חוק הפטנטים, התשכ"ז - 1967
PATENTS LAW, 1967 - 5727

217176

בקשה בינלאומית לפטנט - שלב לאומי

INTERNATIONAL PATENT APPLICATION - NATIONAL PHASE

29-12-2011

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NAMES OF INVENTORS: שמות הממציאים:

בעל אמצאה מכח העברה ששמה הוא By Assignment
Owner, by virtue of of an invention, the title of which is:

(בעברית) (Hebrew): סמן גידול חדש

(באנגלית) (English): NEW TUMOR MARKER

*בקשה חלוקה - Application for Division	*בקשת פטנט מוסף - Application for Patent of Addition	*דרישה דין קדימה Priority Claim		
מבקשת פטנט from Application	*לבקשה/לפטנט to Patent/Appl.	מספר.סימן Number/Mark	תאריך Date	מדינת האיגוד Convention Country
No._____.מס	No._____.מס	200910158747.9	July 7, 2009	CHINA
dated_____יום	dated_____מיום			
*יפוי כח: כללי/מיוחד - רצוף בזה / עוד יוגש P.O.A.: general / specific - attached / to be filed later - Has been filed in case_____הוגש בענין				
המען למסירת הודעות ומסמכים בישראל Address for Service in Israel סנפורד ט. קולב ושות' ת.ד 2273 רחובות 76122				
תתימת המבקש Signature of Applicant בשם המבקש סנפורד ט. קולב ושות' DATE: December 29, 2011 תאריך:		INT. APP. PCT/CN2010/075896 INT. FIL. DATE August 11, 2010 INT. PUBL. WO 2011/003369 A1		מס' בקשה בינ"ל תאריך הגשה בינ"ל מס' פרסום בינ"ל

YOUR REFERENCE:75267 סימוכין שלך:

* מחק את המיותר Delete whatever is inapplicable

משרד המשפטים

מסמך זה הינו העתק שנסרק בשלמותו ביום ובשעה המצוינים,
בסריקה ממוחשבת מהימנה מהמסמך המצוי בתיק,
בהתאם לנוהל הבדיקות במשרד המשפטים.

על החתום

משרד המשפטים (חתימה מוסדית).

We claim:

1. An isolated polypeptide comprising or consisting of the amino acid sequence of SEQ ID No.1.

2. The polypeptide according to claim 1, wherein one or more amino acid residues in the amino acid sequence of SEQ ID No.1 selected from the group consisting of Thr90, Ser231, Ser263, Tyr309 and a combination thereof are phosphorylated.

3. The polypeptide according to claim 2, wherein the Thr90 is phosphorylated.

4. Use of an agent capable of specifically binding to the polypeptide of anyone of claims 1-3 in manufacture of a kit for determining the presence, stage and/or metastasis of a tumor by detecting the level of a polypeptide comprising or consisting of the amino acid sequence of SEQ ID No.1 in a plasma sample.

5. Use of an agent capable of specifically binding to the polypeptide of anyone of claims 1-3 in manufacture of a kit for screening the presence of a tumor in a high-risk population by detecting the level of a polypeptide comprising or consisting of the amino acid sequence of SEQ ID No.1 in a plasma sample.

6. Use of an agent capable of specifically binding to the polypeptide of anyone of claims 1-3 in manufacture of a kit for determining the prognosis of a patient having a tumor by detecting the level of a polypeptide comprising or consisting of the amino acid sequence of SEQ ID No.1 in a plasma sample.

7. Use of an agent capable of specifically binding to the polypeptide of anyone of claims 1-3 in manufacture of a kit for determining the efficiency of a surgery, radiotherapy or chemotherapy treatment to a patient having a tumor and/or for determining when the treatment should be ceased by detecting the level of a polypeptide comprising or consisting of the amino acid sequence of SEQ ID No.1 in a plasma sample.

8. The use according to anyone of claims 4-7, wherein the tumor is selected from the group consisting of lung cancer, liver cancer, gastric cancer, esophagus cancer, osteosarcoma, pancreatic cancer, lymphoma, colorectal cancer, breast cancer, prostate cancer, ovarian cancer, oral cancer, nasopharyngeal carcinoma, cervical carcinoma, leukemia, malignant melanoma, sarcoma, renal carcinoma, and cholangiocarcinoma.

9. The use according to anyone of claims 4-8, wherein the agent is an antibody specific for

the polypeptide.

10. The use according to claim 9, wherein the antibody is a monoclonal antibody or an antigen binding fragment thereof.

11. The use according to claim 10, wherein the antigen-binding fragment is selected from the group consisting of scFv Fab Fab' and F(ab')2.

12. The use according to claim 10, wherein the antibody is MAb E9 or D10 produced by the cell line deposited under CGMCC No. 2903 or 2904, respectively.

13. The use according to claim 9, wherein the antibody specifically binds to the polypeptide present in plasma.

14. The use according to claim 9, wherein the antibody specifically binds to a phosphorylated form of the polypeptide, said phosphorylated form of the polypeptide contains one or more phosphorylated amino acid residues in the amino acid sequence corresponding to SEQ ID No.1 selected from the group consisting of Thr90, Ser231, Ser263, Tyr309 and a combination thereof.

15. The use according to claim 14, wherein the antibody specifically binds to the polypeptide which is phosphorylated at Thr90.

16. Use of an inhibitor of the polypeptide of anyone of claims 1-3 in manufacture of a pharmaceutical composition for preventing or treating cancer metastasis.

17. The use according to claim 16, wherein the inhibitor is an antibody specific for a polypeptide comprising or consisting of the amino acid sequence of SEQ ID No.1.

18. The use according to claim 17, wherein the antibody is a humanized antibody or an antigen binding fragment thereof.

19. The use according to claim 17, wherein the antibody specifically binds to a phosphorylated form of the polypeptide, said phosphorylated form of the polypeptide contains one or more phosphorylated amino acid residues in the amino acid sequence of SEQ ID No.1 selected from the group consisting of Thr90, Ser231, Ser263, Tyr309 and a combination thereof.

20. The use according to claim 19, wherein the antibody specifically binds to the polypeptide which is phosphorylated at Thr90.

21. The use according to claim 16, wherein the antibody is MAb E9 or D10 produced by the cell line deposited under CGMCC No. 2903 or 2904, respectively.

22. The use according to anyone of claims 16-23, wherein the cancer is selected from the group consisting of lung cancer, liver cancer, gastric cancer, esophagus cancer, osteosarcoma, pancreatic cancer, lymphoma, colorectal cancer, breast cancer, prostate cancer, ovarian cancer, oral cancer, nasopharyngeal carcinoma, cervical carcinoma, leukemia, malignant melanoma, sarcoma, renal carcinoma, and cholangiocarcinoma.

23. A method of inhibiting cancer invasiveness and metastasis, comprising the step of inhibiting the phosphorylation of intracellular Hsp90α in tumor cells.

24. The method according to claim 23, comprising the step of inhibiting the phosphorylation of Hsp90α at Thr90 in tumor cells.

25. The method according to claim 23 or 24, comprising the step of overexpressing a nucleic acid molecule encoding protein phosphatase 5 in tumor cells.

26. The method according to claim 25, wherein the overexpression of the nucleic acid molecule is achieved by gene introduction.

27. The method according to claim 25, wherein the protein phosphatase 5 comprising the amino acid sequence of SEQ ID No.5.

For the Applicant,

Sanford T. Colb & Co.
C: 75267

משרד המשפטים

מסמך זה הינו העתק שנסרק בשלמותו ביום ובשעה המצוינים,
בסריקה ממוחשבת מהימנה מהמסמך המצוי בתיק,
בהתאם לנוהל הבדיקות במשרד המשפטים.

על החתום

משרד המשפטים (חתימה מוסדית).

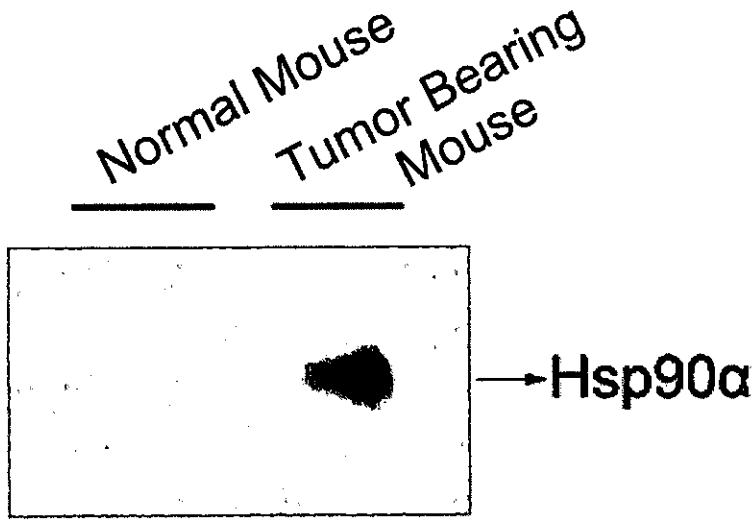

Normal Mouse
Tumor Bearing Mouse
Hsp90α

Fig.1

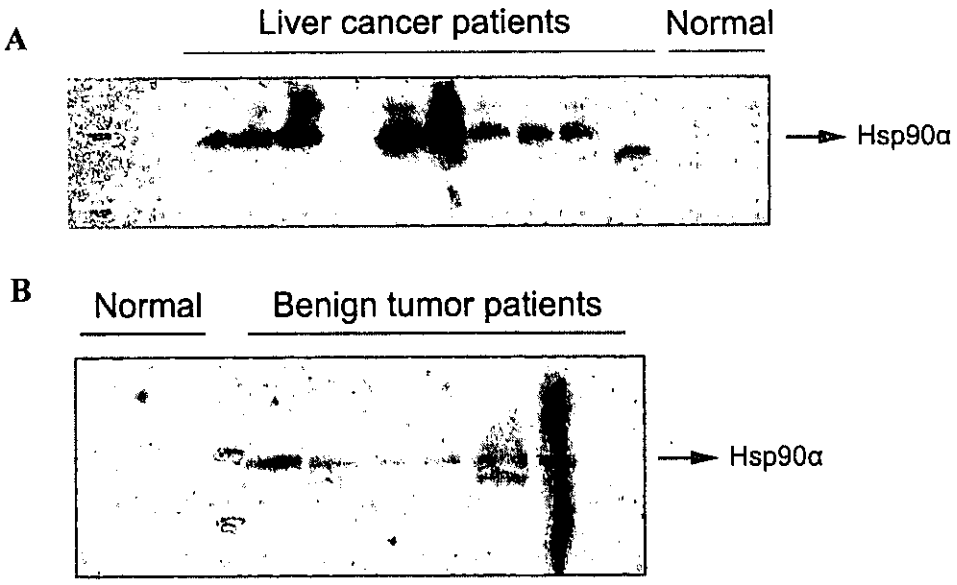

A
Liver cancer patients
Normal
Hsp90α
B
Normal
Benign tumor patients
Hsp90α

Fig.2

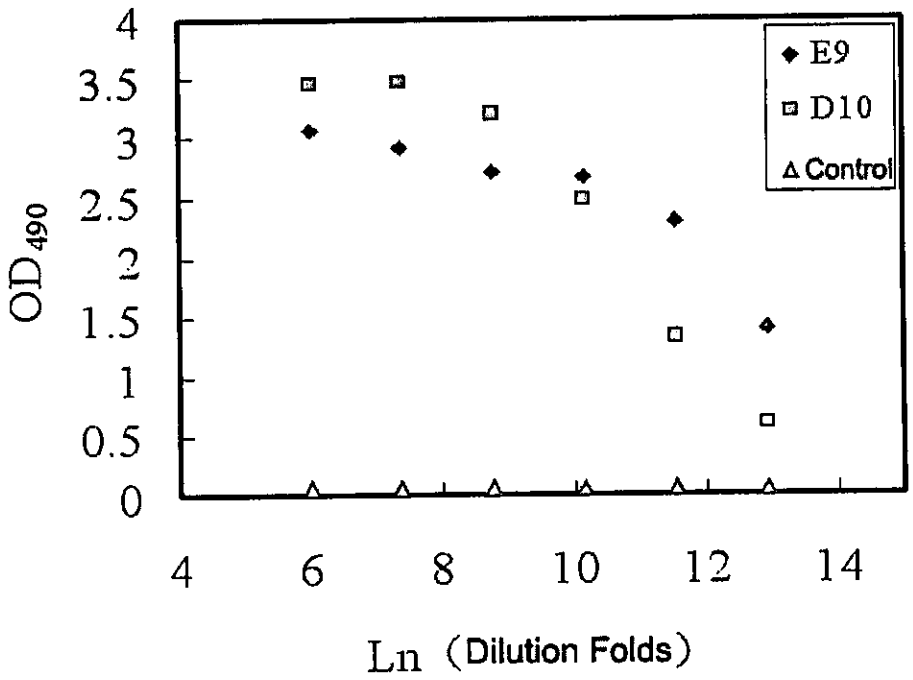

4
3.5
3
2.5
2
1.5
1
0.5
0
OD490
E9
D10
Control
4
6
8
10
12
14
Ln (Dilution Folds)

Fig.3

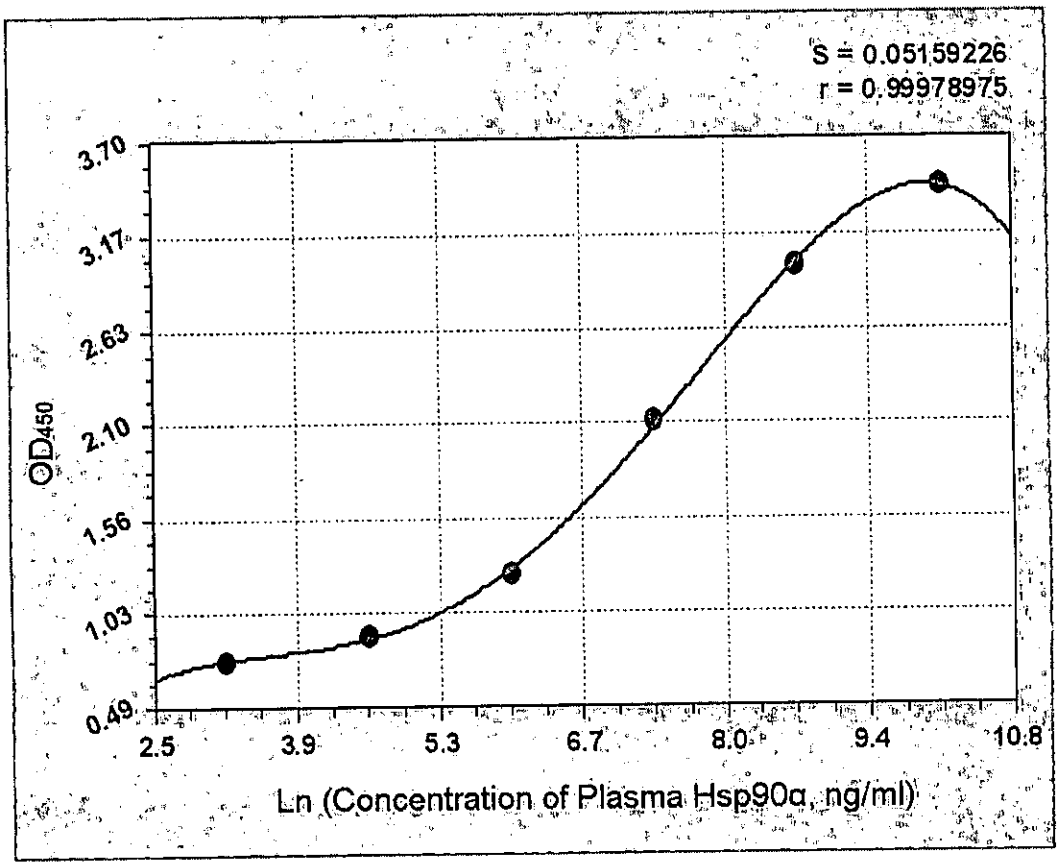

S = 0.05159226
r = 0.99978975
3.70
3.17
2.63
2.10
1.56
1.03
0.49
OD450
2.5
3.9
5.3
6.7
8.0
9.4
10.8
Ln (Concentration of Plasma Hsp90α, ng/ml)

Fig.4

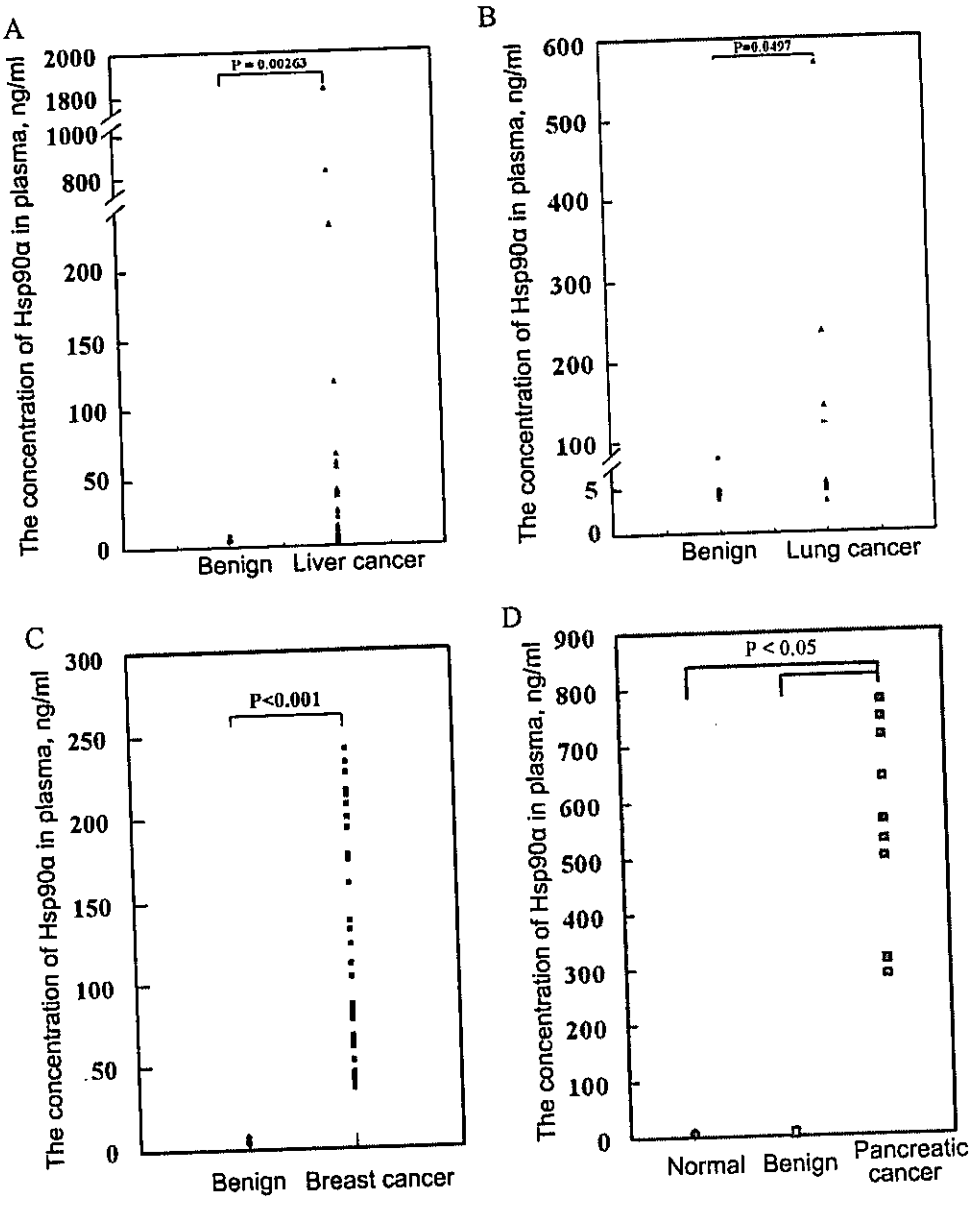

A
The concentration of Hsp90α in plasma, ng/ml
2000
1800
1000
800
200
150
100
50
0
P = 0.00263
Benign
Liver cancer
B
The concentration of Hsp90α in plasma, ng/ml
600
500
400
300
200
100
5
0
P=0.0497
Benign
Lung cancer
C
The concentration of Hsp90α in plasma, ng/ml
300
250
200
150
100
50
0
P<0.001
Benign
Breast cancer
D
The concentration of Hsp90α in plasma, ng/ml
900
800
700
600
500
400
300
200
100
0
P < 0.05
Normal
Benign
Pancreatic cancer

Fig.5

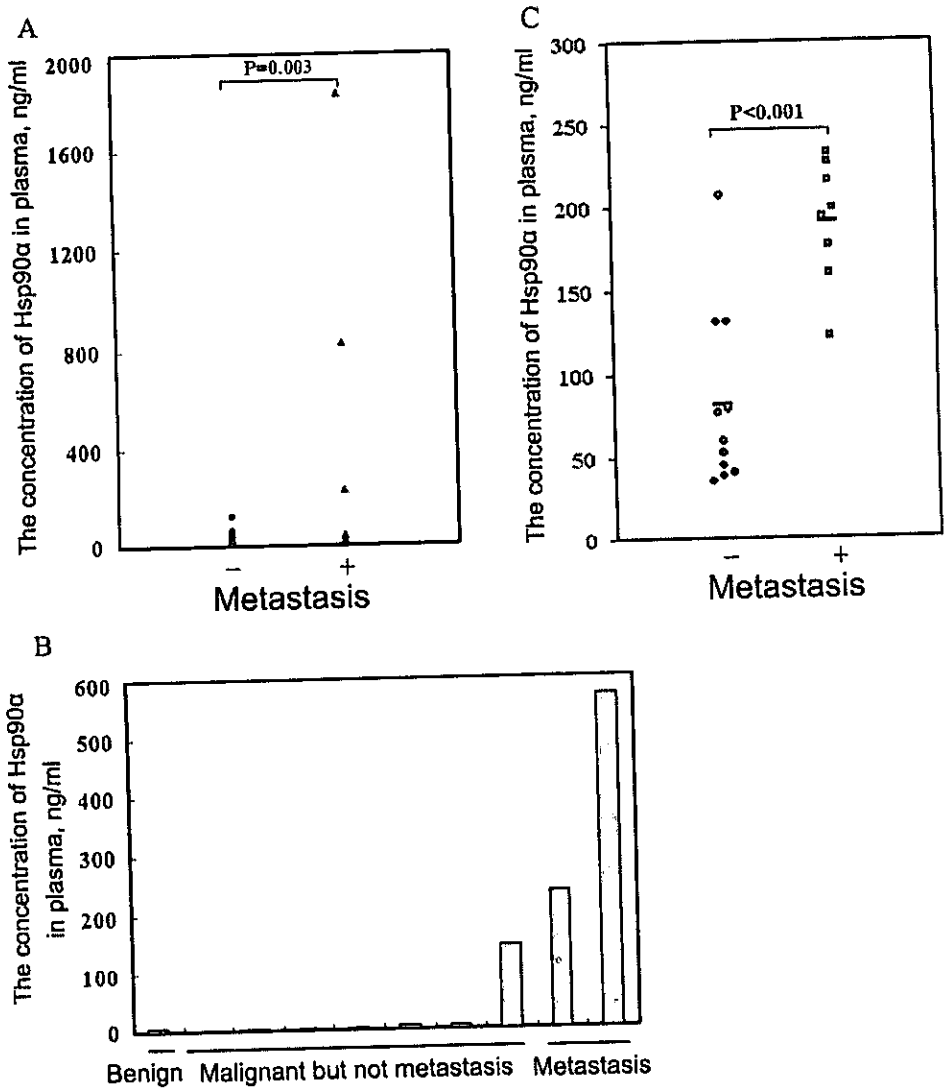

A
The concentration of Hsp90α in plasma, ng/ml
2000
1600
1200
800
400
0
P=0.003
−
+
Metastasis
C
The concentration of Hsp90α in plasma, ng/ml
300
250
200
150
100
50
0
P<0.001
−
+
Metastasis
B
The concentration of Hsp90α in plasma, ng/ml
600
500
400
300
200
100
0
Benign
Malignant but not metastasis
Metastasis

Fig.6

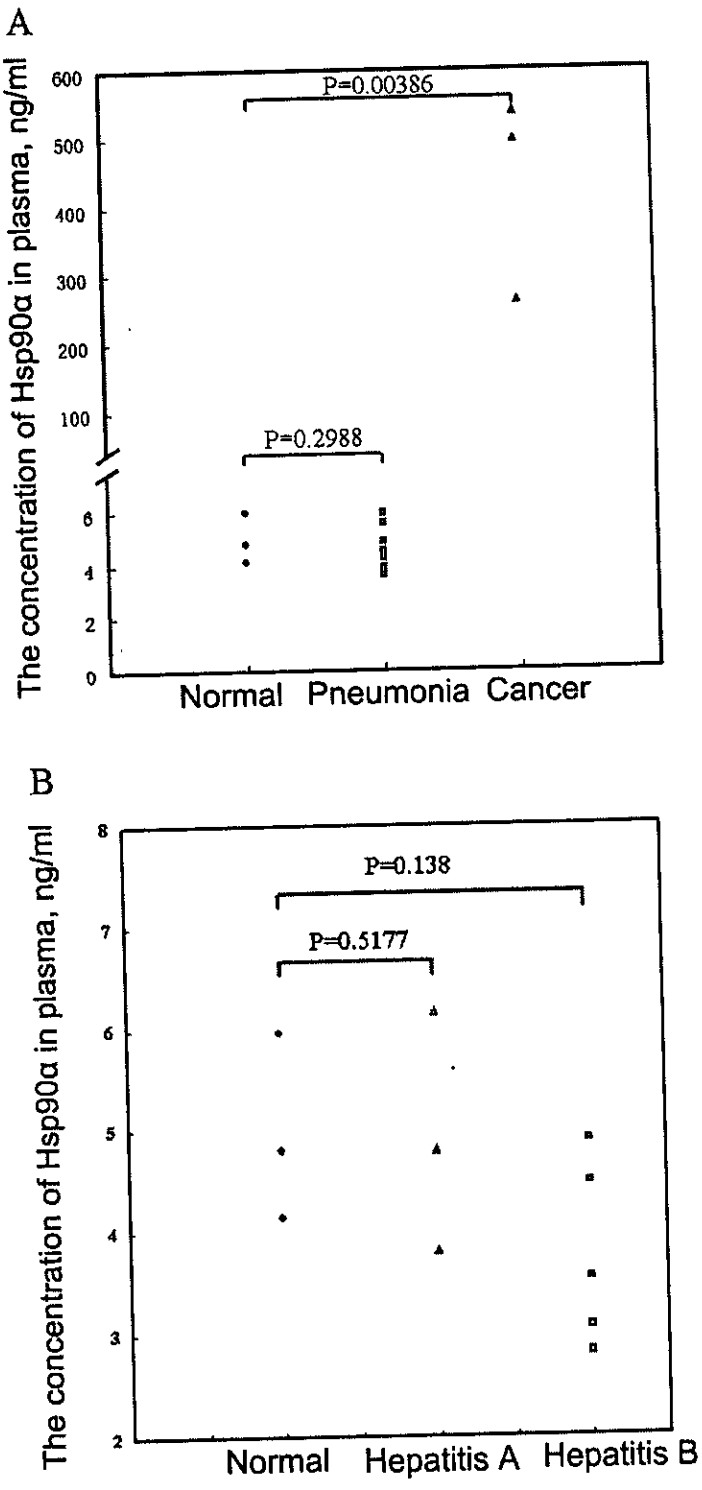

A
The concentration of Hsp90α in plasma, ng/ml
600
500
400
300
200
100
6
4
2
0
P=0.00386
P=0.2988
Normal
Pneumonia
Cancer
B
The concentration of Hsp90α in plasma, ng/ml
8
7
6
5
4
3
2
P=0.138
P=0.5177
Normal
Hepatitis A
Hepatitis B

Fig.7

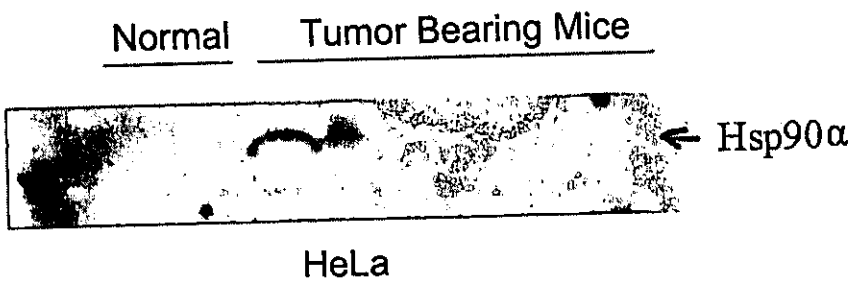


Fig.8

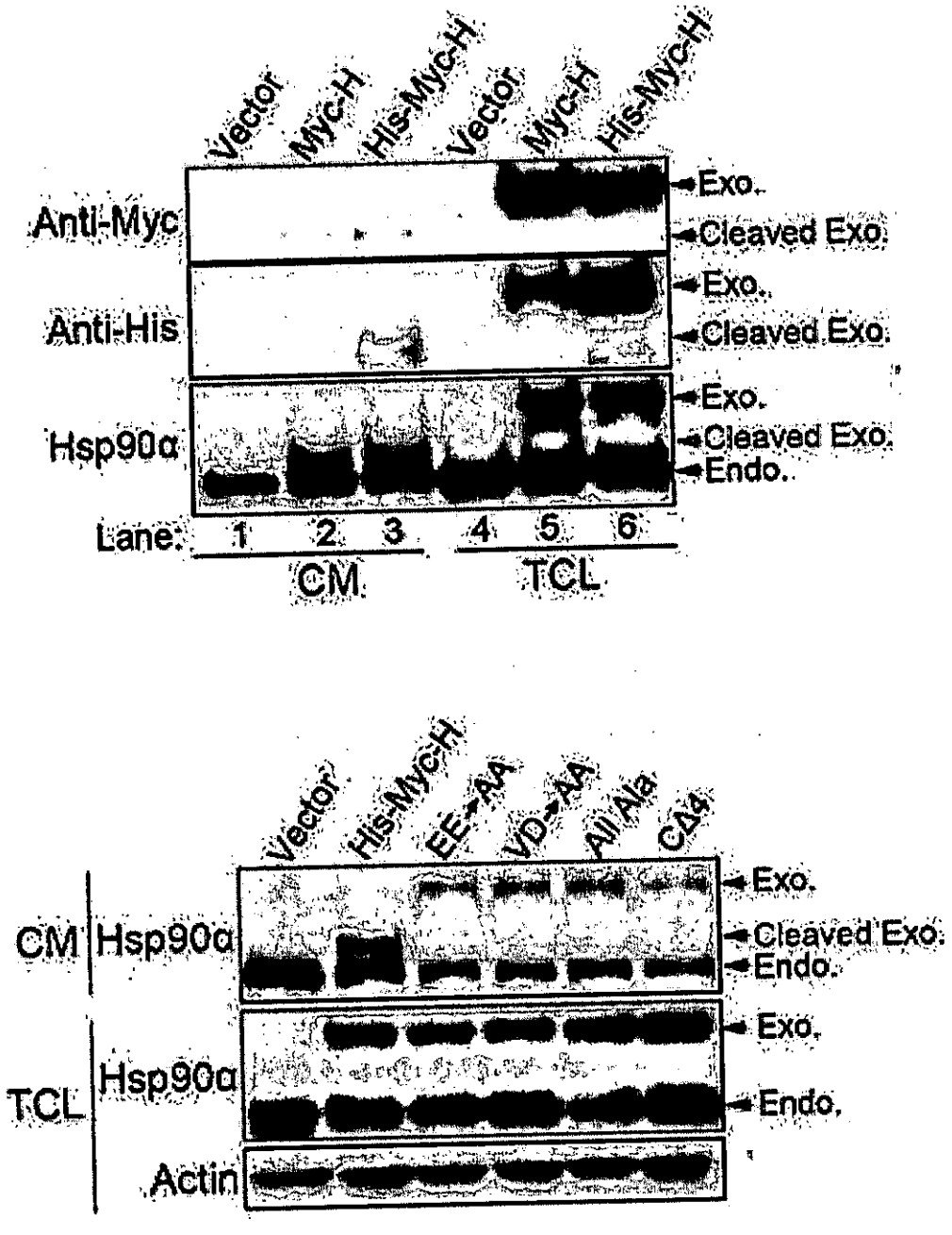

Vector
Myc-H
His-Myc-H
Vector
Myc-H
His-Myc-H
Anti-Myc
Exo.
Cleaved Exo.
Anti-His
Exo.
Cleaved Exo.
Hsp90α
Exo.
Cleaved Exo.
Endo.
Lane: 1 2 3 4 5 6
CM
TCL
Vector
His-Myc-H
EE→AA
VD→AA
All Ala
CΔ4
CM
Hsp90α
Exo.
Cleaved Exo.
Endo.
TCL
Hsp90α
Exo.
Endo.
Actin

Fig.9

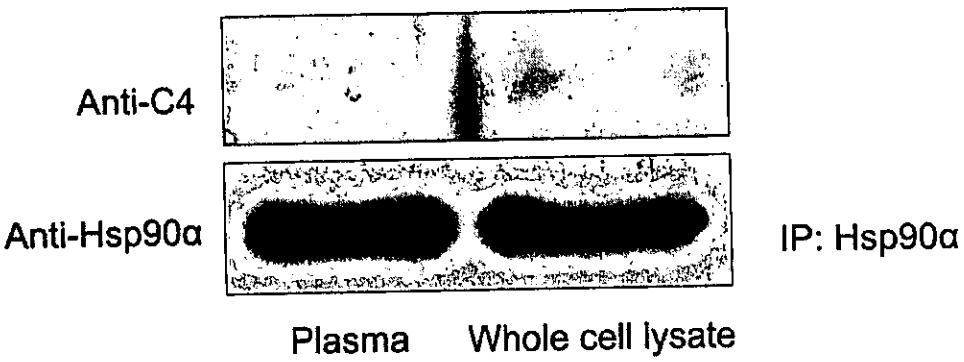

Anti-C4
Anti-Hsp90α
IP: Hsp90α
Plasma
Whole cell lysate

Fig.10

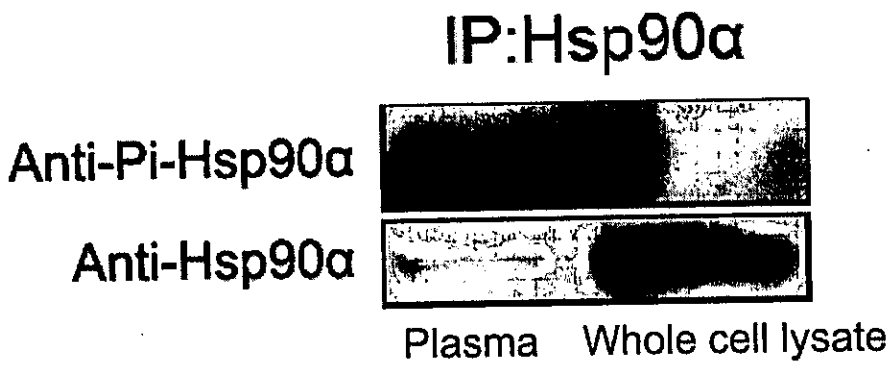

IP:Hsp90α
Anti-Pi-Hsp90α
Anti-Hsp90α
Plasma
Whole cell lysate

Fig.11

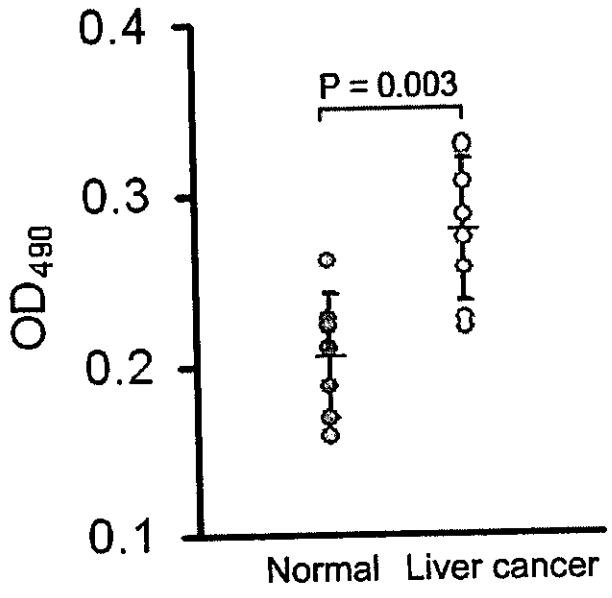

0.4
0.3
0.2
0.1
OD_{490}
P = 0.003
Normal
Liver cancer

Fig.12

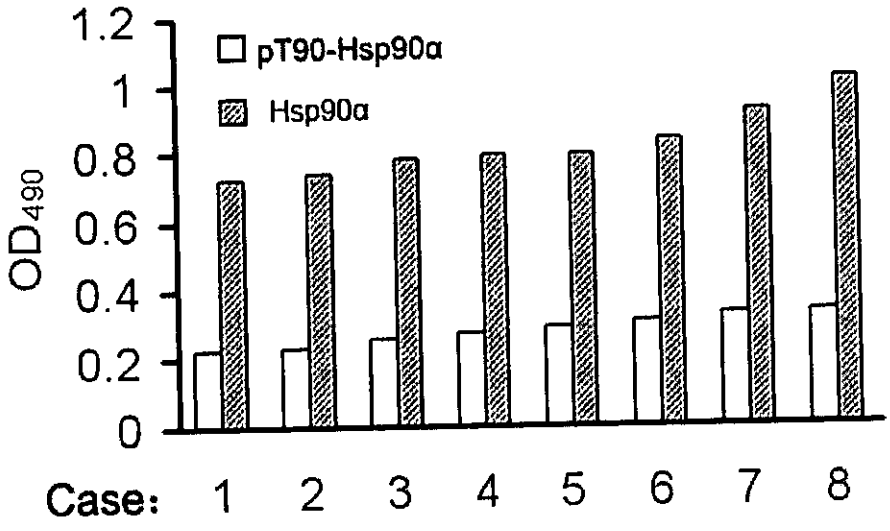

1.2
1
0.8
0.6
0.4
0.2
0
OD_{490}
pT90-Hsp90α
Hsp90α
Case: 1 2 3 4 5 6 7 8

Fig.13

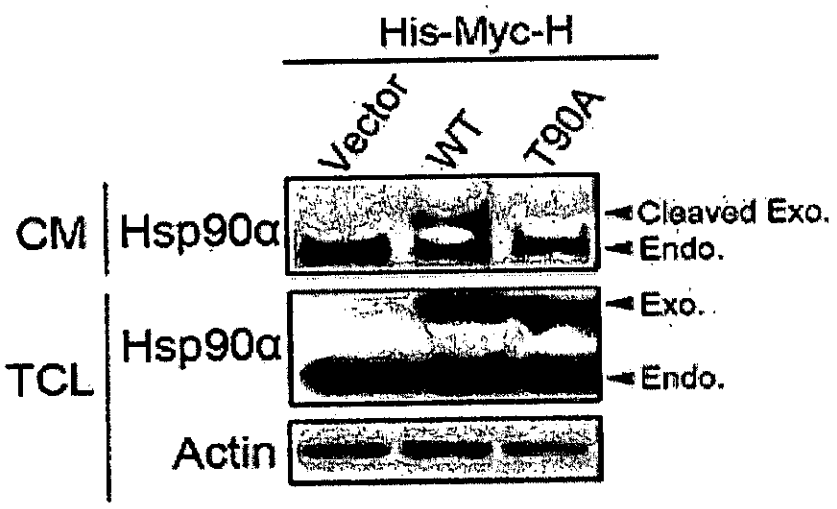

His-Myc-H
Vector
WT
T90A
CM
Hsp90α
Cleaved Exo.
Endo.
TCL
Hsp90α
Exo.
Endo.
Actin

Fig.14

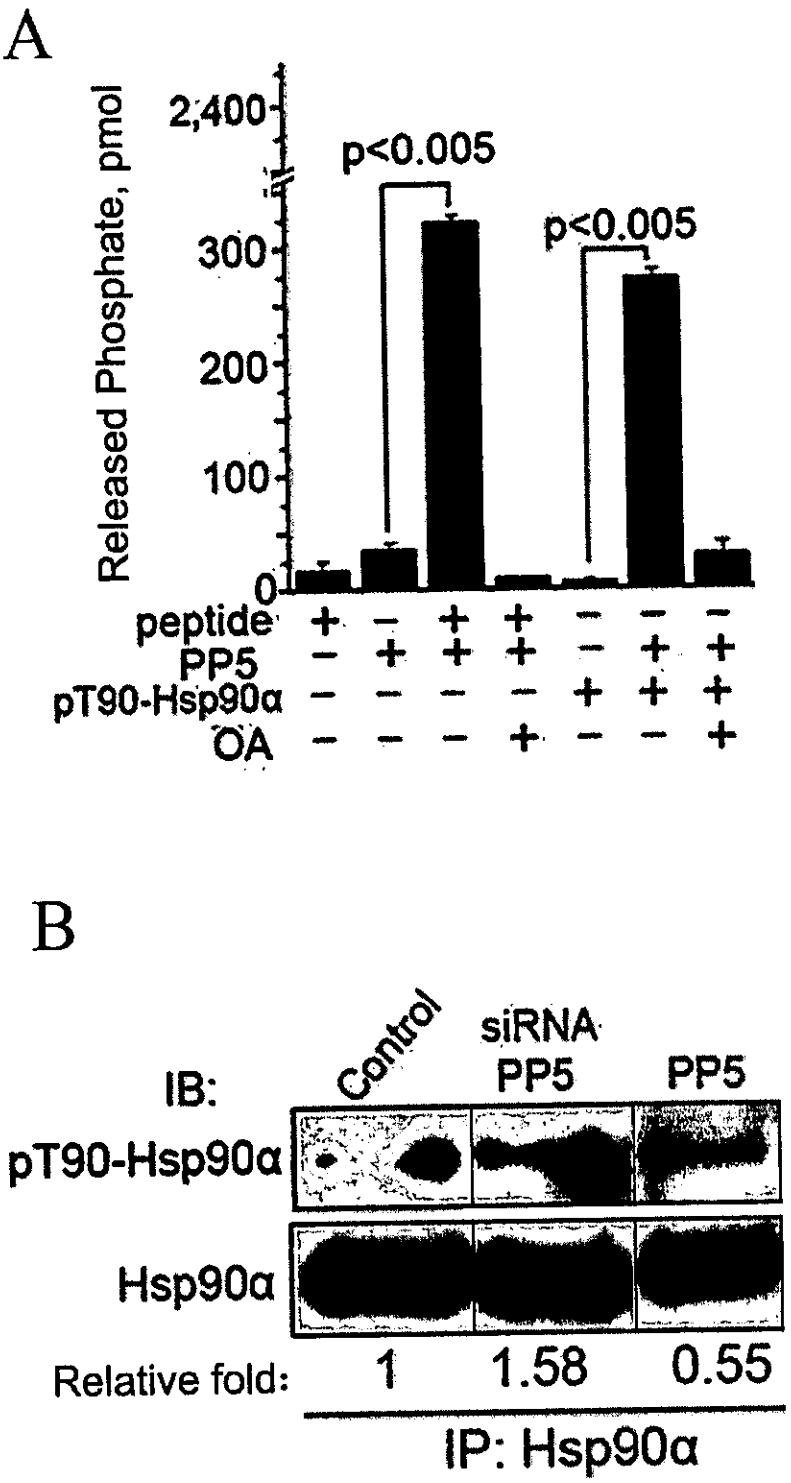

A
Released Phosphate, pmol
2,400
300
200
100
0
p<0.005
p<0.005
peptide + − + + − − −
PP5 − + + + − + +
pT90-Hsp90α − − − − + + +
OA − − − + − − +
B
Control
siRNA PP5
PP5
IB:
pT90-Hsp90α
Hsp90α
Relative fold: 1 1.58 0.55
IP: Hsp90α

Fig.15

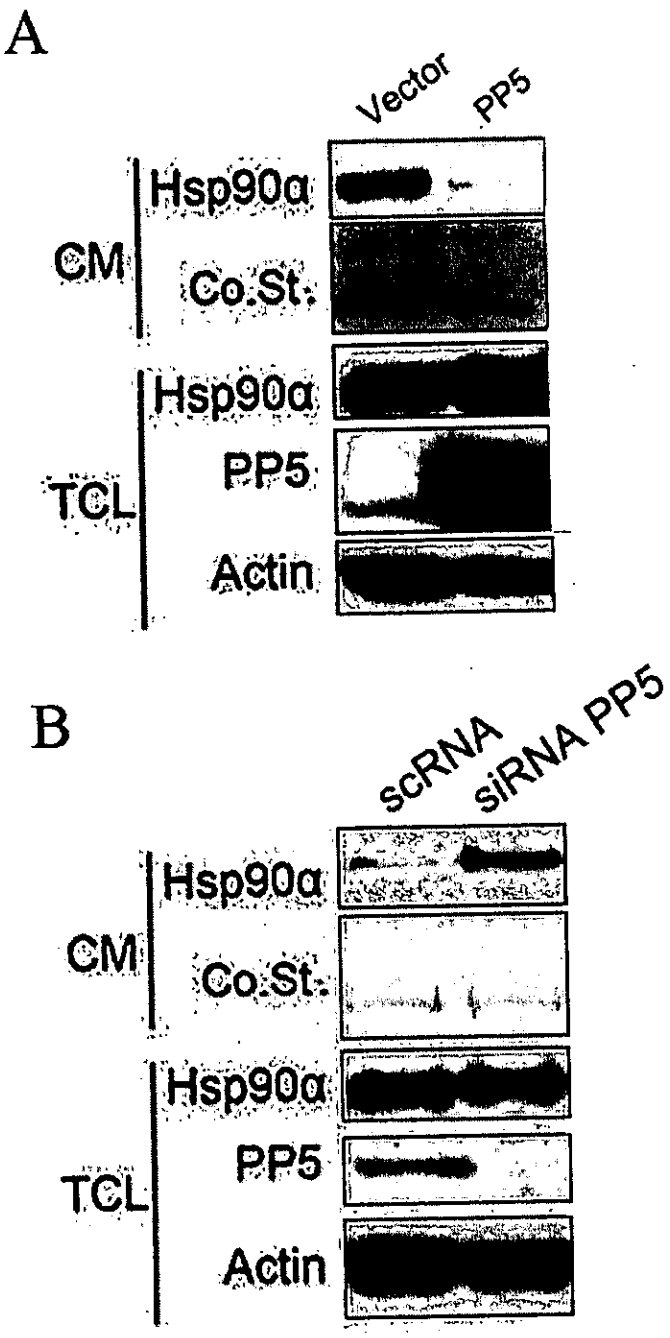

A
Vector
PP5
CM
Hsp90α
Co.St.
TCL
Hsp90α
PP5
Actin
B
scRNA
siRNA PP5
CM
Hsp90α
Co.St.
TCL
Hsp90α
PP5
Actin

Fig.16

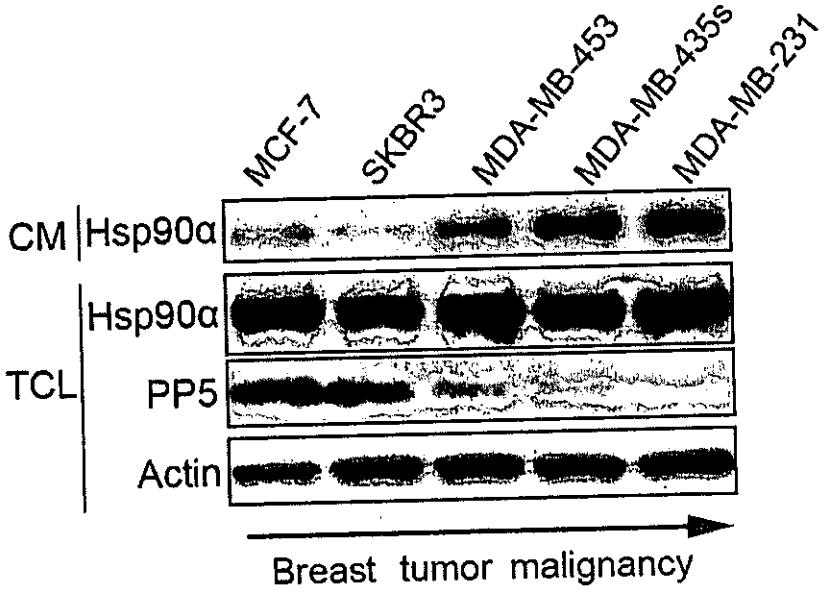

MCF-7
SKBR3
MDA-MB-453
MDA-MB-435s
MDA-MB-231
CM
Hsp90α
TCL
Hsp90α
PP5
Actin
Breast tumor malignancy

Fig.17

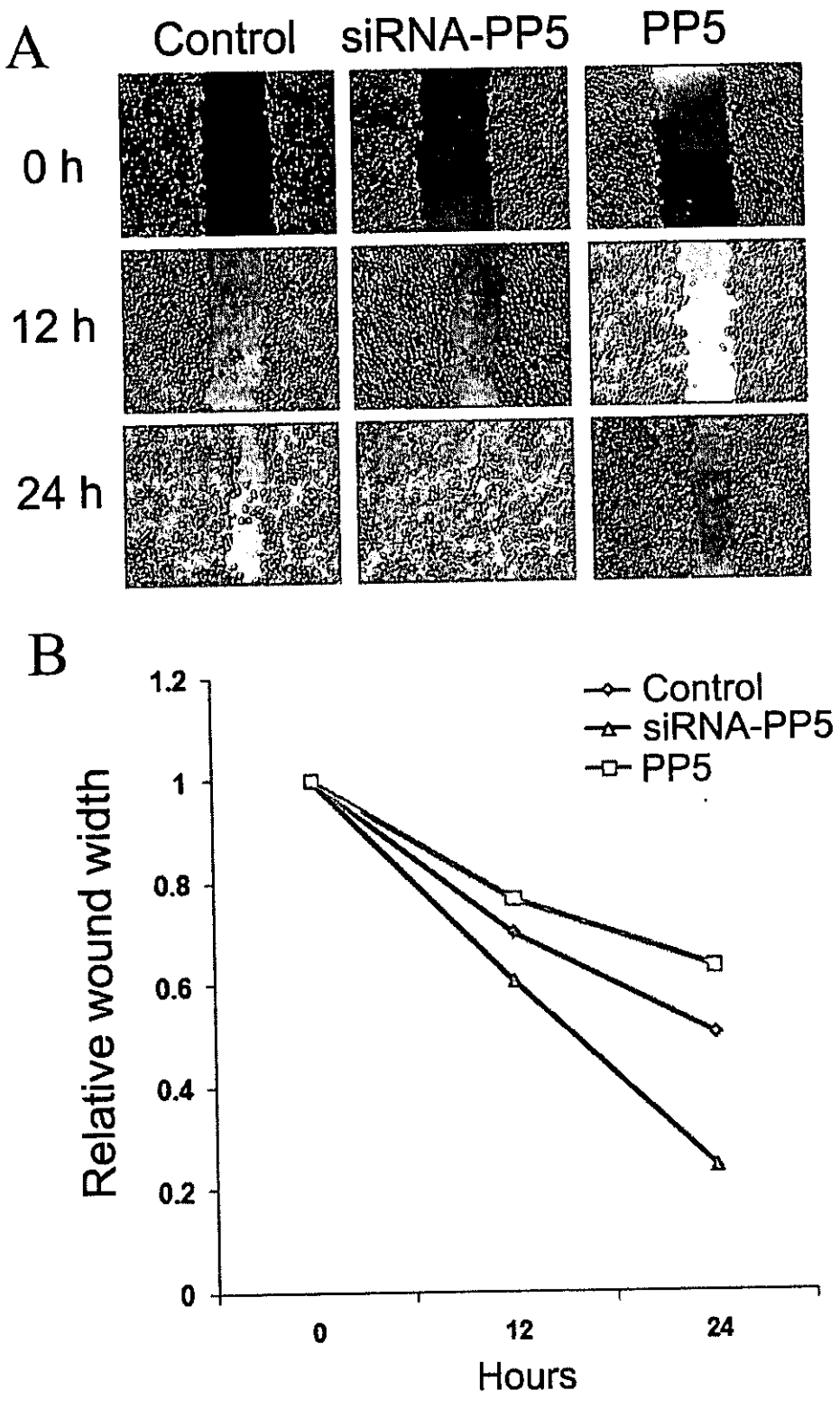
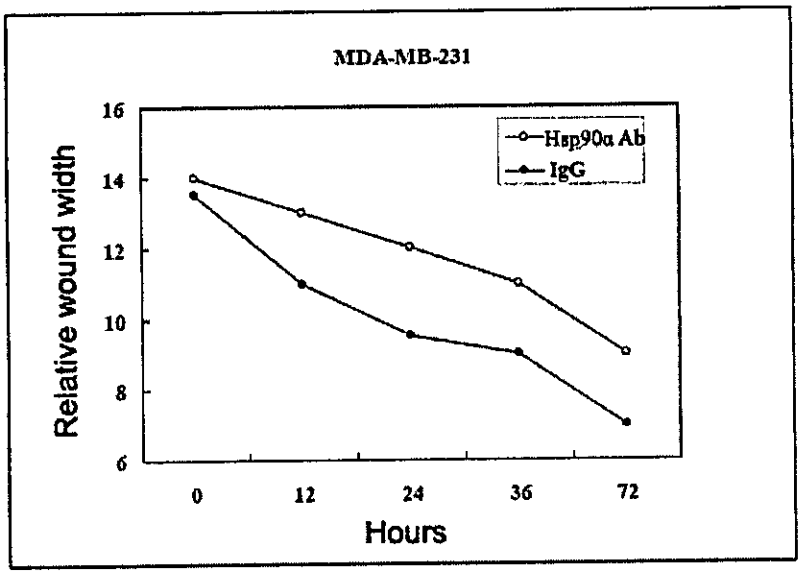

A
Control
siRNA-PP5
PP5
0 h
12 h
24 h
B
Relative wound width
1.2
1
0.8
0.6
0.4
0.2
0
0
12
24
Hours
Control
siRNA-PP5
PP5

Fig.18

A


MDA-MB-231
Relative wound width
16
14
12
10
8
6
0
12
24
36
72
Hours
Hsp90α Ab
IgG

B

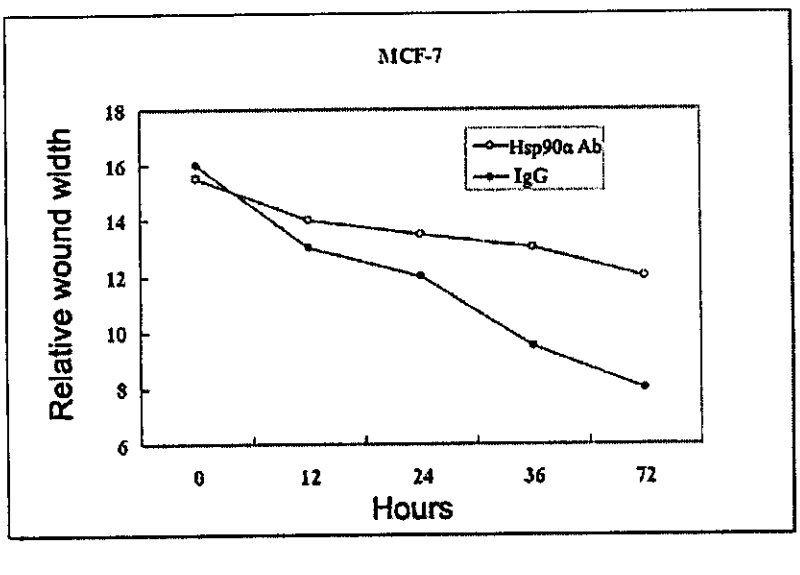
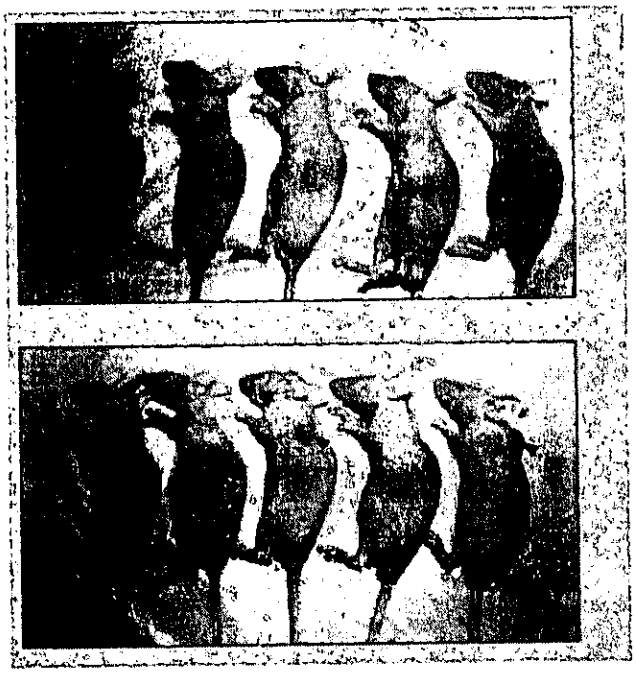

MCF-7
Relative wound width
18
16
14
12
10
8
6
0
12
24
36
72
Hours
Hsp90α Ab
IgG

Fig.19

A

IgG

Hsp90α Ab



B

IgG

Hsp90α Ab

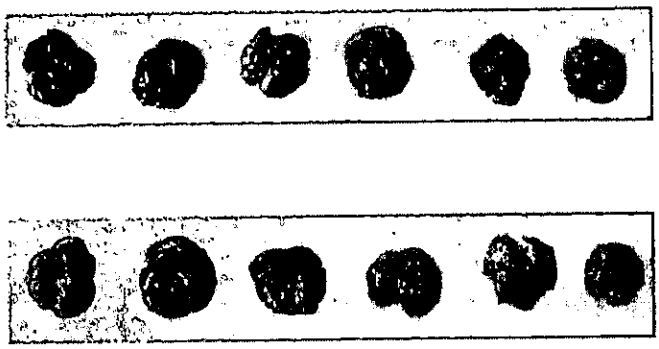


Fig.20

משרד המשפטים

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