

行政院國家科學委員會專題研究計畫 期中進度報告

粒線體融合素/GTP 酶在非小細胞肺癌病人中過度表現的預
後意義(1/3)

計畫類別：個別型計畫

計畫編號：NSC91-2314-B-039-018-

執行期間：91年08月01日至92年07月31日

執行單位：中國醫藥學院醫學系

計畫主持人：許南榮

計畫參與人員：周寬基 姜淑芬 關智尤 林景屏

報告類型：精簡報告

處理方式：本計畫可公開查詢

中 華 民 國 92 年 5 月 14 日

關智尤
林景屏

成果報告類型： 精簡報告

執行單位：中國醫藥學院附設醫院 胸腔外科與醫學研究部

中 華 民 國 92 年 5 月 14 日

研究計畫上所列預期完成之工作項目：

第一年，我們所要完成之工作項目 (91年08月01日至 92年07月31日)：

- (1) To determine the expression of the respective mitofusin/GTPases by RT-PCR;
- (2) To subclone the respective mitofusin/GTPase gene into an expression vector, to deliver recombinant DNA into bacterial cells to express recombinant mitofusin/GTPases.

進度結果

1. Comparison of amino acid sequences between GTPase and mitofusins

- (1) GTPase (HSU95822); (2) Mitofusin1 (AF329637); (3) FLJ20693 fis (AK000700);
- (4) Mitofusin2 (NM_014874)

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(1) -----
-----
(2) -----
-----
(3) -----
-----
(4) -----MSLLF
SRCNSIVTVK
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```
(1) -----
-----
(2) ----MAEPVS PLKHFVLAKK GITAIFDQLL EFVTEGSHFV EATYKNPELD RIATEDDLVE
MQGYKDKLSI
(3) ----MAEPVS PLKHFVLAKK AITAVFDQLL EFVTEGSHFV EATYKNPELD RIATEDDLVE
MQGYKDKLSI
(4) KNKRHMAEVN ASPLKHFVTA KKKINGIFEQ LGAYIQESAT FLEDTYRNAE LDPVTTEEQV
LDVKGYLSKV
```

```
(1) -----
-----
(2) IGEVLSRRHM KVAFFGRTSS GKSSVINAML WDKVLPSGIG HITNCFLSVE GTDGDKAYLM
TEGSDEKKSV
(3) IGEVLSRRHM KVAFFGRTSS GKSSVINAML WDKVLPSGIG HITNCFLSVE GTDGDKAYLM
TEGSDEKKSV
(4) RGISEVLARR HMKVAFFGRT SNGKSTVINA MLWDKVLPSG IGHTTNCFLR VEGTDGHEAF
LLTEGSEEKR
```

```
(1) -----
-----
(2) KTVNQLAHAL HMDKDLKAGC LVRVFWPKAK CALLRDDLVL VDSPGTDVTT ELDSWIDKFC
LDADVFVLVA
(3) KTVNQLAHAL HMDKDLKAGC LVRVFWPKAK CALLRDDLVL VDSPGTDVTT ELDSWIDKFC
LDADVFVLVA
(4) SAKTVNQLAH ALHQDKQLHA GSLVSVMWPN SKCPLLKDDL VLMDSPGIDV TTELDSWIDK
FCLDADVFVL
```

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(1) -----
-----TVNQLAHA LHMDKDLKAG
CLVRVFCPKA
(2) NSESTLMNTE KHFFHKVNEW LSKPNIFILN NRWDASASEP EYMEDVRRQH MERCLHFLVE
ELKVANALEA
(3) NSESTLMNTE KHFFHKVNER LSKPNIFILN NRWDASASEP EYMEDVRRQH MERCLHFLVE
ELKVVANALEA
(4) VANSESTLMQ TEKHFFHKVS ERLSRPNIFI LNNRWDASAS EPEYMEEVRR QHMERCTSFL
VDELGVVDRS
```

(1) KCALLRDDL V LDSPGTDV T TELDSWIDKF CLDADVFLV ANSESTLMNT EKHFHFKVNE
RLSKPNIFIL

(2) QNRIFVSAK EVLSARKQKA QGMPESGVAL AEGFHARLQE FQNFQIFEE CISQSAVTK
FEQHTIRAKQ

(3) QNRIFVSAK EVLSARKQKA QGMPESGVAL AEGFHARLQE FQNFQIFEE CISQSAVTK
FEQHTIRAKQ

(4) QAGDRIFVSA AKEVLNARIQ KAQGMPEGGG ALAEGFQVRM FEFQNFERRF EECISQSAVK
TKFEQHTVRA

(1) NNRWDASASE PEYMEDVRRQ HMERCLHFLV EELKVVNALE AQNRIFVSA KEVLSARKQK
AQGMPESGVA

(2) ILATVKNIMD SVNLAEDDKR HYSVEEREDQ IDRLDFIRNQ MNLLTLDVKK KIKEVTEEVA
NKVSCAMTDE

(3) ILATVKNIMD SVNLAEDDKR FHVQ

(4) KQIAEAVRLI MDSLHMAARE QQVYCEEMRE ERQDRLKFD KQLELLAQDY KLRKQITEE
VERQVSTAMA

(1) LAEGFHARLQ EFQNFQIFE ECISQSAVKT KFEQHTIRAK QILATVKNIM DSVNLAEDK
RHYSVEERED

(2) ICRLSVLVD EFCSEFHPNP VLKIKYSELN KHIEDGMGRN LADRCTDEVN ALVLQTQOEI
IENLKPLLP

(4) EEIRLSVLV DDYQMDFHPS PVVLKVYKNE LHRHIEEGLG RNMSDRCTA ITNSLQTMQQ
DMIDGLKPLL

(1) QIDRLDFIRN QMNLTLVDK KIKEVTEEV PNKVSCAMTD EICRLSVLVD EFCSEFHPNP
DVLKIKYSLP

(2) GIQDKLHTLI PCKKFDLSYN LNYHKLCSD FQEDIVFRFSL GWSSLVHRFL GPRNAQRVLL
GLSEPIFQLP

(4) PVSQRSQIDM LVPRQCFSLN YDLNCDKLCA DFQEDIEFHF SLGWTMLVNR FLGPKNSRRA
LMGYNDQVQR

(1) RSLASTPTAP TTPATPDNAS QEELMITLVT GLASVTSRST MGIIIVGGVI WKTIGWKLLS
VSLTMYGALY

(2) RSLASTPTAP TTPATPDNAS QEELMITLVT GLASVTSRST MGIIIVGGVI WKTIGWKLLS
VSLTMYGALY

(4) PIPLTPANPS MPPLPQGLT QEEFMVSMVT GLASLTSRST MGILVGGVV WKA
WRLIA LSFGLYGLLY

putative transmembrane

seq

(1) LYERLSWTH AKERAFKQF VNYATEKLRM IVSSTSANCS HQVKQIATT FARLCQQVDI
THKQLEEEIA

(2) LYERLSWTH AKERAFKQF VNYATEKLRM IVSSTSANCS HQVKQIATT FARLCQQVDI
TQKQLEEEIA

(4) VYERLTWTTK AKERAFKRQF VEHASEKLQL VISYTGNSCS HQVQELSGT FAHLCQQVDV
TRENLEQEIA

(1) RLPKEIDQLE KIQNNSKLLR NKAVQLENEL ENFTKQFLPS SNEES

(2) RLPKEIDQLE KIQNNSKLLR NKAVQLENEL ENFTKQFLPS SNEES

(4) AMNKKIEVLD SLQSKALLR NKAGWLDSEL NMFTHQYLQP SR

2. DETERMINATION OF PRIMER SEQUENCES FOR MITOFUSIN1

5MFN1M: 5'-ATGGCAGAACCTGTTTCTCCACTGAATCAC-3' (nts
1-26)

3MFN1M: 5'-CCTTTTATCTTCAGCTGCCAGGTTTACT-3' (nts
1098-1070)

5MFN1: 5'-CTCCCTAGATCTTTAGCTTCTACTCCCA-3' (nts
1664-1690)

3MFN1: 5'-TTAGGATTCTTCATTGCTTGAAGGTA-3' (nts 2226-2201)

Mf1M: 5MFN1M to 3MFN1M: 1098 – 0 = 1098 bp

Mf1S: 5MFN1 to 3MFN1: 2226 – 1663 = 563 bp

Mf1L: 5MFN1M to 3MFN1: 2226 – 0 = 2226 bp

3. DETERMINATION OF PRIMER SEQUENCES FOR MITOFUSIN2

5MFN2a: 5'-ATGTCCCTGCTCTTCTCTCGATGCAA-3' (nts 323-348)

3MFN2a: 5'-TGGCCACCAGCACAAACACATCAGC-3' (nts1004-980)

5MFN2b: 5'-CTGTGACAAGCTGTGTGCTGACTTCC-3' (nts 1909-1934)

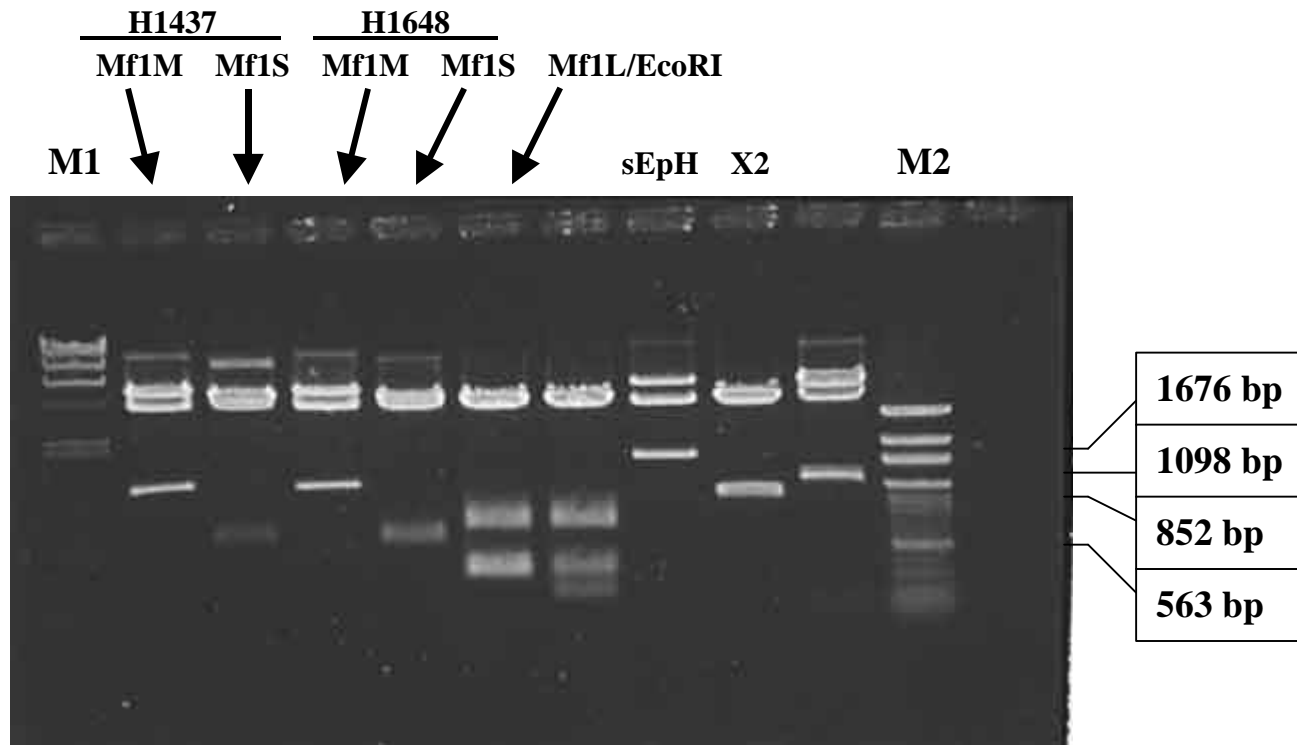
3MFN2b: 5'-CTATGTGCTGGGCTGCAGGTACTGGTGTGTGA-3' (nts 2596-2565)

5MFN2a to 3MFN2a: 1004 – 322 = 682 bp

5MFN2b to 3MFN2b: 2596 – 1908 = 688 bp

5MFN2a to 3MFN2b: 2596 – 322 = 2274 bp

4. Expression of mitofusins detected in lung cancer cells: lung cancer cells only expressed Mfn1, but not Mfn2. The full-length Mfn1 cDNA contained an endogenous Eco RI site.



5. Confirmed RT-PCR results by sequence determination

(1) Full length MF1 (Mf1L) expressed in lung cancer cells

Clone name: MF1-SP6, DNA sequence determined by ABI

TCAAGCTTGGTACCGAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTCGCCCTTATGGCA
 GAACCTGTTTCTCCACTGAATCACTTTGTGCTGGCTAAGAAGGCGATTACTGCAATCTTTGACCAGTTA
 CTGGAGTTTGTACTGAAGGATCACATTTTGTGGAAGCAACATATAAGAATCCGGAACCTTGATCGAATA

GCCACTGAAGATGATCTGGTAGAAATGCAAGGATATAAAGACAAGCTTTCCATCATTGGTGAGGTGCTA
TCTCGGAGACACATGAAGGTGGCATT TTTTGGCAGGACAAGCAGTGGGAAGAGCTCTGTTATCAATGCA
ATGTTGTGGGATAAAGTTCTCCCTAGTGGGATTGGCCATATAACCAATTGCTTCCTAAGCGTTGAAGGA
ACTGATGGAGATAAAGCCTATCTTATGACAGAAGGATCAGATGAAAAAAGAGTGTGAAGACAGTTAAT
CAACTGGCCCATGCCCTTCACATGGACAAAAGATTTGAAAAGCTGGCTGTCTTGTACGTGTGTTTTGGCCA
AAAGCAAAATGTGCCCTCTTGAGAGATGACCTGGTGTTAGTAGACAGTCCAGGCACAGATGTCACTACA
GAGCTGGATAGCTGGATTGATAAGTTCTGCCTAGATGCTGATGTCTTTGTTTTGGGTCGCAAAACTCTG
AAATCANCACCTATTGAATACCGGGAAAACCCCTTTTTTTTTCACAAGGGTGAGTGACCGGCTTTCCCAGC
CCTAAATTTTCATTCCCAATATTCTGTGGGAGTCCCTGCATGAGCCCGAATTTGAAAACGTCGACGCAT
ACGAGACAATCTGGGGAAGTGGTGAAGTGAGAACAACCTCTNNGTTCACGGANTNGTAAAAAGGATA
AGTGCCGAGCCANATACTAAGTCGAGCACGTGCATCCTCCTGTCTCCGCGACATATCGCTGCATATAC
CCCGTAAA

NCBI blast ID: 1017650931-6968-32156

>gi|12744895|gb|AF329637.1|AF329637 Homo sapiens mitofusin 1 mRNA,
nuclear gene for mitochondrial protein, complete cds Length = 2226; Identities =
601/613 (98%)

Query: 6 atggcagaacctgtttctccactgaatcactttgtgctggctaagaaggcgattactgca 65
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Sbjct: 1 atggcagaacctgtttctccactgaagcactttgtgctggctaagaaggggattactgca 60

Query: 66 atctttgaccagtactggagtttgttactgaaggatcacattttgttgaagcaacatat 125
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Sbjct: 61 atctttgaccagtactggagtttgttactgaaggatcacattttgttgaagcaacatat 120

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Sbjct: 121 aagaatccggaacttgatcgaaatagccactgaagatgatctggtagaaatgcaaggatat 180

Query: 186 aaagacaagctttccatcattgggtgaggtgctatctcggagacacatgaaggtggcattt 245
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Sbjct: 181 aaagacaagctttccatcattgggtgaggtgctatctcggagacacatgaaggtggcattt 240

Query: 246 tttggcaggacaagcagtggaagagctctgttatcaatgcaatggttgggataaagtt 305
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Sbjct: 241 tttggcaggacaagcagtggaagagctctgttatcaatgcaatggttgggataaagtt 300

Query: 306 ctcctagtgggattggccatataaccaattgcttccctaagcgttgaaggaactgatgga 365
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Query: 366 gataaagcctatcttatgacagaaggatcagatgnnnnnnngagtgtgaagacagttaat 425
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Sbjct: 361 gataaagcctatcttatgacagaaggatcagatgaaaaaagagtgtgaagacagttaat 420

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Sbjct: 421 caactggcccatgcccttcacatggacaaaagatttgaagctggctgtcttgtactgtgtg 480

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 Sbjct: 481 ttttggccaaaagcaaaatgtgccctcttgagagatgacctgggtgtagtagacagtcca 540

Query: 546 ggcacagatgtcactacagagctggatagctggattgataagttctgcctagatgctgat 605
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 Sbjct: 541 ggcacagatgtcactacagagctggatagctggattgataagtttgcctagatgctgat 600

Query: 606 gtctttgttttgg 618
 |||
 Sbjct: 601 gtctttgttttgg 613

Clone name: MF1-T7

TAGATGCATGCTCGAGCGGCCGCCAGTGTGATGGATATCTGCA**GAATTC**GCCCTTTTAGGATTCTTCAT
 TGCTTGAAGGTAGAAACTGCTTAGTAAAATTTCTCCAGCTCATTTTCAAGTTGAACAGCTTTATTTCTTA
 AGAGCTTTGAATTGTTTTGTATTTTCTCCAAGTGTATCTATTTCTTTGGGTAATCTAGCAATTTCTTCTT
 CCAGCTGTTTTTGGAGTAATATCAACTTGTGGCACAGGCGAGCAAAAGTGGTAGCTATTTGTTGTTTTA
 CTTGGTGACTGCAGTTTGCAGTTCGTGGAGCTAACAATCATCCTCAGTTTTTTCAGTTGCATAGTTTACAA
 ACTGCTGTTTTAAAGGCTCGCTCCTTGGCATGGGTGGTCCAGCTCAGTCTTTCATAAAGATACAAGGCTC
 CATACATAGTTAATGAAACAGATAGGAGTTTTCCAGCCTATAGTTTTCCAAATCACTCCTCCAACAATAA
 TGATGCCCATAGAAGTTCTAGATGTAACGGACGCCAATCCTGTTACTAATGTAATCATGAGTTCTTCCCT
 GTGATGCATTATCTGGCGTTGCTGGAGTGGTAGGAGCAGTGGGGTAGAAGCTAAAGATCTAGGGAGACC
 TTTATATATTTTTAATACATCTGGGATTAGGATGAAACTCTGAACAAAATCATCACCCAAACAGACAGT
 CGACAATTTTCATCTGTCATTGCCATGAACTTGGTTGCACCTCTCGTACTCCTGAATTTTCTTACATCAG
 GTAAGGTACTGGTCGATAGTAGTNCANTGCACCGAAGGCACGAATGCCTATTTCCCGCGCAGGC

NCBI blast ID: 1017651107-8454-781

>gi|17447224|ref|XM_003183.6| Homo sapiens mitofusin 1 (MFN1), mRNA Length = 2221

Identities = 562/564 (99%), Gaps = 1/564 (0%)

Query: 6 ttaggattcttcattgcttgaaggtagaactgcttagtaaaattctccagctcattttc 65
 |||
 Sbjct: 1255 ttaggattcttcattgcttgaaggtagaactgcttagtaaaattctccagctcattttc
 1196

Query: 66 aagtgaacagctttatttcttaagagcttgaattgttttgatatttctccaactgatc
 125
 |||
 Sbjct: 1195 aagtgaacagctttatttcttaagagcttgaattgttttgatatttctccaactgatc
 1136

Query: 126 tatttctttgggtaatctagcaatttcttctccagctgttttgagtaatatcaacttg
 185
 |||
 Sbjct: 1135 tatttctttgggtaatctagcaatttcttctccagctgttttgagtaatatcaacttg
 1076

Query: 186 ttggcacaggcgagcaaaaagtggtagctatftgtgttttacttggtgactgcagtttgc
 245
 |||
 Sbjct: 1075 ttggcacaggcgagcaaaaagtggtagctatftgtgttttacttggtgactgcagtttgc
 1016

 Query: 246 actcgtggagctaacaatcatcctcagttttcagttgcatagtttacaactgctgttt
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 |||
 Sbjct: 1015 actcgtggagctaacaatcatcctcagttttcagttgcatagtttacaactgctgttt
 956

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 Sbjct: 955 aaaggctcgctccttggcatgggtggccagctcagtccttcataaagatacaaggctcc
 896

 Query: 366 atacatagttaatgaaacagataggagttccagcctatagttttccaaatcactcctcc
 425
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 Sbjct: 895 atacatagttaatgaaacagataggagttccagcctatagttttccaaatcactcctcc
 836

 Query: 426 aacaataatgatgccatagaagttctagatgtaacggacccaatcctgttactaatgt
 485
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 Sbjct: 835 aacaataatgatgccatagaagttctagatgtaacggacccaatcctgttactaatgt
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 545
 |||
 Sbjct: 775 aatcatgagttcttctctgtgatgcattatctggcgttgctggagtggtaggagcagtggg
 716

 Query: 546 -gtagaagctaaagatctaggag 568
 |||
 Sbjct: 715 agtagaagctaaagatctaggag 692

(2) Full length MF2 (Mf2L) only expressed in esophageal cancer cells

Clone name: 81A M13F

CGACGGCCAGTGAATTGTAATACGACTCACTATAGGGCGAATTGGGCCCTCTAGATGCATGCTCGAGCG
 GCCGCCAGTGTGATGGATATCTGCA**GAATTC**GGCTTATGTCCCTGCTCTTCTCTCGATGCAACTCTATC
 GTCACAGTCAAGAAAAATAAGAGACACATGGCTGAGGTGAATGCATCCCCACTTAAGCACTTTGTCAC
 GCCAAGAAGAAGATCAATGGCATTTTTGTGACAGCTGGGGGCCTACATCCAGGAGAGCGCCACCTTCCTT
 GAAGACACGTACAGGAATGCAGAACTGGACCCCGTTACCACAGAAGAACAGGTTCTGGACGTCAAAGGT
 TACCTATCCAAAGTGAGAGGCATCAGTGAGGTGCTGGCTCGGAGGCACATGAAAGTGGCTTTTTTTGGC

CGGACGAGCAATGGGAAGAGCACCGTGATCAATGCCATGCTCTGGGACAAAGTTCTGCCCTCTGGGATT
GGCCACACCACCAATTGCTTCCTGCGGGTAGAGGGCACAGATGGCCATGAGGCCCTTCTCCTTACCGAG
GGCTCAGAGGAAAAGAGGAGTGCCAAGACTGTGAACCAGCTGGCCCATGCCCTCCACCAGGACAAGCAG
CTCCATGCCGGCAGCCTAGTGAGTGTGATGTGGCCCAACTCTAAGTGCCCACTTCTGAAGGATGACCTC
GTTTTGATGGACAGCCCTGGTATTGATGTCACCACAGAGCTGGACAGCTGGATTGACAAGTTTTGTCTG
GATGCTGATGTGTTTTGTCTGGNGGCAAAGCCCNANTNCAA

NCBI blast ID: 1019091191-016662-20362

>gi|7662003|ref|NM_014874.1| Homo sapiens mitofusin 2 (MFN2), mRNA

Length = 4550

Identities = 672/680 (98%)

Query: 6 atgtccctgctcttctctcgatgcaactctatcgtcacagtcaagaaaaataagagacac 65
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Query: 66 atggctgaggatgaatgcatccccacttaagcactttgtcactgccaagaagaagatcaat 125
|||||
Sbjct: 383 atggctgaggatgaatgcatccccacttaagcactttgtcactgccaagaagaagatcaat 442

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|||||
Sbjct: 443 ggcatTTTTgagcagctgggggcctacatccaggagagcgccaccttcttgaagacacg 502

Query: 186 tacaggaatgcagaactggaccccgttaccacagaagaacaggttctggacgtcaaaggt 245
|||||
Sbjct: 503 tacaggaatgcagaactggaccccgttaccacagaagaacaggttctggacgtcaaaggt 562

Query: 246 tacctatccaaagtgagaggcatcagtgaggtgctggctcggaggcacatgaaagtggcn 305
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Sbjct: 563 tacctatccaaagtgagaggcatcagtgaggtgctggctcggaggcacatgaaagtggct 622

Query: 306 nnnnnngccggacgagcaatgggaagagcaccgtgatcaatgccatgctctgggacaaa 365
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Sbjct: 623 tttttggccggacgagcaatgggaagagcaccgtgatcaatgccatgctctgggacaaa 682

Query: 366 gttctgccctctgggatggccacaccaccaattgcttctcgggtagagggcacagat 425
|||||
Sbjct: 683 gttctgccctctgggatggccacaccaccaattgcttctcgggtagagggcacagat 742

Query: 426 ggccatgaggcctttctccttaccgagggtcagaggaaaagaggagtccaagactgtg 485
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|||||
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Sbjct: 923  cctggattgatgtcaccacagagctggacagctggattgacaagttttgtctggatgct 982

Query: 666  gatgtgtttgtgctggnggc 685
|||||
Sbjct: 983  gatgtgtttgtgctggnggc 1002

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Clone name: Kbb M13R

TTTAGGTGACACTATATAACTCAAGCTATGCATCAAGCTTGGTACCGAGCTCGGATCCACTAGTAAC
GGCCGCCAGTGTGCTGGAATTCGGCTTCTATGTGCTGGGCTGCAGGTAAGTGGTGTGTGAACATGTTGAG
CTCACTGTCCAACCAACCGGCTTTATTCTGAGCAGCTTTGCTTTGCTCTGAAGTGAGTCAAGAACCTC
AATTTTCTTGTTCATGGCGCAATTTCTGCTCCAGGTTCTCCCGGGTGACGTCAACTTGCTGACACAG
ATGAGCAAAGGTCCCAGACAGTTCTGCTGGACTTGGTGGCTGCAGTTGGAGCCAGTGTAGCTGATGAC
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CCAGGTGACAGCTCATAGACGTAGAGGAGGCCATAGAGCCCAAAGGAGAGGGCAATGAGCCGCCAGCC
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CTTGGGGCCAGGAACCTATTACCAGCATGGTCCATCCGAGAGAGAAATGGAACCAATGTCTTCTG
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NTTANAGGCCCAATTCCTAAGGGAGTCTATTAATTAAGTGGCCG

NCBI blast ID: 1018489490-12259-12970

>gi|7662003|ref|NM_014874.1| Homo sapiens mitofusin 2 (MFN2), mRNA Length = 4550

Identities = 664/666 (99%)

```

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 Sbjct: 2296 ctcttggccttgggtggccaggtcagacgctcatagacgtagaggaggccatagagccc 2237

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Query: 424 aatgcccatggaggctcctggatgtcaaggaggccagccggtaacctggaaacctgaa 483
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 Sbjct: 2176 aatgcccatggaggctcctggatgtcaaggaggccagccggtaacctggaaacctgaa 2117

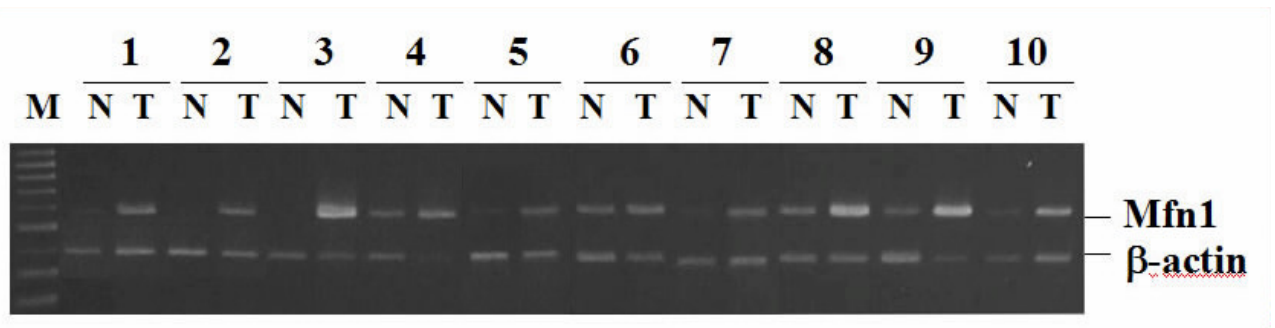
Query: 484 ctctcctgggtgagcgagccctgtggcagtgggggcatgctggggttggtggcgctcag 543
 |||
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Query: 544 agggatgggacgctggacctggtcattgtaacctcaaggcccagcggctgttcttggg 603
 |||
 Sbjct: 2056 agggatgggacgctggacctggtcattgtagcccatcaaggcccagcggctgttcttggg 1997

Query: 604 gccaggaacctattcaccagcatggtccatccgagagagaaatggaactcaatgtcttc 663
 |||
 Sbjct: 1996 gccaggaacctattcaccagcatggtccatccgagagagaaatggaactcaatgtcttc 1937

Query: 664 ctggaa 669
 |||||
 Sbjct: 1936 ctggaa 1931

6. Expression of mitofusin1/GTPase in NSCLC determined by RT-PCR



Amplified DNA fragments were extracted from the gel and sequence determined.

90% of NSCLC overexpressed Mfn1 as determined by RT-PCR. However, there was no extraneous band appeared below the authentic fragment, and therefore no aberrant splicing of Mfn1 gene was detected in the tumor nest.

7. Subcloning of mitofusin1/GTPase gene into an expression vector, and to deliver recombinant DNA into bacteria to express recombinant mitofusin1/GTPases.

Mf1S gene translate Protein sequence :

Theoretical pI: 8.51 Mw (average mass): 22933.10

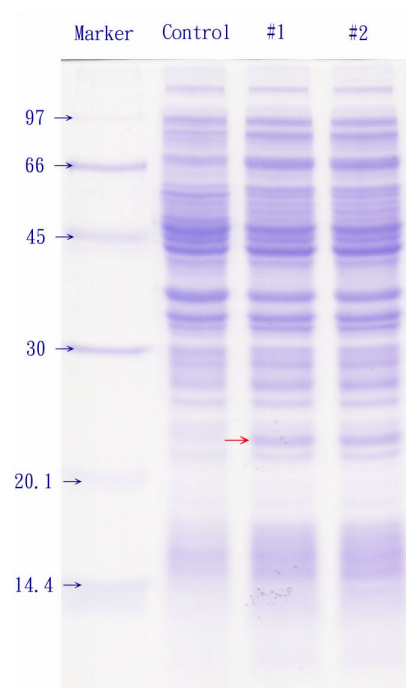
1	11	21	31	41	51		
1	MRGSHHHHHH	<u>GSDDDDK</u> LPR	SLASTPTAPT	TPATPDNAKE	ELMI TLVTGL	ASVTSRTSMG	60
61	IIIVGGVIWK	TIGWKLLSVS	LTMYGALYLY	ERLSWTTHAK	ERAFKQQFVN	YATEKLRMIV	120
121	SSTSANCSEQ	VKQQIATTF	RLCQQVDITR	KQLEEEIARL	PKEIDQLEKI	QNSKLLRNK	180
181	AVQLENELEN	FTKQFLPSSN	EES				

Mf1S sequence 中藍色部分為主要蛋白序列部份，紅色部分為包含表現載體與設計之蛋白酵素切割部位(底線標示之 DDDDK 序列)所增加之序列，預計表現蛋白質之大小為 23 kDa。

下圖：為 13% SDS-PAGE gel。

Control 組為未經 IPTG 誘導的菌體全蛋白。

#1 及 #2 分別為兩株不同的菌株，經 1 mM IPTG 誘導 4 hrs 後之全蛋白，在紅色箭頭所指區域有明顯的新增蛋白出現，分子量與所預期 23 kDa 相吻合。



由表現結果看來，蛋白質表現已無問題，然而其表現量偏低，需大量表現並進行純化進度及抗體之製備。