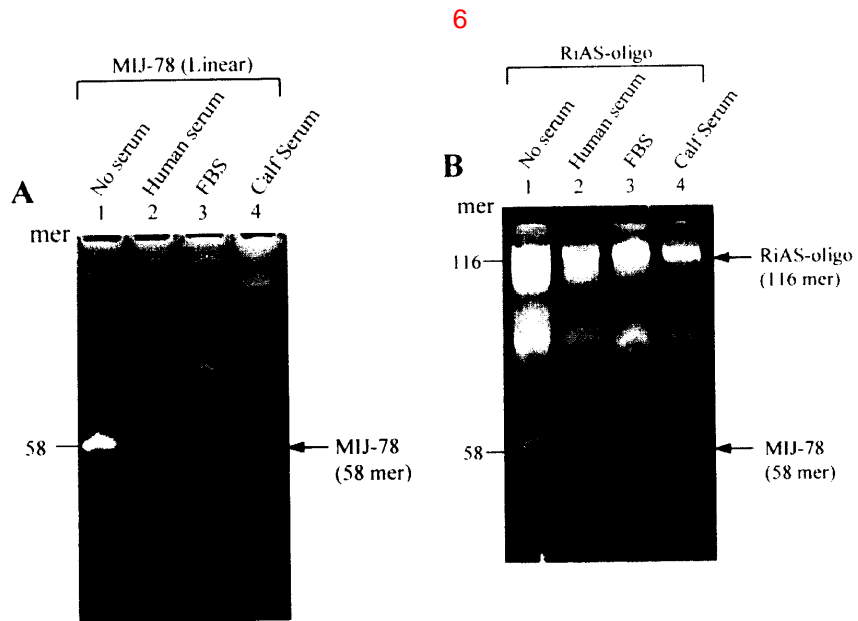
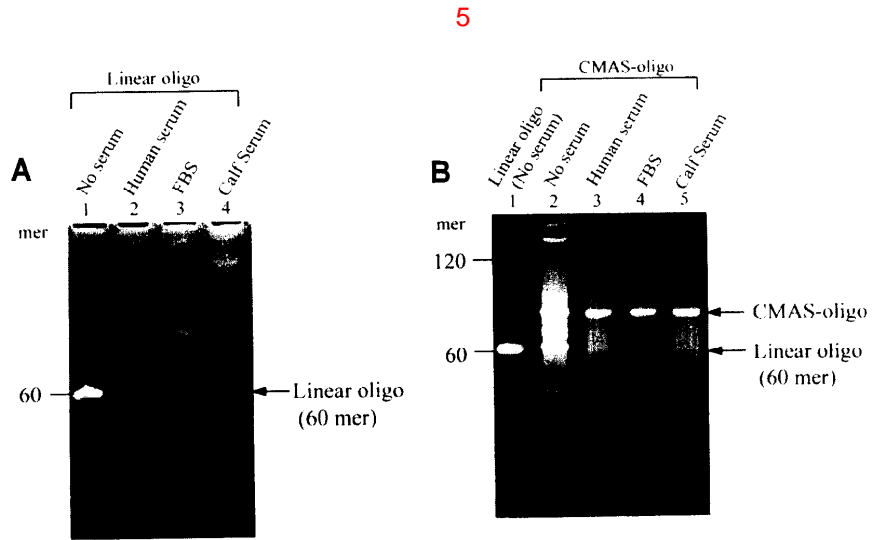


3

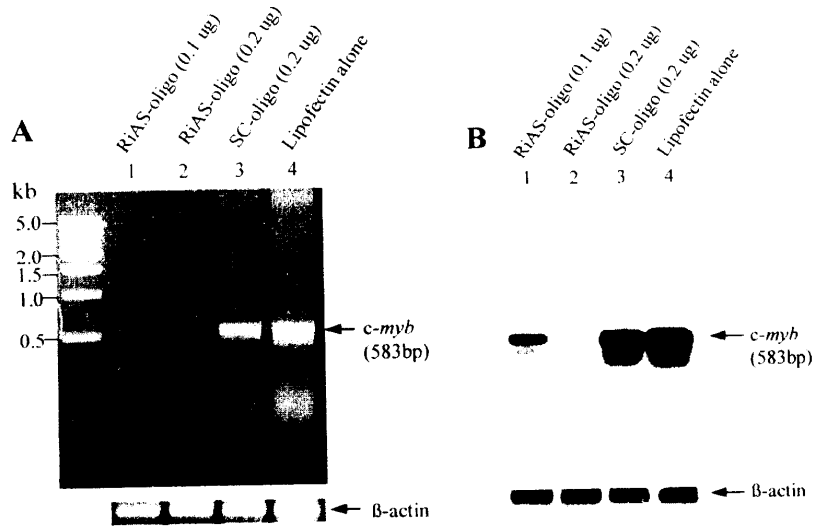


4

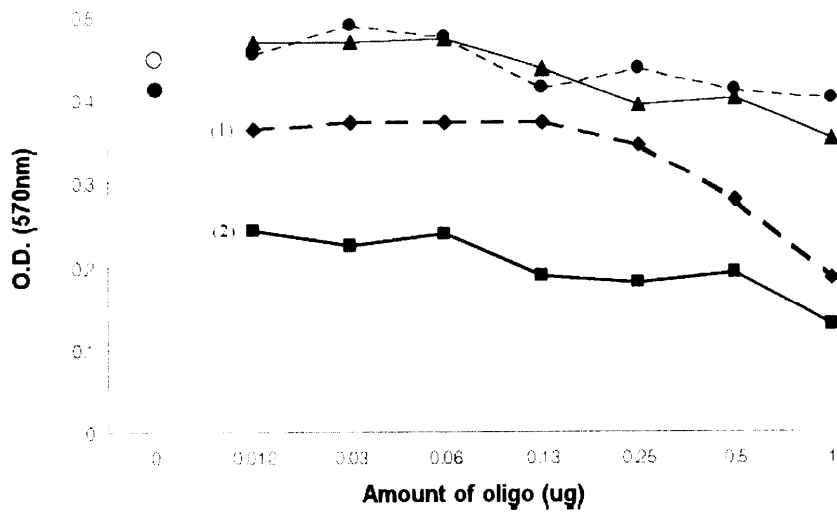




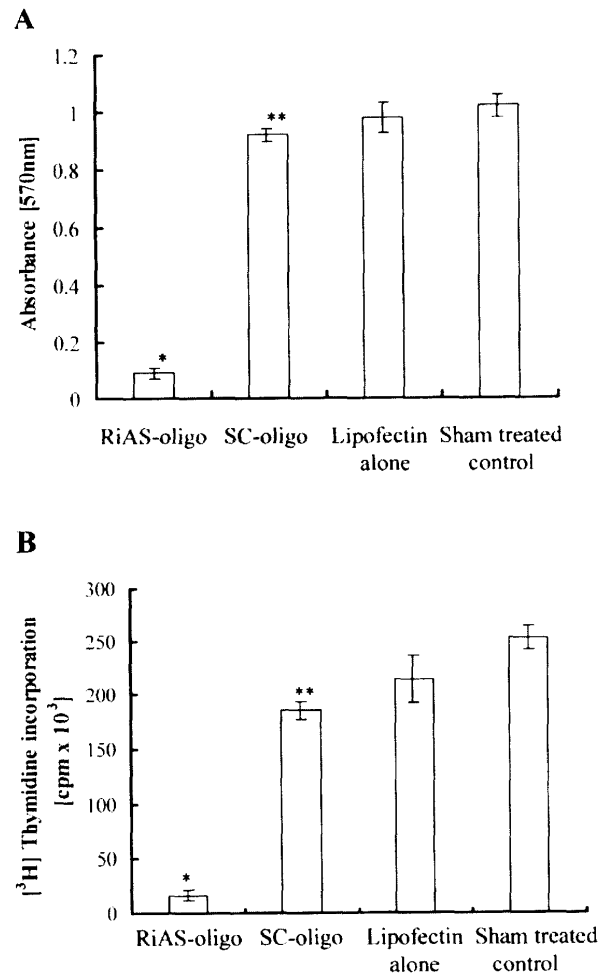
8



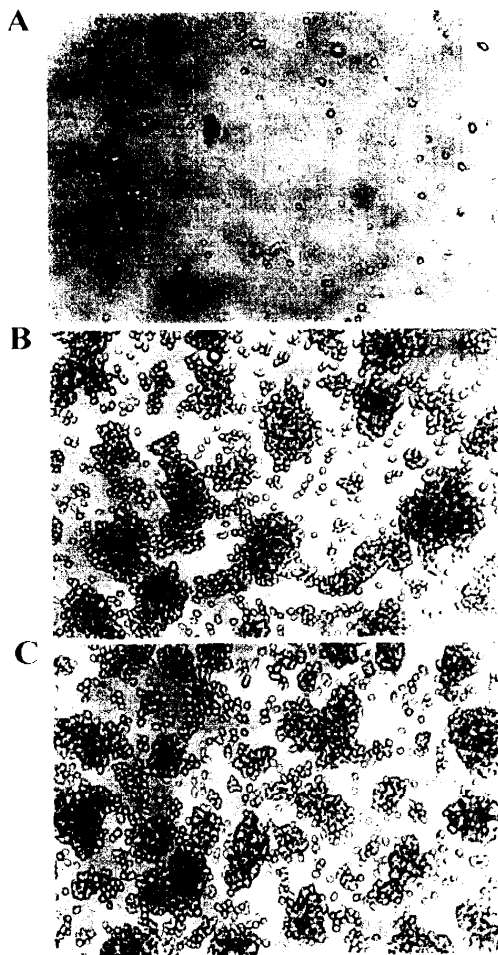
9



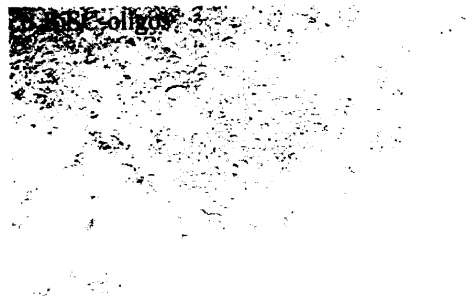
10



11



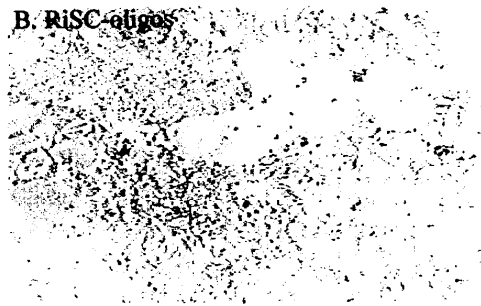
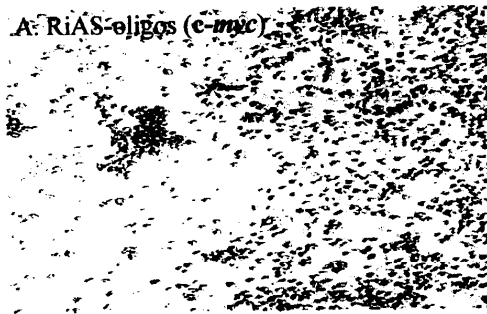
12



C. Cationic Liposome alone



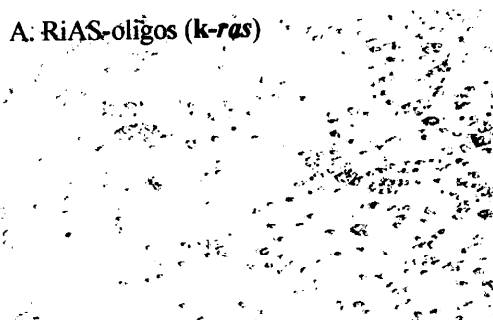
13



C. Cationic Liposome alone



A. RiAS-oligos (*k-ras*)



B. RiSC-oligos (Scrambled)



C. Cationic Liposome alone



<110> PARK, Jong-Gu <120> The novel antisense oligos with better stability and antisense
 effect <130> 0fpo-03-29 <150> KR 99-122917 <151> 1999-04-08 <160> 11 <170> KopatentIn 1.7
 1 <210> 1 <211> 15 <212> DNA <213> Homo sapiens <400> 1 tcagtttttc atcct
 15 <210> 2 <211> 15 <212> DNA <213> Homo sapiens <4
 00> 2 tgatcttctt ctttg 15 <210> 3 <211>
 15 <212> DNA <213> Homo sapiens <400> 3 gctttgcgat ttctg 15 <210> 3 <211>
 15 <210> 4 <211> 15 <212> DNA <213> Homo sapiens <400> 4 accgtattta
 atttc 15 <210> 5 <211> 18 <212> DNA <2
 13> Homo sapiens <400> 5 ggtcttcac tc attatagt
 18 <210> 6 <211> 60 <212> DNA <213> Artificial Sequence <220> <223> antisense oligonucle
 otides <400> 6 tcagtttttc atcctgcttt gcgacttctg tgatcttctt ctttgaccgt atttaatttc 60
 60 <210> 7 <211> 58 <212> DNA
 <213> Artificial Sequence <220> <223> antisense oligonucleotides <400> 7 gatccgcgct tcacattat
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