

# ΑΕΕ ΕΕΕΟΑÇÑΕΥΕΑÇÏ ΑΑΪ Αζ

Ε ¾ÄÇÜ¾ÄÛ ÖÛÄÆÏ ð ÆÛ É ÛË ÄÏ ÖÄÇ¾ÄÏ

Mucins and Lung Cancer

ÇÏ × ÖÖNSC88-2314-B-002-083-M39

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## ÇÏ ¾¾ÇÇ

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 Í ÖË ÛÖÇÛÛ ÆÛ É ÛË ¾ÛÇÖÏ ,  
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 Ì ÖÄÇ¾ÄÈ ÄÄÇÛÛ É ÛÆÏ ð R ,  
 Ì ÄÇ» ¾ÄÆÄÇÜ¾ÄÛÛ É Û  
 » sialomucin»ÄÏ ÖÛÄÖÄÄÏ ¾ÛÄÖ  
 ¾ÄÛÛ» Ä MUC5AC È Ä¾ÄÏ ÖÄÇ¾  
 ÛÏ ÛÄÆÈÛÛ É ÛÏ ÄÛÛ» ÄÄÖR  
 Muc5AC ¾ÄËË ÖÈ ÄÄÏ ¾ÄÈ ð ÆÄÇ  
 ÛÆÏ ÄÖÛ¾Ä ¾Ë ¾ÖÖÖËÆÆÆÏ  
 MUC5ÛÛ É ÛË ÄÄÇÛÛ ¾ÇÇÄ ¾T  
 ÄÇÇË ÈÏ ¾Muc5AC cDNA ÄÇÛÛ  
 Ä» ÖÏ Ö0KD ¾Muc5AC ÛÄÏË ð ËÏ  
 ¾È ÄÄÛ¾È ÖÄÖ¾ÖÏË ð ÄÏ¾È  
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modulate the immune recognition phenomenon of cancer cells. Only few studies have addressed the prognostic implications of mucin and mucin associated antigens in lung cancer. Our previous investigations had discovered the association among lung adenocarcinoma, sialomucin and Muc5AC expression. Therefore, to study the regulation of Muc5AC protein expression should be an important topic in investigating lung cancer. In order to study the protein expression of Muc5AC in lung cancer, 20 kD fusion protein of Muc5AC nontandem repeat sequence was made, injected into animals (chicken, and mice), and successfully generated anti-Muc5AC polyclonal and monoclonal antibodies. These antibodies were applied in the immunohistochemistry, western blotting of variable specimens of lung cancer, as well as ELISA assay. The results are unsatisfactory.

## ÇÏ ÇÇÇÇ

Aberrant expression of mucins and mucin related antigens are noted to be poor survival factors in cancer. Experimentally, mucins had been demonstrated to promote tumor cell invasion, metastasis and

Keywords» Mucin glycoprotein, mucin gene, monoclonal antibody, polyclonal antibody, lung cancer

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SDS-PAGE I AXEW NI AI UP AI L I B 3/4 DE OZ AY YPTG OZ EB AI L B R YNi++ charged column E UH [ L B DA AO VAI - b z AV.1% Coomassie blue AE A 30 3/4 B U WR VA A 30 3/4 B U O 3/4 E SA A UAI L AO band z z UY O 3/4 B U band 3/4 U S A Model 422 Electro-Eluter (Bio-Rad) 3/4 3/4 Y Y A B M A X O O E I L L O VAI - b I z A Y L A N O L Y B C B U A detone N O A -20 1/4 [ 3/4 E B I L A O » A C U B Q A O W A O A A 70% E O A 3/4 O » R A U Y 3/4 N O O D X Centricron-10 (Amicon) z B A U I A A C C O B A N O X R protein assay kit (Bio-Rad) I D I L I X U A S T A N A B A Y A O I a

A E 200 ug A C A O R I L O B U E 4 A reund complete adjuvant z Sonicator I A A A A E 1 ml » R 4 U L z 1/4 L E S A U A z O X E b e [ 3/4 A 2-24 D 3/4 1/4 U » R O L " D 3/4 A O oost (100 ug) » R I O A O W A O { 3/4 O A A I B R [ O A -20 1/4 1/4 3/4 T

UAAOYA (Affinity chromatography) E U ANAS

Muc5AC peptide E 1/4 (coupling) CNBr activated-Sepharose 4B column (Pharmacia) » O U R I L A X E S A A Y A e U I D U 0.01M Tris HCl (pH 7.6) E 1/4 C U I P U W A S A peptide-sepharose column A O 3/4 O Y R A U 0.01M Tris-HCl » 0.5M NaCl (pH 7.6) A A O A R 0.1M glycine (pH 2.5) I A A B » R E S h YIM Tris 3/4 A » E I O z YELISA I O A B 3/4 A 3/4 A S T

I NAS 3/4 U A X W A O I a

A E 100 ug N O L z C C O B A 3/4 U A I I L O B B U E 4 A reund complete adjuvant

z Sonicator I A A A A E 1 ml » R O W Y I S E A 4-6 D 3/4 1/4 A ALB/c O A T-3 D A W A A O Z O Z E » A D X D A W R A U U E A 3/4 50 ug N O L z C C O B A 3/4 A L A e E A I » R 3 3/4 A A A p B A E O I B O A X W A S T

AXE E A S (ELISA)

A 5-10 ug 3/4 U R I L A E A E O N O A 10 ml A O 0.05 M O O B N O X 4 H 9.6) » R 96 3/4 1/4 N D I A S 1/4 B R 1/4 L 1/4 [ 0.1 ml » R A 42 1/4 1/4 A B 1/4 A Q e 3/4 E S R A E O h I S R A 1/4 1/4 O 1/4 1/4 0.125ml A O 0.1% BSA N O PBS » D A 37 1/4 1/4 A B 1/4 3/4 A Q e 3/4 E A U X E O E X E S A O R S 1/4 [ 0.1ml A E 3/4 U A X E O L z R 37 1/4 1/4 A Q z e 3/4 E A U X E O E X E S A O R L 1/4 O E 3/4 a O O X A O O A X E b e L I G » R 7 1/4 1/4 A Q e 3/4 E O X X E O E X E S A O U I 3/4 O E 3/4 a A X A U 1 ml » R S A B Y z A U Y 1:8 3/4 A U A h O A N O 0.1ml E E 3/4 3/4 A R Y D A B A A S T

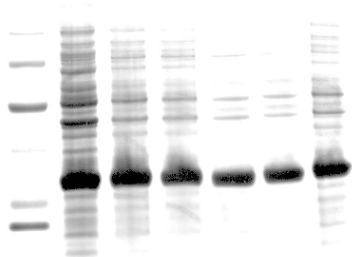
A 3/4 O U O B

A A C U 1/4 U B A A 3/4 A L X » A Z A O B 4 h B C E O L z S A O B 4 h B C I e A I S D A O VAI - b z O A S D A A A O O VAI - b z U O A Nitrocellulose membrane » Membrane E A 5% skim milk 30 3/4 B U R L 1/4 L P B S I P U 000X B U E A O O X E Y A A B » R 4 1/4 A Q A O { 3/4 e R 0.1 % P B S - T I X E S A O R A O 3/4 U P U A O a P B S I P U 000 C B U E 4 A R P conjugated anti-rabbit IgG A 37 1/4 A Q e 3/4 f E S A A A I X E A W R Y-chloro-1-naphthol 3/4 e 0 ml 30% H2O2 A A A B U S T

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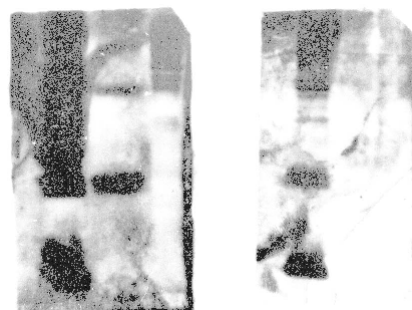
Í ¼ [pRSETC-MUC5AC ÒB ¾Á  
 JM109 Í È » ÀÌ 'MUC5AC ÁÇÄÏÆ,,



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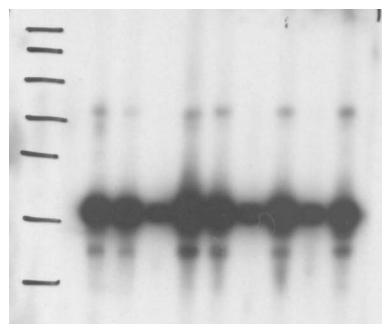
**AÑÁÖÄÿÄÖf à**

ÄÉ 200 µg ÄÖÄÍ È Í ÆÄÛ È ÛÉ  
 ¾Ä Äreund complete adjuvant» È ¼ÄÜÄÇ  
 ¾Ä Ò í ÖÄÖost ÄÖ Ö¼ÇÖÄÄÍ È R  
 Í Í ÖÄ-20¼È¼ÖÜÄÄÖÄÄÉÛÖR



ÛÈ iMUC5AC ¾ÄÑÁÖ» ÖÖ»T  
 Í ÑÁÖ¾Äf à

ÄÖ MUC5AC ÇÄÏÆ¾ÄÄÖÖÛ  
 ÝÉÄ¼KLHÄÜÄ ÖÄÖÖÈ È Ö¼ ½  
 È ÄMuc5AC ÉÖ ÄÖÁÖ» ÄÄÖÄÇÄ  
 ÜÖÆ¾ÄÄÍ È ÄÖÖÖf È ¼É  
 ÖÄÄÑÁÖ» ÖÖ»T



Muc5AC ÁÖÓÌ Ü¼ÖÁÖÖÁ¾Ä  
 ÖÖÖ»È ÜÆÄÖELISA ¾ÄÄÄä

ÄÄÇÜ¼ÜÜÖÄÄ¾ÄÉÈ » ÖÄÖÜ  
 ¾Ö ÖÇÉÖÈ » ÖÄÖÖÖÖ ÖÇf Ä  
 Í »ÖÄÍ È ¼ÄÄÄÄÖÖ» È Y17Q2  
 ¾Ä Muc5AC ÇÄÏÆ¾ÄÄÑÁÖ  
 (CLH2)Æ Öpositive control» Í È ÖÖÄÍ  
 Öf ÖÁÖÄÄ¾ÖÖÖ» È ÜÆÄÖÈ  
 ELISA ÄÖÑÁÖÉÖ ÄÖÈ f7Q2 ¾È  
 CLH2» T

**ÉÖÖ**

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