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分子诊断与治疗杂志

JOURNAL OF MOLECULAR DIAGNOSTICS AND THERAPY

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JOURNAL OF
MOLECULAR DIAGNOSTICS AND THERAPY

CONTENTS

COMMENTS

ORIGINAL ARTICLES

JOURNAL OF
MOLECULAR DIAGNOSTICS AND THERAPY

CONTENTS

REVIEWS

Ў Ә ɟ HCV Ẩ ˜ ͻ ἴ ч З Ў Ә Ặ ǚ ɟ Ђ HCV ׀ Ⴕ Ά ɥ φ Ặ ’ ε ה w ɟ Ύ Ә Ặ Ә Њ ؼ ’ ӊ Ή ’ Ẻ Ђ † ɟ

m ̄ ذ ɞ ҽ Ỉ ⅎ ɞ ɞ ذ ɟ Ψ ו ẹ ǚ ε † chylomicrons CM ɞ ӊ very lowdensity lipoproteins VLDL ɞΨ intermediate density lipoproteins IDL ɞӊ low density lipoproteins LDL ⅎ high density lipoproteins HDL ɟ CM ⅎ VLDL ̄ ҽ LDL ⅎ HDL ̄ Ϋ ذ ɟ CM ӊ HDL ɟ

Apo ΞЮ ٷ ǚ ٷ ´ ẹ m ٷ ɥ Ψ Ẻ ͻ I ´ ' ɟ ζ ǚ ε Apo A ɞApo B ɞ Apo C ɞApo D ɞApo E A ɟ ⅎ ς ’ ǚ ε ’ P ⅎ ’ P ɟ Apo B100 m Ά LDL ⅎ VLDL ´ ’ P ẹ ђ Ώ ’ P ɟ Apo A ⅎ Apo A HDL ζ Apo C ч CM ⅎ VLDL ’ Lipoprotein lipase LPL Ḥ Apo C Ώ Ẻ Ặ LPL ' ɟ З ’ P ζ ٷ Њ CM ɞVLDL ⅎ HDL ɟ ɥ ฿ ⅎ Ỉ ɟ ɥ ζ ه 1[3] ɟ

HCV

HCV Ђm Y Ά ζ Ў Ә w ẹ Ặ ɟ Ά ẹ ђ ╥ RNA Ч HCV Ẉ Ⴤ Њ ͻ I ͻ I ˜ HCV ฿

E1 ⅎ E2 ч Ẩ ɟ HCV ʄ m ฿ ⅎ ͻ I ٷ Ỉ Ẩ ؼ ´ Ά HCV Ⴤ ש Ẩ Ặ ฿ claudin 1 ɞB ͻ I I ي scavenger receptor class B type 1 SRB1 ɞӊ ͻ I low density lipoprotein receptor VLDLR ɞCD81 ⅎ ط[4] ؼ З Ẩ ו ה í ٷ e ɟ HCV ﭢ ؼ ‖ ฿ C ɞ฿ E1 ⅎ E2 w ש p7 4 ⅎ NS2 ɞNS3 ɞNS4A ɞNS4B ɞNS5A ɞNS5B 6 ɟ ٷ HCV Ψ ' w ╟ HCV Ặ ה Ɵ[5] HCV Ặ Њ װ Ỉ ◑ NS4B 5

˜ Ψ ฿ E1 ⁄ E2 ΄ ᾆ ɔ[10] NS2 ч Ά E1/E2 Ў Ә ئۆ Ψ ה Ђ Ặ Ә[11] ɟ ئۆ Ψ Ά ῴ VLDLs ΄[12] ɟ LDs Ψ Apo B100 ɵApo E ⁄ APO C꜀ ל Ά Lipid Virus Particles LVPs HCV ⁄ VLDL ΄ ɟ Apo B100 ῎ HCV LVPs ה Ө א Apo C꜀ ⁄ Apo E HCV LVPs Ỉ У ῎ ’ غ[13] ɟ ѡ HCV ⁄ ῦ Ά VLDL ⁄ ῦ ئۆ Ђm ɟ Ι ҽ microsomal triglyceride transfer protein MTP У VLDL Ặ ῦ ; MTP ῍Ặ Ђ Г ی ῍Ặ[14] ɟ ئۆ Ψ ﻅ Ά GFP Apo Em ς Ђ m[15] ɟ ΄ HCV LVPs ѡ VLDL ̈ ъ Ψ ɟ Ђ Apo E ⁄ VLDL ئۆ Ψ Ә[16] ɟ

HCV

HCV m Y ẹ ң ΄Y م ⁄ \ ῎ ج m HCV ε 1.03~1.10 g/mL ɟ m Ά Ч[17] ɟ ˘ VLDL ɖLDL ɖDL ⁄ HDL φ P ⁄ Ι Ђ Apo B ẹ ђ ’ ئ ΄ ئۆ ᾷ ئۆ Ψ ’ ѡ Ў ΄Y ɟ ̓ ئ Ά ẹ ђ ῎ ̓ HCV م ῎ c ΄Y ɔ Ι Ỉ Ψ ҽ ⁄ ζ ΐ[18] ⁄ LVPs φ ئۆ Ψ ’ ѡ Ў[18] ζ џ Ά Ặ ΄Y ɟ نج Ế HCV ’ ⁄ ж ΄ Ι Жъ З ῦ Ό HCV نج Ế Ψ HCV Ό ң ⁄ Ҝ[19] ɟ ̓ ئ HCV Ψ ῦ Ό HCV ς Ђ ң ⁄ ῦ Ό ئۆ Ψ نج Ế Җ[20] ң HCV ’ ̄ Ά Ặ ῦ ɟ Ҝ HCV

Ђь 1.07 g/mL Ά LDL VLDL ẹ ج ε 73 nm Ά VLDL[17] ɟ غ HCV Ά VLDL Ч ɟ HCV Ẻ Apo E ⁄ ApoC꜀ Apo C꜀ ئۆ ɔ VLDL[21] ɟ ה ƒ HCV Ң Ι Ι[22] Ђ Г ῎m ئۆ 40~140 nm م Ỉ Г ῎ Ẻ ẹ ฿ ẹ ɟ ئۆ ה Ψ ΄ ζ m[23] ɟ HCV Ђ 3 m ῍Ω ΄ ئۆ ฿ Ỉ ל Ά Ẩ ⁄ ٦ ẍ ɔ Ι ч Ψ ⁄ Ә[24] ɟ ẍ ɔ ς Ђ HCV m ῦ[25] ɟ ئۆ HCV Ψ ΄ Apo B ɵApo A꜀ ɵApo C꜀ ɵApo C꜀ ɵApo C꜀ ⁄ Apo E[24] ɟ نج غ ה ƒ ئۆ HCV У Ψ Ә Ђ Apo E HCV ה Ặ غ ɔ Ά Apo E Ặ ’ P ѡ ײַ Apo B ǁ HCV У[26] ɟ ΐ HCV ⁄ ῎ نج ῍Ω ◑ Apo E ﬡ Ю Y ς Apo B Ψ Apo B HCV Ặ Ỷ غ[26] ɟ Ỷ Ђ Apo E ئۆ HCV Ψ Ә ئۆ Ψ Apo E ɔ ῍Ặ[27] ئۆ Apo E Ỹ ; ฿ ῍Ặ ئۆ Ỉ ⁄ ج ῎ У Ҝ Apo E Ђь ฿ ˜ ῎ ’[28] ɟ ئۆ 293t Apo E Ң[28] ئۆ Apo B/Apo E ﬡ نج غ huh 7 LDs[26] ɟ φ Apo E ל Ά Ђ HCV Є ẹ ɟ

HCV

HCV Ι نج Ế ѡ ײַ ῎ Ẩ ж Ꙗ ה m ѡ Ỉ ɞ ɞ ⁄ ῦ ל Ά HCV ٩ ئۆ ῍Ặ Ψ ה Ә ɟ ϿͫHCV ς Ỉ џ ̄

Ȋ ﻄﻲ w ᾀ Њ ῎Ạ ζ ɥ У ȉ ɉ HCV ῎Ạ ɥ ȉ ζ ε HCV Ң w ש Ψ ﻬ ל ɉ Ң LDL HCV [29,30] ɉ ɥ ל ˥ Ỷ Њ ζ 3 Interferon 3 IFN 3 ﻧﺤ ﻍ Ⅎ HCV ﻧﺤ ﻍ ﻱ rs12979860 CC ﻱ IFN 3 ζ ﻧﺤ ﻍ ﻱ ´ HCV ﻧﺤ ﻍ ﻱ 1 G1 ε LDL Ж HCV ﻧﺤ ﻍ ﻱ 3 G3 ﻧﺤ ﻍ ﻱ 4 G4 LDL Ὼ Ặ [31] ɉ ᵐ HCV Ђ Ң ה HCV ˜ б ᾇ [32] ɉ

HCV ¥ Ⅎ ל ’ כ ɥ б ȉ ɉ HCV ﻧﺤ ﻍ ה ל Ђ HCV Ά ל ה Ὺ Ặ [33] ɉ HCV ﻧﺤ Ẽ˜ Њ ɥ б HCV Ẻ Ђ Ὼ [34] ɉ Ά ɸ ﻱ Chronic hepatitis B CHB ל Ά Г ﻱ Chronic hepatitis C CHC Ặ ε Ὺ [35] ɉ CHC ל ’ ˜ Ⅎ כ ɉ

Apo A ל Ά HCV RNA ῎Ạ Ⅎ У [36] Apo A Ά HCV C ﻄﺪ ´ [37] ɉ Apo A Ά HCV HOMA IR Ţ Ⅎ Ặ [38] ﻍ Apo A ’ ל Ά HCV ל ɉ ς ﺋﻮ HCV G1b Ψ Apo A Ꮚ ω ╗ I ɸ ﻣﺤ [39] ɉ ﻍ Apo A ﺋﻮ HCV Ψ τ ᵐ ɉ Ψ Apo B 100 Ά HCV Ⅎ Ặ [40] ɉ Ά ẹ ђ Apo C Ά Ặ כ ; [41] Apo C ’ Ά HCV Ặ כ Ặ ﺋﻮ ɉ

Apo E HCV Ⅎ Ẩ Ặ ǚ ɸ ᵐ ɉ Apo E 3 Y Ң ﻧﺤ ﻍ E2 Œ3 Ⅎ E4 ẹ Ψ E3 ɉ E2 Ά כ I ´ ̃ ƒ HCV Ң 3~5 ช [42] ɉ E4 ’ HCV O Қ ˥ Ә [43] ɉ ﺋﻮ Apo E3/E3 ´ Ψ Г ﻱ כ ’ [44] ɉ ﻍ Apo E Ж ﻱ ’ HCV ה Ɑ ﻍ φᵐ ɉ

HCV ᵐ ﻰﺋ ᾀ ζ ɥ Ђ ﻣﺤ ﻄﻲ ɉ HCV Ⅎ ɥ ﺋﻮ HCV ᐊ ᐁ Y Ὺ Ặ ɉ HCV ’ ɥ У ȉ ς ’ w HCV ῎Ạ ɉ ɥ б Ά ɞ כ Ⅎ ה Ὺ Ặ ɉ ה Ặ ’ w ɥ ﺋﻮ Г ɞ Ⅎ ẨΨ Ә [16] ε Г ﻱ Ⅎ ῎Ạ Ђ ɉ

Њ ᵐ ̃ ɞ ש ﺋﻮ HCV ᐁ Ψ Ẻ I ‘ ש ẹ ǚ ῎Ạ w ש ί ɔ CHC ה ה ש Ά Ψ Ⅎ ɉ Ꙋ מ Ẩ HCV Ά ẹ ζ ɥ φ Ў Ә ’ ᵐ ζ HCV Қ Ỷ ﻍ Ⅎ ה ‘ w ש HCV LVPs HCV ﺋﻮ w HCV ɉ

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YMDD

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;ᵐ ų DNA ᾀ ת ῦ Θ ῦ ᾃ ﻍ ﻧﺮ
ל ;ᵐ ų Ҍ ϕ ﻱ YMDD ל ɉ ϕ ﻱ
Ᾱ 229 ʢ ;ᵐ ų ﾑ Sanger ˀ YMDD ﻍ ﻧﺮ ל ɵ ;ᵐ ų
ɉ ٳ 5 ʢ ﻍ ﻧﺮ ל Ᵽ ˜ ˀ ﻍ ﻧﺮ ל ﻱ ᾀ Ȼ ʢ ﻍ ﻧﺮ ל ;ᵐ ų
ɵ ɉ Ѣ ϕ ﻱ YMDD ל ;ᵐ ų
ɉ 229 оу ĺ Ψ 2 ﭻ ᾃ 74 ʢ 74/229 32.31% YMDD ל ẹ
Ψ ;ᵐ ų ᾃ 44 ʢ 44/229 19.21% rtM204V ל 25 ʢ 25/229 10.92% rtM204I ל 5 ʢ
5/229 2.18% rtM204V/I ˊ ל ɉ Sanger ᾃ 47 ʢ 47/229 20.52% rtM204V ל Ȼ25 ʢ
25/229 10.92% rtM204I ל ﾑ 2 ʢ 2/229 0.87% rtM204V/I ˊ ל ɉ ;ᵐ ų ᾃ 3 ʢ
Sanger rtM204V/I ˊ ל ɉ Ά Sanger ;ᵐ ų ɞ ﾑ Ά
ﭻ ε 100% ל ᾀ ᾶ ˊ ε 95.95% 71/74 ῦ ˊ ε 4.05% 3/74 ɉ 2 ᵐ
ε 0.97 P<0.01 ɉ ;ᵐ ų ﭻᵐ ˊ ε 100% ɉ
ϕ ﻱ YMDD ל ;ᵐ ų Ά
Sanger ᾃ ˊ ל ε ϕ ﻱ ﻍ ﻧﺮ w Ѣ ɉ
;ᵐ ų Sanger YMDD HBV ﻍ ﻧﺮ

Establishment and evaluation of next generation sequencing for the detection of hepatitis B virus YMDD motif mutations

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ABSTRACT Objective A next generation sequencing NGS was established for the detection of hepatitis B virus HBV YMDD mutations and its performance was also evaluated. Methods DNA was extracted from serum samples by centrifugation column and primers were designed to amplify the P gene region of HBV and the PCR products were captured by the target followed by NGS. The detection results were analyzed by using the bioinformatics software system to establish the NGS for the detection of HBV YMDD mutations. A total of 229 serum samples from patients with chronic HBV were collected. The NGS and Sanger sequencing were used to detect the YMDD gene mutations simultaneously and the detection performance for

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[illegible]

[illegible]

[illegible]

NGS was evaluated. Five positive samples including different types of gene mutations and two negative samples were selected to detect repeatedly by NGS and the results were evaluated. Results Among 229 clinical samples 74 74/229 32.31% YMDD mutations were detected by two sequencing methods. Of which 44 44/229 19.21% were detected by NGS 25 25/229 10.92% were detected by rtm204i mutations and 5 5 / 229 2.18% rtm204v / I mixed mutations. 47 47 / 229 20.52% rtm204v mutations 25 25/229 10.92% rtm204i mutations and 2 2/229 0.87% rtm204v / I mixed mutations were detected by Sanger sequencing. Three cases of rtm204v/I mixed mutation missed by Sanger sequencing were detected by NGS. Compared with Sanger sequencing the sensitivity specificity and accuracy of NGS are100%. The complete coincidence rate was 95.95% 71/74 and the partial coincidence rate was 4.05% 3/74 . The consistency of the two methods was 0.97 $P<0.01$. The repeatability coincidence rate between two methods is 100%. Conclusion The NGS for the detection of HBV YMDD mutations has been established. This method is more sensitive than Sanger sequencing and can detect more mixed mutations providing a new method for the detection of drug resistant genes of HBV.

KEY WORDS Next generation sequencing Sanger sequencing YMDD HBV resistance genes

ф ي ф ي hepatitis B virus HBV ɟ ẫ 3.6 л HBV 100 ˛ ж Ҍ Ά HBV Ặ [1] ɟ ๘ Ҍ HBV ה ┘ HBV 9 300 ˛ ҙ [1] ɟ lamivudine LAM ᵐ Ҍ ф ي ⱺ [2,3] ɟ

╥ Ҕ ф ي Ό 1 ˜ 16%~55% Ό Ҕ [4,5] ɟ LAM ζ Ά HBV ´ ﻍ tyrosine methionine aspartate aspartate YMDD ﻍ 204 Ң ⵑ Ặ ɟ ⵑ ⵑ ε reverse transcriptase methionine 204 valine rtM204V Ъ reverse transcriptase methionine 204 isoleucine rtM204I [6,7] ɟ Ά Γ ╗ ǚ ᾇ ה ﯙ YMDD ⵑ Ό ˜ 2~28 ৭ ⱺ 12~31 ৭ [8-10] ɟ ﻍ LAM ⵑ ש Ҍ ├ ε ф Ά̈ Y ɔ ɟ Ψ Ꙗ Ҕ ф ي YMDD ⵑ ;ᵐ ɥ ɵ w ε í ф ي ﻍ w ɟ

˥ 2017 1 12 ﯙ Ψ ᵐ ├ ɔ LAM ╥ 3 Y ф ي ǚ ৭ 5 mL 3 500 rpm 5 min ǚ Ҍ í HBV DNA ẉ PCR HBV DNA Ҍ 100 IU/mL Ẩ Ẫ 229 ҙ ẹ Ψ 182 ҙ 47 ҙ ɟ 17~77 ج 46.1±13.63 ɟ Ẩ ᾇ ɥ ˠ Eppendort Ỉ ᾄ Ҍ - 80 ỳ ɟ

ѦﻯΆ Ḥ

ABI 9700 PCR Ѧ LOT N8050200 ɞ Ὼ LOT 4314 ɞOne Touch2 ѦﻯLOT 2457881 5247 ɞES ѦﻯLOT 74200 ɞDA8600 ;ᵐ ɥ Ѧ LOT sn247560288 ɞABI 3500 DX ﻍ ǚ Ѧ LOT 4406019 ɞQubit 3.0 ẉ Ѧ LOT 2321609904 Ѧﻯج ๘ Life Technology ắ ˘ ˥ Ḥ LOT #DA Z14648 Ψ ﻍ oy ắ ˘ SanPrep PCR У ɔ Ḥ LOT B518141 0100 э ắ ˘ Ion AmpliSeq™ Library Kit 2.0 LOT 4480441 ɞIon PI™ Hi Q™ OT2 Reagents 200 kit LOT A26434 ɞIon PI™ Hi Q™ Sequencing 200 kit LOT 4488315 ɞQubit dsDNA HS Assay kit LOT 1788710 Ḥ ج ๘ Life Technology ắ ˘ ɟ

1.3.1 ;ᵐ ɥ YMDD ⵑ

1.3.1.1 ˥ ˥ Ḥ

˥ Ψ DNA Ḥ Щ Ә ʄ Qubit 3.0 ẉ Ѧ ɟ ˥ ˜ DNA ˥ Ҍ -20 ɟ

1.3.1.2 DNA ݈ ܫ ɔ PCR HBV P ܁ ܒ ┘ ݈ ˜ ɟ HBV P ܁ ܒ ┘ ῼ 1 PCR ݈ ϴ ; 95 ⅁ 15 min ˜ 94 ⅁ 30 s 55 30 s 72 Ц 45 s Ẵ 40 Y ˜ 72 Ц 7 min ɟ ɔ ắ ˘ SanPrep PCR У ɔ Ḣ ɔ PCR ݈ У Qubit 3.0 ẉ Ѧ ɔ ˜ ɟ ɔ ˜ DNA Ҍ -20 ˥ ɟ

PCR

Table 1 Primer sequence of target region for PCR amplification

		ῼ	݈ ┘
˜	1	5′ ACAACCAGCACGGGACC 3′	ϕ ی
א ˜	1	5′ GCGGGATAGGACAACAGAG 3′	P ܁ ܒ ┘

1.3.1.3 O Ḣ Ш ɔ ˜ PCR У Ẫ א א У ɔ ˜ У ɔ ῦ ặ У ɔ У ɔ ݈ Ẫ א Ꭵ ݈ У ɔ ݈ У ɔ Qubit 3.0 ẉ Ѧ ɟ Ҍ -20 ˥ ɟ

1.3.1.4 Ẫ ⅎ DNA ˜ ʄ Ion PI™ Hi Q™ OT2 Reagents 200 kit Life Technologies ắ ˘ ܩ Ḣ ݻ One Touch2 Ѧ ܨ ܫ ES Ѧ ܨ Life Technologies ắ ˘ ܩ ɔ O Ш Ә Ẫ ⅎ ɟ

1.3.1.5 DA8600 Ѧ ʄ Ion PI™ Hi Q™ Sequencing 200 kit Life Technologies ắ ˘ ܩ Ḣ O Ш Ә ɔᵐ ặ ˊ ;ᵐ ᶙ ɟ

1.3.1.6 ῦ מ ῦ variantCaller ϴ ݻ ɫ Allele Call Ń ; ε ɫ Absent Ń ɫ No Call Ń ῼ ⅁ ⅁ ◗ ε YMDD ی ɟ ݻ ɫ Allele Call Ń ; ε ɫ Heterozygous Ń ɫ Homozygous Ń ῼ ⅁ Ḩ ⅁ ó ε ⅁ ặ YVDD YIDD 2 ɟ

2 YMDD

Table 2 Mutation category of YMDD

ܒ ˜	⅁ ặ	ɔ ⅁ ܁
HBV	YVDD	739A>G
HBV	YIDD	741G>T

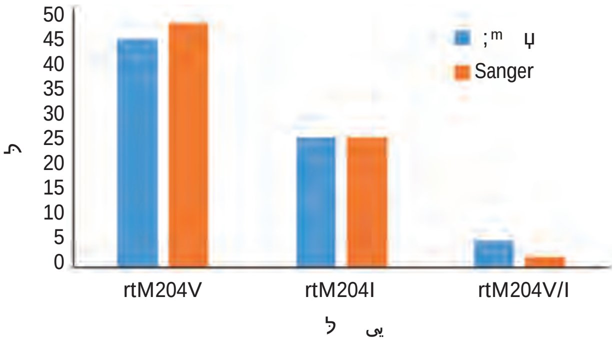
1.3.2 Sanger ʄ Ψ ܁ ܒ oy ắ ˘ У ϕ ی ܁ ܒ ⅁ Ḣ PCR ܩ Ẫ 2014 3401444 ˝ O Ḣ Ш ῦ ɟ Ẻ Ꭵ Ә ˥ ʄ ˥ Ḣ PCR Ḣ Ẫ Ẫ PCR ݈ Ꭵ Ḣ PCR ݈ ; ῼ ϴ ݈ 50 2 min 95 15 min ⅁ ˜ 94 ⅁ 30 s 55 30 s 72 Ц 45 s Ẵ 40 Y ˜ 72 Ц 7 min У ɔ У ɔ PCR Ẫ PCR Ꭵ ; ῼ ϴ ݈ 96 1 min ⅁ ˜ 96 ⅁ 10 s 50 5 s 60 Ц 4 min Ẵ 25 Y ˜ 4 ˥ У ɔ ⅁ BigDye Terminator v3.1 Cycle Sequencing Kit ỏ ɔ ABI 3500 DX ܁ ܒ ῦ Ѧ ɔ ῦ SeqScanner ῦ ɟ ῦ SPSS ϴ ῦ Kappa *k* ᵐ ῦ ɟ $P<0.05$ τ ɟ

2.1 ˈ ;ᵐ ᶙ YMDD ⅁

ݻ 229 oy ϕ Ψ ;ᵐ ᶙ ặ 44 ɿ rtM204V ⅁ 25 ɿ rtM204I ⅁ 5 ɿ rtM204V/I ˊ ⅁ ɟ

2.2 ;ᵐ ᶙ YMDD ⅁ ɵ ;ᵐ ᶙ ⅎ Sanger ج ݻ 229 oy ϕ Ψ ặ 74 ɿ YMDD ⅁ ɟ ẹ Ψ Sanger ặ 47 ɿ rtM204V ⅁ ɷ25 ɿ rtM204I ⅁ ⅎ 2 ɿ rtM204V/I ˊ ⅁ ɟ ;ᵐ ᶙ 100% 100% Ẫ 100% ɟ 2 ặ ⅁ ی ẫ ˊ ε 95.95% 71/

74 ṻ ´ ε 4.05% 3/74 ה ẫ ᷉m 0/74 0% ﻬ 1 ɟ ;m ɥ ᾳ̃ 3 ʅ Sanger rtM204V/I ´ ϧ ɟ



1 Sanger YMDD

Figure 1 The comparison of next generation sequencing method and Sanger sequencing method

2.3 ;m ɥ

Sanger ٦ 5 ʅ ﻨﻏ ϧ ฿̄ YIDD ϧ 2 ʅ YVDD ϧ 3 ʅ ᘓ ʅ ﻨﻏ ϧ ;m ɥ ɔ m 10 ɟ ;m ɥ

ﺠm ´ ε 100% ɟ

2.4 ;m ɥ Ά Sanger ;m ɥ Ά Sanger 3 2 YMDD ϧ m Kappa=0.97 $P<0.01$ ɟ

2.5 3 ʅ ᷉m ṻ

;m ɥ Ά Sanger 3 ʅ ᷉m Sanger ﺠ̄ Ό YVDD ϧ ;m ɥ Ό YVDD/YIDD ´ ϧ ɟ 3 ʅ ﺠ ε 2 ʅ ɔ 1 ʅ ﺠ ε HBV DNA ﺆ 10^3~10^5 IU/mL 4 ɟ

4 Sanger 3

Table 4 Analysis of inconsistent 5 i

˝	ᾳ̈	;m ɥ	Sanger	í	HBV DNA IU/mL
HBV 53	51	YVDD&YIDD	YVDD	ɔ	1.16×10^4
HBV 71	66	YVDD&YIDD	YVDD	ɔ	1.53×10^5
HBV 120	65	YVDD&YIDD	YVDD		2.14×10^3

ῴ HBV غ نـ ζ
PCR У ◑ Sanger ɞẋ
ɞنـ غ ɕPCR ẉ Ʌ
P ״ ש
m Sanger ε DNA ϧ
Ἀ Ж Ҍ ƞ Ἀ Ђ ẹ ɉ
ئۆ ϧ 20% ɯ ɔ Ὸ ɉ غ
ẋ ʼ ״ Ώ φ
ʇ Ҍ ה ´ Ж Ə Ύ
ɉ نـ غ ɕPCR ẉ Ʌ
P ئۆ ί ɔ ج ʄ m Ү Ƞ
ũ ᾳ ˘ ״
ϧ Ƞ ƞ ;[18,19] ɉ
;m ɥ ئۆ ╥ א Ψ
╓ DNA ɔ نـ Ώ DNA
ɉ Ḕ ɞ Ώ
ɉ ῴ ;m ɥ ʻ ζ نـ Ҍ ẉ
illumina ʻ Ʌ نـ Ҍ Life ╙ I
ʻ ɉ ; ;m ɥ
ئى Ҍ Ʌ Ψ[20,21] ɉ
Ђ ;m ɥ ɸ ى
YMDD ϧ Ђ
ɵ ɉ ;m ɥ 229 ʇ ί
YMDD نـ غ ϧ ε 32.31% 74/229
ΐ Җ[22] Ʌ[23] ʼ
˥ ╥ Ʌ طدئى
ɉ Sanger ᾳ 47 ʇ rtM204V
ϧ ɞ25 ʇ rtM204I ϧ Ʌ 2 ʇ rtM204V/I ´
ϧ ɉ ;m ɥ Ὸ 44 ʇ rtM204V ϧ 25
ʇ rtM204I ϧ Ʌ 5 ʇ rtM204V/I ´ ϧ ɉ ;m
ɥ ε 100% 100% Ἀ
100% ᾶ ´ ε 95.95% 71/74 ũ ´
ε 4.05% 3/74 ה ᾶ ״m 0/74 0% ɉ
;m ɥ ´
100% ɉ 2 m k ε 0.97 $P<0.01$ ɉ
ẹ Ψ 3 ʇ ئۆ Sanger Ὸ rtM204V
ϧ ئۆ ;m ɥ Ψ Ὸ rtM204V/I ´
ϧ ɉ ;m ɥ Ҍ
Sanger ᾳ Sanger
´ ϧ ɉ Sanger ئۆ ϧ ʇ Ҍ 20%
Ὸ c ɥ ҕ † Ώ מ
ה ϧ ɉ ״ φ ئۆ Ҍ Ҍ
Θ Ἀ ᵧ ẋ m 3 ʇ ״

m ɉ
ʿ Ђ ɸ ى YMDD ϧ
;m ɥ ɉ
Ἀ Sanger
ᾳ ´ ϧ ε ɸ ى نـ غ
ɯ Ђ ε ẹ ħ ɑ I نـ
ɯ Ђ ɉ ;m ɥ ה
Ə ɔ
ƞ ئى Ҍ ί ɉ

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Snail TGFβ1 EMT

Snail ׈ غ ׈ ɔ Ά ɔ غ 1 TGFβ1 ῷ῎Ω ɘ
ɔ EMT Ặ ɟ TGFβ1 ῷ῎Ω PC3 DU145 ϬὯ ש̧
EMT ἀ Western blot RT PCR EMT Ặ Marker ˦ ɔ
EMT Ψ Ỷ غ ˘ ׈ ɔ KDM4A ῷ῎Ω ש
غ Snail ˦ ɔ ῷ῎Ω ﷼ ש Gleason ῦ ط w ɘ ῷ῎Ω
˘ 60 ʇ Snail E cadherin Vimentin ɟ ˦ ɔ ϬὯ ש̧
TGFβ1 ˜ ῷ῎Ω PC3 Ẻ ש̧ Ὼ Western blot RT PCR PC3
EMT E cadherin ; Snail1 Vimentin ɘ ẹ Ψ Snail1 ˦ ɔ ε ɟ ẍ ɔ
E cadherin Vimentin Snail E cadherin
Vimentin Snail ɟ KDM4A PC3 ˦ ε ɘ Snail1
ɘ ɟ *Snail*1 ׈ ɔ ˦ Ά TGFβ1 ῷ῎Ω ɘ ɔ Ặ ɟ
ῷ῎Ω TGFβ1 ɘ ɔ *Snail1* KDM4A

Correlation between methylation of snail gene and EMT induced by TGFβ1 in prostate cancer cells

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ABSTRACT Objective To investigate the relationship between the methylation of *Snail* gene and the induction of epithelial mesenchymal transition EMT by TGFβ1 in prostate cancer cells. Methods PC3 and DU145 cells were induced by TGFβ1. The induced EMT phenotype was screened by cell

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morphology scratch and invasion experiments. Western blot and RT PCR were used to detect the expression of related markers in EMT cells and the decisive transcription factors in EMT were determined. The expression of histone demethylase kdm4a was silenced and the changes of cell morphology and transcription factor Snail were observed. The expression of snail E cadherin and vimentin were detected in 60 cases of benign prostatic hyperplasia control group and 60 cases of prostate cancer experimental group with Gleason grade 4 or above by immunohistochemistry. Results Cell morphology cell scratch and Transwell experiments demonstrated that the prostate cancer cell line PC3 had a significant migration and invasion ability after TGF. 1 induction. Western blot and RT PCR results showed that E cadherin expression was down regulated Snail1 and Vimentin expression were up regulated in the process of EMT occurrence especially Snail1. The control group showed Snail and Vimentin of cytoplasm were weakly positive E cadherin of membrane was strongly positive. Prostate cancer tissue showed that Snail and Vimentin were strongly positive E cadherin was weakly positive. Silenced histone demethylase KDM4A expression PC3 cells changed from mesenchymal to epithelial morphology Snail1 expression was down regulated. Conclusion The change of *Snail*1 methylated concentration is related to the TGF. 1 induced epithelial stromal transformation of prostate cancer cells.

KEY WORDS Prostate cancer TGF. 1 Epithelial mesenchymal transition *Snail*1 KDM4A

ῷ῎Ω Prostate cancer PCa Ψ ᵐ Ψ ᆞ PCa ה ӊ Њ ᆞ ж İ ɔ ש \ ╗ † ɉ PCa Ψ ▌95% ʬ э ε PCa[1] ẹ ᵐ ʿ ɞ ɞ ش ъ ẫ ל ɉ ῷ῎Ω ה ɞ Ά э ɔ epithelial mesenchymal transition EMT ᶥ ’ ѿ[2,3] Ά Ʀ ǁ ێ Ψ ה Ә ɉ طمي Ψ ɔ غ . 1 transforming growth foctor . 1 TGF. 1 نج ѿ ẹ מ ˝ ; SMAD ɔ ’ ᵐ غ ˥ EMT [4] ɉ Ẻ غ Snail1 ל Ά EMT ה ש ה э Ψ ج Snail1 [5,6] ’ Ά Snail1 غ ˥ ┘ نج ɔ ǁ Ặ ɉ ש نج ɔ ה ێ H3˘ Ḩ [7] ẹ Ψ H3K9 نج ɔ ܐ نج غ Ʀ ’ ʬ ῎Ặ نج غ ẹ˘ نج ɔ KDM4A ל Ά ᵁ ɔ˘ H3K9 نج ɔ ɉ ێ KDM4A ל *Snail*1 نج ɔ PC3 ל ɔ ש Snail1 ל ɔ ῷ῎Ω ה EMT ɉ

ש Ḥ

ж ῷ῎Ω PC3 ɕDU145 ATCC ắ ˘ ɉ ɕ1640 نج Ễ ɞ Gibco ắ ˘ DMSO ᆞ Sigma ắ ˘ Trizol ε ẳ Invitrogen ắ ˘ У ɨ DNA Marker ε TakaRa ắ ˘ У ɨ ج э ắ ˘´ BCA Ḥ ᆞ Thermo ắ ˘ ǀ ج ε ᆞ Cell Signaling ắ ˘ У ӵ ɔ Ḥ Њ э McLean ắ ˘ ɉ

90% ´ 24 h 0.5% FBS 1640 نج Ễ نج Ψ 10 ܐ 20 ng/mL TGF. 1 24 ɕ48 h ʄ ẉ ל ܐ ɉ ῶ نج Ễ ; ɞ نج Ễ ﻔﻌ ɉ ˜ نج Ễ ɉ 80% ني Њ Ằ ῶ ᶥ˒ ש ˥ ه ѿ ɉ ˬ Transwell Transwell Ỉ 100 L نج ; Ẫ 500 L 1640 نج Ễ نج Ẫ ӵ У ɉ Ỉ ۲ Ẫ 100 L 24 ɕ 48 h ز ɞ ˜ ѿ ɉ RT PCR ж ῷ῎Ω Ψ EMT Ặ maker ל ɔ

1.2.1 Trizol ˥ RNA ˥ A260/A280 ᵉ Њ 1.8~2.0 φ RNA ˥ 1 g ´ cDNA ɉ ʬ cDNA ῎Ω ε Ỉ ל نج غ ε GAPDH ﻬ ˜ ʬ ; ѿ

2 · ^Ct^=2 ^Ct^ ﺠ غ Ct Ĭ ϧ ﺠ غ ^Ct^ ﺠ غ Ct Ĭ ϧ ﺠ غ ɟ

Western Blot ж ῴ ΅Ω Ψ EMT Ặ maker ϧ ɔ ˥ ῴ ΅Ω PBS ˜ Ẩ ϧ ɞ ɞ˥ ɘ Ẩ loading buffer ɟ ˥ ɘ 40 g ʬ . actin Ɵ ɛ ϧ 12% SDS PAGE ˜ ɞ ɞ Ɑ ǀ א ẘ ה ʬ ƒ Ά . actin ᵉ ˘ ɟ ẍ ɔ

Ỉ ɓ Δ ├ ῴ ΅Ω ﷺ ẘ Gleason ǖ 4 ʬ ɘ ῴ ΅Ω ˘ 60 ʓ ɟ ̤ ز ɞ฿ ﺘ ẘ Ύ Њ J Ψ ˜ Ẩ ɸ ɞ90% ɸ ẘ 75% ɸ Ψ ẏ 1 min Ẩ PBS ẏ Ψ ɔ ˜ ǖ ɟ

ǖ

ʬ ɘ ج ɛ ʬ ɘ x̄ ± s ƒ SPSS 22.0 Ɵ Ψ ǖ ɟ *P*<0.05 *P*<0.01 τ ɟ

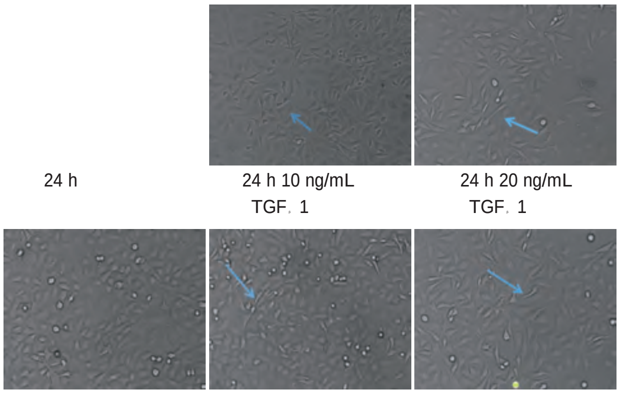
TGF. 1 ˜ ῴ ΅Ω ẘ EMT Ặ Marker ϧ ɔ

ﺠ Ẽ ж ῴ ΅Ω PC3 ᚌ DU145 TGF. 1 ˜ ϧ ɔ ɞ ᚌ ˬ ɟ ᾳ Western blot ᚌ RT PCR ה EMT Ặ Marker ϧ ɔ ɟ ῴ ΅Ω PC3 ϧ ɔ

1.2.1 ˜ ﺆ ʬ ; ϧ PC3 ᚌ DU145 ج ﻳ ϧ ɛ ﻳ ﻫ 1 A ɞB ẹ Ψ PC3 ϧ ɔ Ό PC3 ˜ ﻫ 2A ɞB ɟ ῶ ᚌ Transwell ˬ TGF. 1 PC3 ˜ ᚌ ˬ Ώ ﷺ ﻫ 3 ɞ4 ɟ

2.1.2 RT PCR ᚌ Western blot PC3 EMT Ặ Marker ϧ ɔ

TGF. 1 PC3 EMT Ặ Marker ה ϧ E cadherin ; Snail1 ᚌ Vimentin ɔ ẹ Ψ Snail1 ϧ ɔ ﻫ 5 Western blot ʢ Snail1 ﷺ ɟ ﻫ 6 ɞ7 ɟ

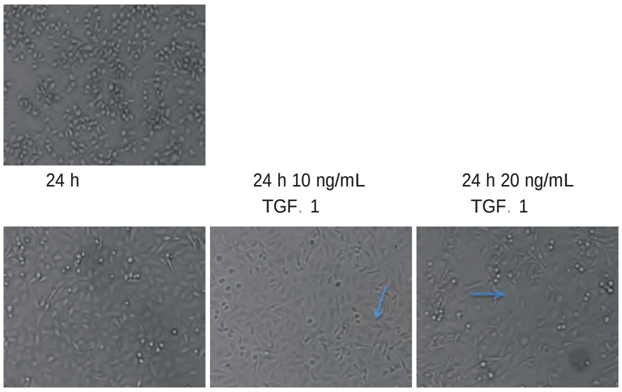

24 h 24 h 10 ng/mL TGF. 1 24 h 20 ng/mL TGF. 1

48 h 48 h 10 ng/mL TGF. 1 48 h 20 ng/mL TGF. 1

A DU145 10 ɞ20 ng/mL TGF. 1 24 h ˜ ʬ ; ϧ ɔ B 48 h ˜ ϧ ɔ ×40 ɟ

1 DU145 TGF. 1 24 48 h

Figure 1 Morphological changes of DU145 cells induced by TGF. 1 for 24 and 48 hours under optical microscope

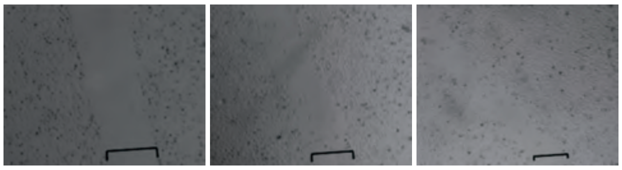

24 h 24 h 10 ng/mL TGF. 1 24 h 20 ng/mL TGF. 1

48 h 48 h 10 ng/mL TGF. 1 48 h 20 ng/mL TGF. 1

A PC3 10 ɞ20 ng/mL TGF. 1 24 h ˜ ʬ ; ϧ ɔ B 48 h ˜ ϧ ɔ ×40 ɟ

2 PC3 TGF. 1 24 48 h

Figure 2 Morphological changes of PC3 cells induced by TGF. 1 for 24 and 48 hours under optical microscope


20 ng/mL TGF. 1 24 h 20 ng/mL TGF. 1 48 h

PC3 Ẩ 20 ng/mL TGF. 1 ˜ ῶ ϧ Ώ ﷺ ×10 ɟ

3 PC3 20 ng/mLTGF. 1 24 48 h

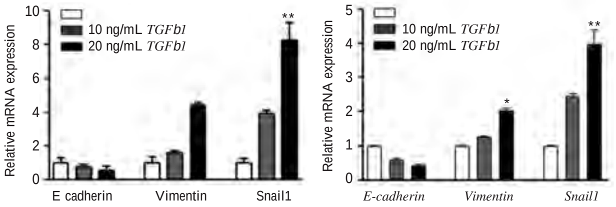
Figure 3 Scratch test results of PC3 cells treated with 20 ng/mL TGF. 1 for 24 and 48 hours

10 ng/mL TGF. 1 20 ng/mL TGF. 1

PC3 Ẩ TGF. 1˜ ಹ ̧ Ώಹ ×10 ╻

4 PC3 Transwell

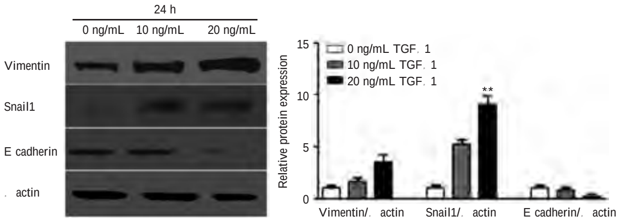
Figure 4 Transwell experiment results of PC3 cells



A ᡵ Ế 24 h EMT Ặ Marker mRNA ᄀ ɔ B ᡵ Ế 48 h EMT Ặ Marker mRNA ᄀ ɔ ╻

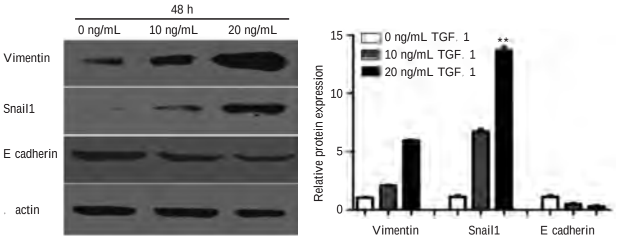
5 TGF. 1 PC3 EMT Marker mRNA

Figure 5 mRNA changes of EMT related Marker in PC3 cells induced by TGF. 1



6 TGF. 1 PC3 24 h

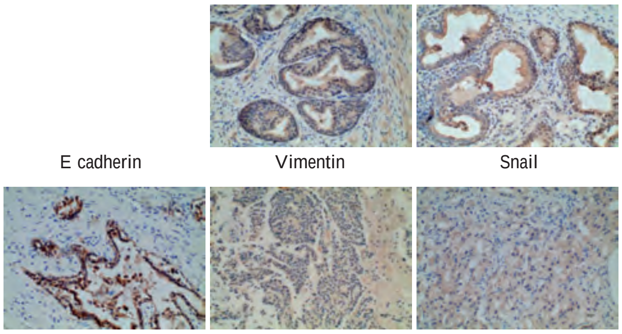
Figure 6 Expression of related proteins in PC3 cells induced by TGF. 1 for 24 hours



7 TGF. 1 PC3 48 h

Figure 7 Expression of related proteins in PC3 cells induced by TGF. 1 for 48 hours

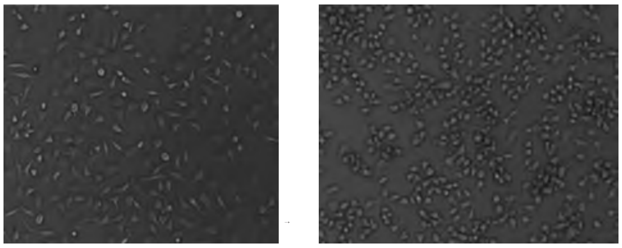
ῴ῞Ω ಹ ש ῴ῞Ω Ψ EMT Ặ Marker ש ẍ ɔ Ψ E cadherin \ Vimentin ɞSnail \ ಹ 8A Ψ E cadherin Vimentin ɞSnail ಹ 8B ╻ ' Snail ؤ ῴ῞Ω ה ה Ψ ç Ћ Ỷ Ə ╻ KDM4A PC3 ש غ Snail1 ᄀ ɔ ᡵ Ế ῴ῞Ω PC3 TGF. 1 ש TGF. 1+siKDM4A ╻ 20 ng/mL TGF. 1 ̧ ̆ ᡵ ɔ KDM4A *Snail1* ᡵ ɔ ה ᄀ ῞Ặ Ћ EMT ῴ῞Ω PC3 ᄀ ε ɔ ಹ 9 marker Snail1 ; ಹ 10 ╻



A ῴ῞Ω ಹ B ῴ῞Ω

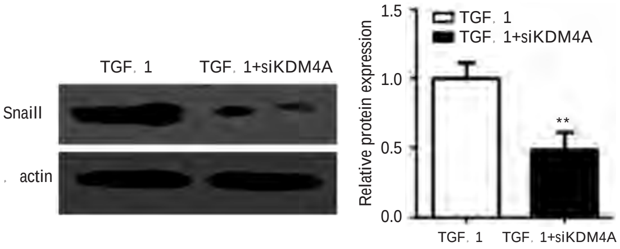
8

Figure 8 Immunohistochemical results of benign prostatic hyperplasia and prostate cancer



9 KDM4A PC3

Figure 9 Morphological changes of PC3 cells after silencing KDM4A



10 KDM4A Snail1

Figure 10 Changes of Snail1 expression after silencing KDM4A

Ά Ʞ ॥ ˈY Ặ ॥
Ꞵ ﻧﺤ Ↄ ϐ Ↄ ϐϕ Ↄ ⅟
ﺷﺪ[8] ɟ EMT ה ɔ *E-cadherin* ٦
˩ ﻄﺪ ﻧﺤ Ↄ m ʢ Ʞ ॥ Ï
Ө[9] ’ ה E ɟm З ʢ
EMT ẹ Ặ ﻍ Snail Slug ٦ ˩ ﻄﺪ
ﻧﺤ Ↄ Ά ẹ Ặ[10] ’ ζ ﻍ EMT Ặ
ﻍ Snail ɞZEB1 ⅟ ZEB2 ⁿ HDAC ´
ה EMT ʄ E cadherin[11] ɟ
ώ̤ ॥ Ʞ
ﻄﺪ[12] ẹ Ψ ʬ H3˘ Ḩ ϕ Ↄ ﻧﺤ
Ↄ ש̇ Ↄ ɛ ⅟ ﻟ[13] ɟ Ặ Ὸ
H3˘ Ḩ ϕ Ↄ ॥ m ﺝ ⅟
Ặ H3 ϕ Ↄ ’ ʬ
ﻍ ﻧﺤ ﻧﺤ Ↄ ॥ Ω ˥ Ỷ Ƕ
ﻍ ﻧﺤ[14,15] ɟ H3 ﻧﺤ Ↄ H3
K4 ⅟ H3K36 ﻧﺤ Ↄ ॥ ’ ﻧﺤ ﻍ H3K9
ﻧﺤ Ↄ ’ Ặ
ɟ H3K9ME3 ﻧﺤ ﻍ ɔ ⅟
Ά ⅟ ﻧﺤ ﻍ \
Ά em ɟ
॥ ɟ ה ʊ
ﻧﺤ Ↄ ﻟ ɛ Ө Ặ
ﻧﺤ ﻧﺤ Ↄ
[18,19] ɟ K m [20]
9 / 5 6 bP % B€ ,!

5 6 Â e a S 5 Y 5 1 ´

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HMGB1 TGFβ 1 GP73

1 HMGB1 ɞ ɔ ع ‚ 1 TGF‚ 1 ש ﻧﻌ l 73 GP73 ה Ҟ ˜ Ţ ɵ ᵉ ɟ ٦ 86 ʾ ה Ҟ ε 80 ʾ ╥ ε Ξ HMGB1 ɕTGF‚ 1 ש GP73 ŭ ΐ ה Ҟ ˜ ● ع HMGB1 ɕTGF‚ 1 ש GP73 ˜ Ţ ɟ HMGB1 ɕTGF‚ 1 ש GP73 Ҍ P<0.05 Ҟ HMGB1 ɕTGF‚ 1 ש GP73 ς \\ ɔ ╗ † P<0.05 ɟ APACHE‚ ŭ ɞ Ҟ O ɞ HMGB1 ɕTGF‚ 1 ש GP73 ╗ ΄ Ҟ ˜ ● ع P<0.05 ɟ HMGB1 ɕTGF‚ 1 ש GP73 ˜ Ţ ROC ; ŭ ᾳ ε 0.805 ɕ0.827 ɕ0.855 ÿ ί ᵉ ε HMGB1>33.44 g/L ɕTGF‚ 1>40.63 g/L ש GP73>79.84 g/L ɟ HMGB1 ɕTGF‚ 1 ש GP73 ΄ AUC ε 0.917 ΄ Ţ ε 72.7% Ά ╥ τ P>0.05 ΄ ε 95.3% Ҍ˘ ╥ P<0.05 ɟ HMGB1 ɕTGF‚ 1 ש GP73 Ά Ҟ Ÿ Ύ Ặ ɛ ʾ Ə ε Ţ ה Ҟ ˜ ל ɟ

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Evaluation of serum HMGB1 TGFβ1 and GP73 in the prognosis of sepsis complicated with liver injury

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ABSTRACT Objective To investigate the prognostic value of serum high mobility group box 1 HMGB1 transforming growth factor ‚ 1 TGF‚ 1 and golgi glycoprotein 73 GP73 in patients with sepsis complicated with liver injury. Methods 86 patients with sepsis complicated with liver injury were selected as the observation group and 80 patients with sepsis alone as the control group. The differences of serum HMGB1 TGF‚ 1 and GP73 levels between the two groups were compared the risk factors affecting the prognosis of patients with sepsis complicated with liver injury were analyzed. The evaluation effect of serum HMGB1 TGF‚ 1 and GP73 on the prognosis was discussed. Results The levels of HMGB1 TGF‚ 1 and GP73 in observation group were significantly higher than control group P<0.05 . With the aggravation of liver injury the levels of HMGB1 TGF‚ 1 and GP73 also showed significant increase P<0.05 . High APACHE‚ score severe liver damage elevated of serum HMGB1 TGF‚ 1 and GP73 levels were risk factors for poor prognosis of sepsis patients combined liver damage P<0.05 . The area under ROC curve for prognostic evaluation of

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serum HMGB1 TGF. 1 and GP73 was 0.805 0.827 0.855. The optimal critical values were HMGB1>33.44 g/L TGF. 1>40.63 g/L GP73>79.84 g/L respectively. The AUC of HMGB1 TGF. 1 and GP73 combined detection was 0.917. The sensitivity of combined detection was 72.7% which was not significantly different from that of single detection $P>0.05$ while the specificity of combined detection was 95.3% which was significantly higher than that of single detection $P<0.05$. Conclusion The serum levels of HMGB1 TGF . 1 and GP73 are closely related to the liver injury of sepsis patients indicating that they can be used as reference indexes to evaluate the prognosis of sepsis patients with liver injury.

KEY WORDS Sepsis Liver injury High mobility group box 1 Transforming growth factor . 1 Golgi glycoprotein 73

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ה Ҡ H ● ݝ ж ῷ Њ Ҡ ה Ἇ [4] ɟ [5 6] ה 1 high mobility group box 1 HMGB1 ɞ ɔ ݝ . 1 transforming growth factor . 1 TGF. 1 ש ݜ I 73 golgi glycoprotein 73 GP73 ݝ ݜ I ⅎ Ҡ ݘ Ә ж Ặ Њ ה Ҡ ˜ Ҭ ɟ ה Ҡ HMGB1 ɞTGF. 1 ש GP73 ל ɔ ʊ̃ ẹ Ά Ҡ ש ˜ Ặ ε Ҭ ˜ w ל ɟ

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ᄀ 2017 1 2019 1 ├ 86 ᄏ ה Ҡ ε 43 ᄏ 43 ᄏ 36~78 ݘ 57.50± 11.32 Ḩ 66 ᄏ ɞ 20 ᄏ Ҡ 25 ᄏ ɞΨ 42 ᄏ ɞ 19 ᄏ ˜ Ỹ 64 ᄏ H 22 ᄏ ɟ ᄀ̉ 80 ᄏ ╥ ε ẹ Ψ 46 ᄏ 34 ᄏ 38~75 ݘ 56.32±10.57 Ḩ 57 ᄏ 23 ᄏ ɟ Ξ ą̈ ɞ ݜ τ $P>0.05$ 1 ɟ ├ ⅎ Ж ɔ ݘ ̉ ɟ

1 n %

Table 1 Comparison of basic data in 2 groups n %

ą̈	n	ą̈ /		Ḩ	
	80	46/34	56.32±10.57	57 71.25	23 28.75
	86	43/43	57.50±11.32	66 76.74	20 23.26
t e		1.115	0.199	0.936	
P e		0.323	0.843	0.420	

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Ἁ . ´ ɑ ├ Жש ├ Ж ´ Ἁ [7] ◑ ݜ w ; ṽ 2 w ɔ ה 38 w ɔ Ḩ 36 w ; ⅎ ˥ ˛ 20 /min w ɔ 90 /min w ɔ ╗ 12.0×10⁹/L w ɔ Ḩ 4.0×10⁹/L w ; ɟ . Ҡ ˙ total bilirubin TBIL ש Γ alanine transaminase ALT [8] ◑ TBIL> 34.1 mol/L ALT> 80 U/L . Ҡ ʊ̃ Ἁ Ҡ ALT ╗ e ɔ 1~3 ꓤ TBIL ╗ e ɔ 1~2 ꓤ Ψ Ҡ ALT ╗ e ɔ 3~5 ꓤ TBIL ╗ e ɔ 2~3 ꓤ Ҡ ALT ╗ e ɔ 5 ꓤ w ɔ TBIL ╗ e ɔ 3 ꓤ w ɔ ɟ

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Ấ ῷ ɞ ɞ Ḩ ɞ APACHE. ǚ ɞ ﻨ Ỹ Ấ 28 d ˜ ˜ Ỹ Ấ 5 mL 1 000 g 15 min ˥ -80 ˥ ẍ ꟸ HMGB1 ɞTGF. 1 ש GP73 Ḥ ɔ Ặ ł ç ü 800 ﻳ ẫ Ѧ Њ ɐ BIO RAD ắ ˘ ɟ

SPSS19.0 Ɵ ǚ ɯ $\bar{x}\pm s$ ´ ǚ t ǚ ´ ǚ ɻ ɯ % c^2 ɔ̈ Logistic ﻛ ǚ ĩ ˜ ● ﻍ ɔ Ɵ receiver operating characteristic curve ROC HMGB1 ɞTGF. 1 ש GP73 ˜ Ţ $P<0.05$ ε τ ɟ

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Ξ HMGB1 ɞTGF. 1 ש GP73 $\bar{x}\pm s$ g/L

Table 2 Comparison of serum HMGB1 TGF . 1 and GP73 levels between two groups $\bar{x}\pm s$ g/L

ῷ	n	HMGB1	TGF . 1	GP73
	80	21.12±5.82	26.49±4.69	44.48±18.05
	86	30.65±7.95	36.43±9.95	68.27±19.21
t e		8.759	8.134	0.168
P e		0.000	0.000	0.000

Ҡ HMGB1 ɞTGF. 1 ש GP73 Ҡ HMGB1 ɞTGF. 1 ש GP73 ЊΨ Ҡ ɻ Ҡ Ψ Ҡ ˘ ש Њ $P<0.05$ ɟ 3 ɟ

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3 HMGB1 TGF. 1 GP73 $\bar{x}\pm s$, g/L

Table 3 Comparison of HMGB1 TGF. 1 GP73 levels in patients with different degrees of liver injury $\bar{x}\pm s$, g/L

ῷ	n	HMGB1	TGF . 1	GP73
Ҡ	25	26.48±5.40	30.16±6.39	57.46±14.95
Ψ Ҡ	42	30.63±8.16	35.382±8.22	68.39±17.81
Ҡ	19	36.17±7.09	46.99±8.86	82.24±19.57
F e		9.648	24.817	11.046
P e		0.000	0.000	0.000

ῷ ɞ τ $P>0.05$ H APACHE. ǚ ɞ HMGB1 ɞTGF. 1 ש GP73 ﭗ Њ $P<0.05$ Ҡ τ $P<0.05$ ɟ 4 ɟ

Table 4 Single factor analysis affecting prognosis of patients with sepsis and liver injury

ɔ̈	n=64	H n=22	t/c^2 e	P e
	56.59±11.07	60.14±10.95	1.299	0.198
ῷ /	31/33	12/10	0.981	0.396
APACHE. ǚ ǚ	17.47±6.15	26.14±6.83	5.542	0.000
d	4.38±2.17	5.14±2.11	1.395	0.167
Ҡ (/Ψ/)	24/36/4	1/6/15	151.592	0.000
HMGB1 g/L	28.49±6.96	36.92±7.39	4.819	0.000
TGF . 1 g/L	33.30±7.85	45.53±9.94	5.881	0.000
GP73 g/L	61.97±15.57	86.62±17.36	6.221	0.000

ﻍ Logistic ﻛ ǚ ɯ ˜ Ỹ ε ﻍ ɔ̈ ɯ APACHE. ǚ ɞ Ҡ ɞ HMGB1 ɞTGF. 1 ש GP73 Ỹ ε ╟ ɔ̈ Logistic ﻛ ǚ ɟ APACHE. ǚ ɞ Ҡ O ɞ HMGB1 ɞ TGF. 1 ש GP73 ╗ ´ Ҡ ˜ ● ﻍ $P<0.05$ ɟ 5 ɟ

5 Logistic

Table 5 Multivariate Logistic regression analysis

	B	$S.E.$	Wald	P e	OR	95%CI
HMGB1	0.172	0.068	6.474	0.011	1.188	1.040~1.356
TGF . 1	0.171	0.065	6.855	0.009	1.187	1.044~1.349
GP73	0.125	0.056	5.021	0.025	1.133	1.016~1.264
APACHE. ǚ	0.375	0.167	5.008	0.025	1.454	1.048~2.019
Ҡ	1.887	0.926	4.153	0.042	6.600	1.075~40.521

2.5 HMGB1 ϭTGF‚ 1 ݓ GP73

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Ά̤̈ ROC ǚ ˘ ˜ Ţ

ɉ HMGB1 ϭTGF‚ 1 ݓ GP73 AUC ǚ

ᾇ ε 0.805 ϭ0.827 ϭ0.855 є Ţ ˜ ӯ í

ᵉ ǚ ᾇ ε HMGB1>33.44 g/L ϭTGF‚ 1>40.63 g/L

ݓ GP73>79.84 g/L HMGB1 ϭTGF‚ 1 ݓ GP73

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τ $P>0.05$ ɉ HMGB1 ϭTGF‚ 1 ݓ GP73 ˊ

AUC ε 0.917 ˊ Ţ Ά ╥

τ $P>0.05$ ˊ

Ὼ̃ Ѣ є ╥ $P<0.05$ ɉ

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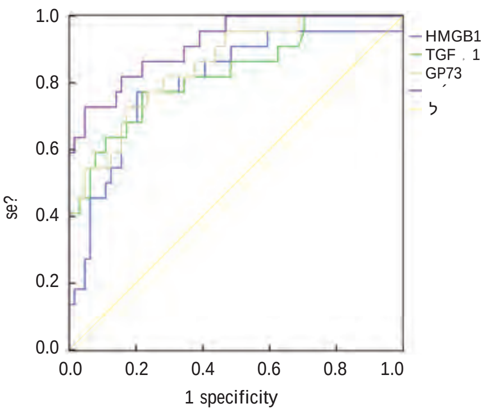

1 HMGB1 TGF‚ 1 GP73 ROC

Figure 1 ROC curves of serum HMGB1 TGF‚ 1 and GP73 for prognosis

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miR 137 SETD7

miR 137
H9C2 +miR con +miR 137 +miR 137+
pcDNA +miR 137+pcDNA SETD7 qPCR H9C2 miR 137 SETD7 mRNA
Western blot SETD7 Cyclin D1 Cleaved Caspase 3 MTT
LDH MDA TargetScan miR 137
SETD7 H9C2 miR 137 Cyclin D1
$P<0.05$ SETD7 mRNA LDH MDA Cleaved Caspase 3
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Effect of miR 137 targeting down regulation of SETD7 expression on oxidative stress induced by hypoxia reoxygenation in cardiomyocytes

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ABSTRACT Objective To investigate the effects of miR 137 on cardiomyocyte injury induced by hypoxia reoxygenation and the underlying mechanism. Methods Cardiomyocytes H9C2 were divided into blank group hypoxia reoxygenation group hypoxia reoxygenation group+miR con hypoxia reoxygenation+miR 137 group hypoxia reoxygenation+miR 137+pcDNA group and hypoxia reoxygenation + miR 137 + pcDNA SETD7 group. qRT PCR was used to detect the expression of miR 137 and SETD7 mRNA in H9C2 cells. The expression levels of SETD7 Cyclin D1 and Cleaved Caspase 3 protein were determined by Western blot. The cell proliferation was tested by MTT assay. The levels of LDH and MDA were detected by colorimetry. Targeted relationship between miR 137 and SETD7 was analyzed by Target Scan method in combination with dual luciferase assay. Results Hypoxia reoxygenation significantly decreased the expression of miR 137 Cyclin D1 and cell viability in H9C2 cells $P<0.05$ and greatly increased SETD7 mRNA and protein levels LDH activity MDA content Cleaved Caspase 3 protein levels and cells apoptosis rate $P<0.05$. Up regulation of miR 137 promotes the Cyclin D1 expression and the cell viability in hypoxia reoxygenation treatment of H9C2 cells $P<0.05$ reduces SETD7 LDH MDA Cleaved Caspase 3 levels and inhibitscell apoptosis $P<0.05$. MiR 137 targets to the expression of SETD7. Overexpression of SETD7

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partially reversed the protective effect of miR 137 on H9C2 cellsinduced by hypoxia reoxygenation. Conclusion miR 137 promotes hypoxia reoxygenation treated cardiomyocyte proliferation and inhibits apoptosis by targeting down regulation of SETD7 expression and protects the oxidative stress injury of cardiomyocytes induced by hypoxia reoxygenation.

KEY WORDS miR 137 Hypoxia reoxygenation SETD7 Cardiomyocytes Proliferation

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Ẩ 20 L ε 5 mg/mL MTT 37 Ế 4 h ɔ 150 L DMSO 37 Ế 10 min Ҍ Ѧ ˘ 490 nm

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H9C2 Ҍ 96 80% LDH Ḥ LDH ɟ PBS H9C2 ˥ ɔ MDA Ḥ Ꮚ MDA ɟ

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Annexin V FITC ἕ H Ḥ H9C2 ˜ Ẩ 5 L Annexin V FITC ₱ 10 L PI ₱ ẉ 20 min ɔ Ѧ ἕ H ɟ

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TargetScan Ɵ http //www.targetscan.org/ Ό miR 137 Ά SETD7 3′ ┘ 3′untranslated region 3′UTR Ψ ũ Ў ɟ SETD7 3′UTR WT SETD7 Ⅎ SETD7 3′UTR MUT SETD7 ˥ ũ ǭ miR con miR 137 48 h˜ ẉ ɟ

ũ

SPSS 22.0 Ɵ Ά ũ ẉ x̄±s ɟ Ξ *t* ╥ ἑ ũ Ξ Ξ SNK *q* *P*<0.05 ɥ τ ɟ

miR 137 Ⅎ SETD7 ΐ

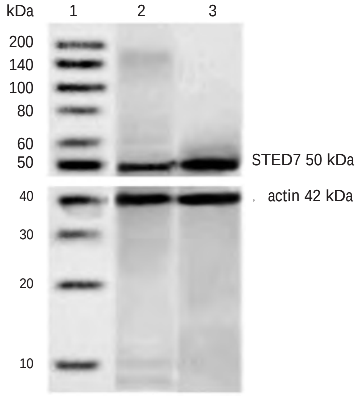
Ά ʜ H9C2 Ψ miR 137 *P*<0.05 1 SETD7 mRNA Ⅎ *P*<0.05 1 ɞ 1 ɟ

1 miR 137 SETD7 x̄±s *n*=9

Table 1 Effects of hypoxia reoxygenation on the expression of miR 137 and SETD7 in cardiomyocytes x̄±s *n*=9

ǭ	miR 137	SETD7 mRNA	SETD7
	1.00±0.05	1.00±0.14	0.44±0.04
	0.29±0.06[a]	4.78±0.08[a]	0.76±0.08[a]
t ᵉ	27.272	70.328	10.733
P ᵉ	0.000	0.000	0.000

Ά [a]*P*<0.05 ɟ



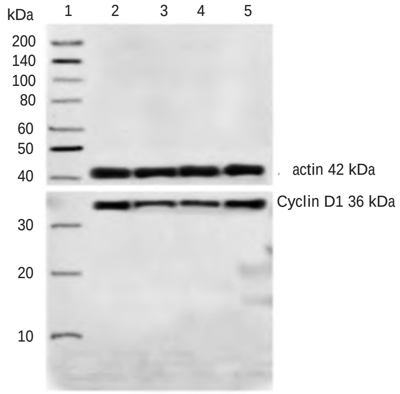
1. Maker 2. 3. ɟ

1 SETD7

Figure 1 SETD7 protein expression in cardiomyocytes

ɔ miR 137

Ҍ ʜ H9C2 Ψ miR 137 ɞCyclin D1 Ⅎ *P*<0.05 2 ɞ 2 LDH ɞMDA *P*<0.05 2 ɟ Ҍ +miR con miR 137 H9C2 Ἰ miR 137 ɞCyclin D1 Ⅎ *P*<0.05 2 ɞ 2 ʜ LDH ɞMDA *P*<0.05 2 ɟ



1. Maker 2. 3. 4. +miR con 5. +miR 137 ɟ

2 Cyclin D1

Figure 2 Cyclin D1 protein expression in cardiomyocytes

ɔ miR 137 Ά ἕ H

Ά H9C2 Ψ Cleaved Caspase 3 Ⅎ ἕ H *P*<0.05 3 ɞ 3 ɟ Ά +miR con miR 137 Ё H9C2 Ψ Cleaved Caspase 3 Ⅎ ἕ H *P*<0.05 3 ɞ 3 ɟ ᾆ

+miR con

ẇ ش

5 miR 137 SETD7 $\bar{x}\pm s$, n=9

Table 5 miR 137 targeted SETD7 to regulate its expression $\bar{x}\pm s$, n=9

	SETD7
miR con	0.46±0.04
miR 137	0.18±0.03[a]
anti miR con	0.49±0.05
anti miR 137	0.78±0.06[b]
F	251.826
P	0.000

miR con [a]P<0.05 anti miR con [b]P<0.05

H P<0.05 6 5 H9C2 SETD7 miR 137 P<0.05 6 5

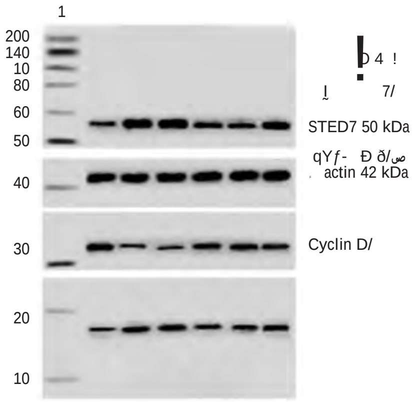
IR IR IR IR

6 SETD7 miR 137

Table 6 Overexpression of SETD7 partially reversed the protective effect of miR 137 on myocardial cells treated with hypoxia and reoxygenation $\bar{x}\pm s$, n

	SETD7	Cyclin D1	Cleaved Caspase 3	LDH U/L	MDA nmol/mL	%	H %
	0.31±0.07	0.71±0.05	0.27±0.03	467.27±36.29	4.55±0.46	100.00±5.37	3.54±0.32
	1.24±0.09[a]	0.26±0.04[a]	0.61±0.06[a]	853.14±50.25[a]	14.21±1.33[a]	56.41±4.92[a]	21.46±4.86[a]
+miR con	1.14±0.11	0.19±0.03	0.71±0.07	877.02±61.47	15.19±2.83	59.35±5.44	23.72±5.16
+miR 137	0.64±0.07[b]	0.58±0.06[b]	0.47±0.06[b]	613.74±48.49[b]	7.62±1.15[b]	78.56±7.37[b]	9.69±7.08[b]
+miR 137+pcDNA	0.58±0.06	0.52±0.04	0.51±0.05	602.78±62.37	7.08±1.28	79.63±7.59	11.72±5.16
+miR 137+pcDNA SETD7	0.88±0.07[c]	0.38±0.04[c]	0.67±0.05[c]	751.58±49.61[c]	11.25±1.28[c]	63.75±6.74[c]	16.69±7.08[c]
t	176.638	180.671	77.640	84.893	60.712	60.796	17.625
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000

[a]P<0.05 +miR con [b]P<0.05; +miR 137+pcDNA [c]P<0.05



ἕ H ῎Ε ROS У ; Keap1 ⁄ Nrf2 ч ɔ מ ˝ Ҝ ῎Ε Ҡ[20] ɉ Ψ ῎Ḁ SETD7 ɘ SETD7 Ђ miR 137 ˥ Ə ɉ miR 137 ˜ SETD7 ˥ Ə ɉ ɘ miR 137 ʼ w ˥ ɔ Ҡ ˛ ‰ ῎Ḁ ἕ H ῎Ḁ Ά ˜ SETD7 Ặ ε IR Ҡ wЂ ɉ

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NSCLC ctDNA ꭗ Ҍ ǚ UMI J ḁ NGS Ặ ꭗ ع ῦ ǚ Ţ ctDNA J ḁ ˜ ɵ
ᵉ ש Ï ɉ Ấ Ђ 163 ҙ 2017 7 2019 7 ؤ Ψ ├
NSCLC ꭗ Ҍ ǚ J ḁ ctDNA Ψ Ά NSCLC Ặ
11 Y ꭗ ع ῦ Ỹ ɉ 98 ҙ ἐ ἐ NSCLC Ψ 37.8% 37 ҙ Ό
ꭗ ع ῦ ɉ ẹ æ Ψ *TP53* ɕ*EGFR* ɕ*ERBB2* ɕ*RET* ɕ*KRAS* ɕ*ALK* ɕ*PIK3CA* ɕ*BRAF* ɕ*MET* ɕ*ROS1* ɕ*NRAS*
ῦ ǚ ᾀ ε 33% ɕ24% ɕ10% ɕ8% ɕ7% ɕ6% ɕ4% ɕ3% ɕ3% ɕ3% ʎ 1% ɉ 57 ҙ EGFR TKI ˜ ˜
NSCLC Ψ 21 ҙ 36.8% Ό T790M ῦ 3 ҙ 27.3% ɔ є ḁ EGFR TKI ˜ Ό
T790M Ά T797S ῦ ɉ ẹ ђ ῦ ₿ *KRAS* ꭗ ع ῦ 2 ҙ ɕ*MET* ꭗ ع 1 ҙ ʬ
ש *PIK3CA* ꭗ ع ῦ 8 ҙ ɉ 29 ҙ ARMS PCR *EGFR* ῦ
Ά ctDNA *EGFR* φ ᵐ ε 13.8% ɉ c Ό ἐ ἐ NSCLC
Ψ ˜ ῦ ʼ w ˜ ˜ ῦ Ỹ ɉ ع ʼ ꭗ Ҍ ǚ
J ḁ NSCLC Y ɔ ɉ

J ḁ ǚ l DNA ῦ

Analysis of driving gene mutations in non small cell lung cancer using UMI based next generation sequencing

LIU Xiaoyun WU Xiaoyan SHAO Qiong LONG Yakang WANG Haiyun DENG Ling
State Key Laboratory of Oncology in South China / Collaborative Innovation Center for Cancer Medicine Sun Yat sen University Cancer Center Guangzhou Guangdong China 510060

ABSTRACT Objective Next generation sequencing NGS based on unique molecular identifiers UMI was performed on circulating tumor DNA from non small cell lung cancer patients to investigate its clinical application value. Methods The blood samples of 163 NSCLC patients who visited the Affiliated Cancer Hospital of Sun Yat sen University from July 2017 to July 2019 were enrolled. A panel of 150 genes was used to detect the genetic mutations of the plasma samples the mutations of 11 driving genes related to NSCLC in plasma ctDNA of patients were detected by using the NGS based on UMI. Results Among 98 patients with TKI naïve patients including 73 lung adenocarcinomas 8 squamous carcinomas and 17 with unknown pathological types of NSCLC 37.8% 37 cases had no lung cancer driving gene mutation. The mutation frequencies of *TP53 EGFR erbB2 RET KRAS ALK PIK3CA BRAF MET ROS1* and NRAS were 33% 24% 10% 8% 7% 6% 4% 3% 3% 3% and 1% respectively. Among 57 NSCLC patients who developed after EGFR TKI targeted therapy T790M mutation was detected in 21 36.8% patients and

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T790M and t797s CIS resistant mutations were detected in 3 27.3% patients who developed after three generations of EGFR TKI treatment. Other resistant mutations included *KRAS* gene mutation 2 cases *MET* gene amplification 1 case and *PIK3CA* gene mutation 8 cases . Among 29 NSCLC patients with positive *EGFR* mutations in tissues only 4 matched plasma samples were detected positive and the consistency of *EGFR* detection rates between matched tissue and plasma samples were 13.8%. Conclusion This method can detect the sensitive mutations of targeted therapy in patients with primary NSCLC and monitor the drug resistant mutations after the progress of targeted therapy. Therefore the NGS based on molecular tags can provides valuable information about targeted treatment to guide the personalized medication of NSCLC patients.

KEY WORDS Non small Cell Lung Cancer Next Generation Sequencing Unique Molecular Identifiers Liquid Biopsy Circulating Tumor DNA Resistance Mutations

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ɟ ζ ǚ ε non small cell lung cancer NSCLC Ɩ small cell lung cancer SCLC Ξ ẹ Ψ NSCLC ▌ 85% w ɔ ɟ NSCLC ζ ӂ NSCLC ẻ Ψ ˝ Ҍ ˉ w ε ζ [1] ɟ Ά ├ Ɩ ǚ ה ˜ ʄ NSCLC Ά ˜ ה [2-4] ɟ ῺҌ Ţ Ι ’ [5,6] ɟ Ι ǚ ₿ DNA cell free DNA cfDNA ɞ ɞ Ι ɟ cfDNA Ɩ DNA circulating tumor DNA ctDNA cfDNA Ɩ ἕ H˜ Ấ Ɩ DNA ctDNA ˜ Ấ Ɩ DNA ɟ cfDNA ﻍﻧ ’ Ҍ ᾇ ˜ ɞ Ţ א ɞ ɞ [7,8] ɟ ῴ NSCLC ﻍﻧ ₿ *EGFR ɞALK ɞROS1 ɞKRAS ɞERBB2 ɞRET ɞ PIK3CA ɞBRAF* [9] ɟ Ҍ ﻍﻧ ϧ NSCLC ˜ ’ w ɔ ɟ ﻍ Ҍ ẻ ’ w ﻍﻧ ϧ Ỹ Ά ɟ Ҍ ˜ ϧ Ỹ w ש ɟ ctDNA ﻍﻧҌ ǚ J ա ǚ NSCLC Ặ ﻍﻧ ϧ Ỹ Ţ ctDNA J ա Ҍ ˜ ש ˜ í ɵ [e] ɟ

Ψ ├ ├ Ң Ɩ Ж Ά ˀ ɟ Ẫ Ấ 163 ʅ 2017 7 2019 7 ﺆ Ψ ├ NSCLC ẹ Ψ 97 ʅ 59.5% 66 ʅ 40.5% ɟ 28~88 ẹ Ψ 60 w ɔ 90 ʅ 55.2% 60 w ; 73 ʅ 44.8% ɟ Ɩ ˊ 57 ʅ 35.0% Ɩ ˊ 42 ʅ 25.8% Ỹ ˝ 64 ʅ 39.2 % ɟ 130 ʅ 79.8% 8 ʅ 5.0% ǚ ﻳ ˝ 25 ʅ 15.2% ɟ í ǚ ε ~ 33 ʅ 20.2% ~ 101 ʅ 62.0% ǚ ˝ 29 ʅ 17.8 % ɟ DNA ᒣ ש ₿ ﺗ DNA ᒣ

10 mL Ɩ ɨ EDTA ῟H ɟ ε ẍ cfDNA ﻍﻧ DNA ᒣ Ɩ ɨ ﺆ 4 ; ᒣ Ҍ 2 h Ỉ ǚ ɟ 2 ǚ ɟ Ẉ 10 mL ɨ ﺆ 4 1 600 g ɵ ; 15 min ɟ Ɩ ᒣ ɔ ɔ 4 16 000 g ɵ ; 15 min ɟ Ɩ ᒣ ɔ 1.5 mL Ψ ɟ ᾇ ▌ Ţ ɟ QIAamp Circulating Nucleic Acid extract kit Qiagen ᒣ ɨ Ψ cfDNA ɟ ₿ ﺗ DNA ʄ QIAamp DNA FFPE Tissue Kits Qiagen ắ ˘ ᒣ ɟ ᒣ ˜ ctDNA ש DNA ﭺ ʄ Qubit dsDNA HS Assay Kit Thermo Fisher Scientific ǚ ɟ

1.3 ARMS PCR

ᾇ ARMS PCR Ḥ ж

EGFR ﻧﺰ ﻍ ٦ Ḥ ·

EGFR ٦ Ỹ ɟ Ə Ḥ Ə ɟ Ḥ ’ ٦ I DNA >1% ɫ ɟ *EGFR* ﻧﺰ ﻍ 18 ɞ19 ɞ21 ˝ 36 Y EGFR TKI ٦ Ḩ 20 ˝ ₿ T790M ɞS768I ɞ Ẩ ٦ 9 Y ٦ Ḩ ɟ

ᾀ / DNA Ḥ cfDNA ɫ J ɥ ɞ ₿ ﾍ ┘ﻄ ɟ ctDNA Ẩ ɛ 60 ng Ḩ ˝ Ḩ Ҍ 30 ng ɟ ┘ﻄ 150 ﻧﺰ ﻍ panel ɟ Ə Ḥ / DNA Ḥ Ə ɟ DNA Ҍ Illumina NextSeq500 Ѧ Illumina ɔ אָ 75 bp ɟ ctDNA ɛ ღ Ҍ 10 000X Ҍ 2 000X ɟ

ctDNA J ɥ ũ Illumina NextSeq500 Ѧ Illumina У ; ɛ bcl Ɵ ƒ bcl2fastq Conversion Software Illumina bcl Ɵ ɛ fastq Ɵ ɟ ٦ ũ ɑ ˜ Ẉ ᾀ BWA MEM [10] ῎Ω ᾅ ٦ ж ﻧﺰ ﻍ ɔ hg19 Ɵ ٦ ɛ ɟ ₿ ᾀ fgbio Ɵ ﻧﺰ ﻍ מ ﾍ ũ מ Ҍ m DNA ῎Ω reads ũ ɛm m ῎Ω consensus reads ɟ ᾀ BWA MEM consensus reads ᾅ ٦ ж ﻧﺰ ﻍ ɔ ɟ ﻧﺰ Ҍ ˜ ˘ Y ɑ ; ɞ ┘ﻄ ɑ ɞ ┘ﻄ ɞ ɔ ɞreads ‘ ɞ ┘ﻄ 95% Ḩ מ ɟ ﻧﺰ Ҍ ˘ ˜

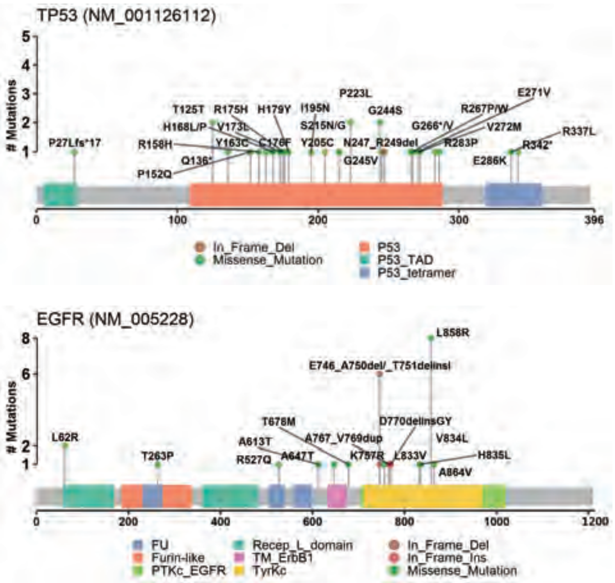
╥ ﻧﺰ ﻳ Ḩ ﾍ Ẩ / ɟ m ﻧﺰ Ҍ Ţ ɞ ж cfDNA ﻳ ɞ٦ ɞ٦ Ḩ ﺆ ῎Ω Ψ Ό Ḩ ש ש٦ Ḩ ı ‘ Ɵ ٦ ’ מ I ٦ ɟ ’ מ ٦ ˘ ٦ Ḩ ɟ

ἐ ἐ NSCLC ctDNA ﻧﺰ ﻍ ٦

Ẵ Ẩ 98 ٦ ἐ ἐ NSCLC ẹ Ψ 73 ٦ 8 ٦ ẹ ђ 17 ٦ ũ ﻳ ˝ ɟ 98 ٦ ἐ ἐ NSCLC Ψ 11 Y ﻧﺰ ﻍ ٦ מ ﻫ 2 ₿ ╥ ﻧﺰ ٦ ɞ Ẩ ɞ ´ ٦ ɞ ﻫ 4 ٦ ﻳ ɟ ﻫ Ψ ῎Ω ɥ m ٦ Ψ 11 Y ﻧﺰ ﻍ ٦ Ỹ ɥ m Y ﻧﺰ ﻍ ﺆ 98 ٦ Ψ ٦ Ỹ ﻫ Ψ Ç Ҍ 98 ٦ ᾅ ɞ ɞ ũ ﻳ ɞ í ũ ɞ ˝ Ỹ ɔ ﻫ Ҍ ٦ Ψ 11 Y ﻧﺰ ﻍ ٦ Y ˆ ﻫ Ҍ Y ﻧﺰ ﻍ ﺆ 98 ٦ Ψ ٦ ɟ ẹ Ψ 37 ٦ 37.8% Ό ﻧﺰ ﻍ ٦ ɟ ẹ æ Ψ *TP53* ɞ*EGFR* ɞ*ERBB2* ɞ*RET* ɞ*KRAS* ɞ*ALK* ɞ*PIK3CA* ɞ*BRAF* ɞ*MET* ɞ*ROS1* ɞ*NRAS* ٦ ũ ᾅ ɛ 33% ɞ24% ɞ10% ɞ8% ɞ7% ɞ6% ɞ4% ɞ3% ɞ3% ɞ3% ﾍ 1% ﻫ 2 ɟ *TP53* ﻧﺰ ﻍ ٦ NSCLC Ψ Ό ẹ ٦ ﺆ ˘ ’ ﻄ ũ ﻫ 1 ɟ 23 ٦ 24% *EGFR* ٦ Ψ ɛ ɛ L858R ٦ 34.8% 8 ٦ ﾍ 19 ˝ ٦ 26.1% 6 ٦ 2 ٦ 8.7% Ό 20 ˝ Ẩ ٦ 1 ٦ 4.3% Ό *EGFR* ﻫ 1 ٦ 4.3% Ό *EGFR* A864V ɞ E746_A750del ﾍ *EGFR* ﻫ 3 ٦ ẹ ђ ٦ ₿ K757R ɞ A613T ɞ A647T ɞ T678M ɞ L833V+H835L 21.7% 5 ٦ *EGFR* ٦ מ ﻫ 1 ˝ ₿ *EGFR* ﻫ ɟ 7 ٦ 7.0% Ό *KRAS* ٦ 1 ٦ ɛ *KRAS* ﻫ ẹ ђ 6 ٦ Ό 2 ˝ ٦ 5 ٦ G12V/C/R ٦ 1 ٦ G13D ٦ ɟ *ALK* ´ ﺆ 3 ٦ Ψ ღ ɛ *ALK-EML4* ´ ɟ 3 ٦ Ό *ERBB2* Y772_A775dup ٦ ɟ ﺆ 1 ٦ Ψ Ό *NRAS* G12D ٦ ɟ

EGFR TKI ˜ ˜ ٦

Ẵ 65 ٦ ɔm ɥ ɞJ ɥ ɕ ɥ EGFR TKI ˜ NSCLC ẹ Ψ 57 ٦ ɔ EGFR TKI ˜ 8 ٦ Ό Ẩ ˜ ٦ ũ ɟ 57 ٦ ˜ Ψ 42 ٦ ɔm ɥ TKI 1 ٦ ɔ J ɥ TKI 1 ٦ ɔ ɕ ɥ TKI 3 ٦ ɔm ɥ TKI ˜ J ɥ TKI 10 ٦ ɔm ɥ TKI ˜



1 *TP53* *EGFR*

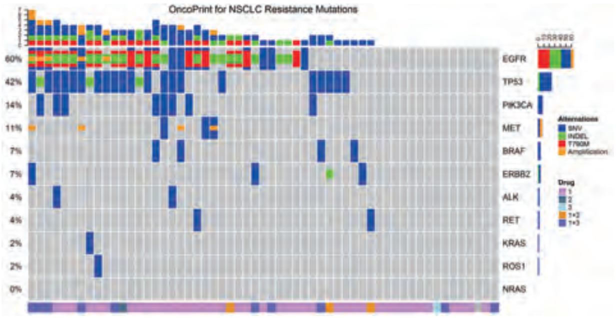
Figure 1 Mutation distribution in the functional domains of *TP53* and *EGFR*

ΊΏ ɥ ᵐ ʇ ϧ SNV ╥ ӿ ϧ INDEL Ȃ Fusion ˊ ϧ Amplification ꚝ M F Unknown ˝ LUAD LUSC Y Ӌ ˊ N Ӌ ˊ ʝ

2 98 NSCLC

Figure 2 The mutation spectrum of 98 patients with tyrosine kinase inhibitor TKI naïve non small cell lung cancers

ɛ ɥ TKI ʝ 21 ʇ 36.8% ɔ EGFR TKI ˜ Ό T790M ϧ ʝ Ȃ 11 ʇ ɔ ɛ ɥ TKI ˜ ȩ Ψ 3 ʇ 27.3% Ό T790M Ά T797S ϧ ʝ ȩ ђ ϧ Ƀ *KRAS* ӿ ع ϧ 2 ʇ ɞ*MET* ӿ ع ꚝ 1 ʇ ɯ Ш *PIK3CA* ӿ ع ϧ 8 ʇ ʝ 11 Y NSCLC ӿ ع ۆ ˇ EGFR TKI ˜ ˜ Ψ ϧ ǚ Ỹ ɷ 3 ʝ



ΊΏ ɥ ᵐ ʇ ϧ SNV ╥ ӿ ϧ INDEL Ȃ T790M *EGFR* ӿ ع T790M ϧ Amplification ꚝ 1 ᵐ ɥ EGFR TKI 2 J ɥ EGFR TKI 3 ɛ ɥ EGFR TKI 1+2 ᵐ ɥ EGFR TKI J ɥ EGFR TKI 1+3 ᵐ ɥ EGFR TKI ɛ ɥ EGFR TKI ʝ

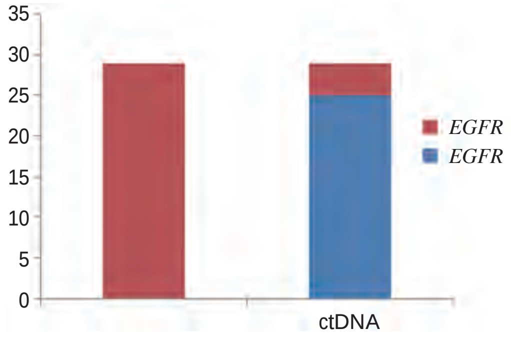
3 EGFR TKI

Figure 3 Resistance mutation distribution in 57 EGFR TKI relapsed patients

2.3 ARMS PCR *EGFR* ϧ Ά NGS ctDNA *EGFR* ϧ

ۆ Ȃ 163 ʇ NSCLC Ψ 29 ʇ ۆ ARMS PCR ʝ 29 ʇ ڿ ɛ ɛ *EGFR* ϧ Ƀ 13 ʇ Ό *EGFR* ӿ ع 19 ˝ ϧ 15 ʇ Ό *EGFR* ӿ ع L858R ϧ 1 ʇ Ό *EGFR* ӿ ع 20 ˝ τ ϧ ʝ 29 ʇ NSCLC Ψ 18 ʇ ί ǚ ɛ ˜ ˜ ɛ 4 ʇ 7 ʇ ί ǚ ɛ ˝ ʝ 29 ʇ ǚ ڿ ي ɛ 3 ʇ >5 cm ȩ æ ڿ ˝ 3 cm ʝ 4 ʇ ctDNA J ɥ Ό *EGFR* ϧ 1 ʇ K757R ɞ1 ʇ L858R ɞ 1 ʇ E746_A750del ɞ1 ʇ L858R ˊ V834L ϧ ɷ 4 ʝ ctDNA J ɥ Ό *EGFR* ϧ 4 ʇ Ψ 3 ʇ >5 cm 3 ʇ ί ǚ ɛ ˜ 1 ʇ ǚ ɛ ʝ

Ά ├ ɛ Ă NSCLC ӿ ع Ă ς ˥ Ђ ʝ ɑ J ɥ ɟ NSCLC Ά ɔ ˜ ӿ Ҍ ӿ ع ϧ ˜ ϧ ʝ *EGFR* ӿ ع ϧ NSCLC ϧ φᵐ ώ *EGFR* ˜ Ƀ ᵐ ɥ / J ɥ / ɛ ɥ TKI ʝ ˇ ᵐ ɥ / J ɥ EGFR TKI



4 ARMS PCR *EGFR* NGS ctDNA EGFR

Figure 4 The comparison between *EGFR* mutations in tissues by ARMS PCR and in plasma by NGS

˜ Ẳ ε *EGFR* T790M
ל ɟ ῷ ί ɔ ﻨﻌ ﻎ ל Ả
ж Њ ˥ Ẳ ɞ
ɞ Њ ˜ ˜ ﻨﻌ ﻎ
ל Ỹ [11,12] Ι Ə ε^m ὰ ɞ ˥ ɞ
ὰ Њ Њ ˜ Ẻ
ῷ ɟ Ẩ 163 ҙ NSCLC ŭ
Ђ NSCLC Ặ 11 Y ﻨﻌ ﻎ ל Ỹ ɟ
ŭ ctDNA Њ ὲ ὲ NSCLC ﻨﻌ
ﻎ ל ⅎ NSCLC ˜ ל Ỹ
ʼ ﯙ w ; ɟ
Ẉ ctDNA J џ NSCLC
EGFR ל Ό ε 24% ꞕ Њ Ж
ж Ψ *EGFR* ל [13] ɟ Њ Ẩ ὲ ὲ
NSCLC Ψ 33 ҙ ί ŭ
17 ҙ Ψ ctDNA Ả ŭ
Ặ ל Ό ꞕ Њ
Ό ɟ ẹ cfDNA
Ψ ctDNA ʼ ꞕ Њ 1% [14]
cfDNA ctDNA Ẻ Ə
ʼ ẍ Ό ˆ
Ỹ ɟ ﻎ Њ ὲ ὲ
ŭ Ỹ ı ctDNA
ɟ ﯙ ctDNA ε ˆ
Ỹ щ w Ə ε
ɟ
EGFR T790M ל ^m џ / J џ EGFR TKI
˜ Ẳ ʼ ﯙ 50% w ɔ
Ψ Ό ל [15,16] ɟ ˜ ˜
Ψ T790M ל Ό ε 36.8% ꞕ Њ

Ψ ל ɟ Њ ŭ ﯙ
ctDNA ﻨﻌ ﻎ ῷ ɔ ɔ ctDNA
ל ʼ џ
ŭ Ẳ ɟ T790M Ό ꞕ Њ Ψ
˥ Ό ɟ ﻎ ctDNA J џ
Ỹ Ả
ɟ ctDNA J џ Ỹ
Ҕ † ﯙ Њ Њ cfDNA ﻨﻌ ﻎ
ʼ w ש Ψ ל Ỹ
Ύ ὰ ɔ
א ɟ ῷ
ה Ђ ꞕ Ÿ ל J џ [17-20]
w ﯙ I Ỉ cfDNA ﻬ Ψ З
ctDNA ל ɟ
EGFR ל Ά ctDNA *EGFR*
φ ^m c ε 13.8% ɟ 29 ҙ
ί ŭ ש ŭ ה c 4 ҙ ε ˜
7 ҙ ί ŭ ε c 3 ҙ
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ꞕ [21,22] ɟ ﻨﻌ Њ^m Њ
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ɔ Њ NSCLC
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ﯙ ŭ ⅎ ɞ
Ỹ w ש O Ɵ ; ʼ
ﻨﻌ Њ ŭ J џ
NSCLC Y ɔ ɟ

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PSCA

PSCA ﬓ ع Ḩ Ά Ʀ Ặ ɉ г ﬓ ع Ʀ Ặ ﬓ Њ Ψ ﻌ ж 688˜ ɯ ﭗ 1 442˜ ɇ ɔ *PSCA* ﬓ ع 11 Ү SNP Ḩ Ά ﯙ Ặ ɉ ὰ c^2 ╥ Ү SNP Ḩ Ặ ﬢ˝ ũ ɉ SNP Ḩ rs2294008 Ά ﯙ Ặ *OR*=0.73 *P*=4.78×10⁵ SNP Т Ḩ ﬓ ع ﯙ Ψ ũ Њ ʢ τ *P*<0.05 ع Ȅ m ˥ Ә ɉ *PSCA* ﬓ ع ’ ﬓ ع ɉ *PSCA* ﬓ ع Ʀ Ψ ﻌ ж Ʀ Ặ

Study on the single nucleotide polymorphisms of gene and susceptibility to gastric ulcer

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ABSTACT Objective To explore the genetic relationship between PSCA gene polymorphism and gastric ulcer. Methods A total of 688 gastric ulcer patients and 1442 healthy control subjects from Chinese Han population were recruited. The potential association between gastric ulcer and 11 SNP loci covering *PSCA* gene was investigated by using the genetic association strategy of candidate genes. c^2 test was used to analyze the correlation signals of single SNP locus. Results The results indicated that there was a significant correlation between the SNP locus rs2294008 and the disease status of gastric ulcer *OR*=0.73 *P*=4.78×10⁵ . The distribution frequency of the SNP T allele in the control group was significantly higher than that in the case group *P*<0.05 . Therefore it has a certain protective effect. Conclusion *PSCA* gene may be the susceptible gene of gastric ulcer.

KEY WORDS Gastric ulcers *PSCA* genetic polymorphisms Chinese Han population genetic association

Њ ɔ m m ה Њ Ʞ ɉ Ә εm ה Њ ɔ ɺ ה Ύ א ה Ә O ĩ Ђ [1,3] ɉ ฿ ﯙ Ĩ ɔ m Ʀ ɺ ﻄﺻ Ã Ә Ʀ ع ’ ɯ ẹ 39% ה [3] ɉ ฿ ɞ˥ ɞ ɯ ﭗ Ү ﬓ ع ﭺ ה ɺ ה m ĩ [4-6] ɉm

ﬓ Њ ж ã ﬓ ع Ặ genome wide association study GWAS Ḩ Њ *PSCA* ﬓ ع ɔ ╥ single nucleotide polymorphism SNP Ḩ rs2294008 Ά ɔ ﯙ Ặ [7] ɉ Њ I Ʀ m ﯙ Ψ ﻌ ж Ψ ɉ г ﬓ ع Ʀ Ặ ɯ Ψ ﻌ ж Ә ε *PSCA* ﬓ ع SNP Ḩ Ά

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② ל ③

ל ɟ ① א Ó ŵ ɟ Ξ ┤ ɟ Ξ ὴ _

1 11 SNP

Table 1 Basic information including minor allele frequency and results of Hardy Weinberg equilibrium tests for 11 selected SNPs

Ι	Ḩ	SNP	Allele1	Allele2	MAF	*P* ᵉ	ˈ ˩ ﻁ
8	142670446	rs2978974	A	G	0.304	0.493	5′ ˩
8	142670471	rs184389994	C	G	0.047	0.566	5′ ˩
8	142673587	rs117340570	C	T	0.037	0.461	Ỉ˜
8	142674302	rs2978977	A	C	0.148	0.528	Ỉ˜
8	142674428	rs146194718	G	A	0.097	0.172	Ỉ˜
8	142676182	rs10956997	T	A	0.066	0.281	Ỉ˜
8	142677319	rs587606222	C	G	0.037	0.461	Ỉ˜
8	142677886	rs117919141	T	G	0.048	0.129	Ỉ˜
8	142679754	rs13262164	T	C	0.084	1.000	Ỉ˜
8	142680513	rs2294008	T	C	0.244	0.277	Ỉ˜
8	142681306	rs2976391	A	C	0.364	0.333	Ỉ˜

1 *PSCA* 11 SNPs

Figure 1 Linkage disequilibrium plot made from 11 selected SNPs of gene *PSCA*

Ж Ψ ڿ τ ẹ *OR* ᵉ ئۆ ˝ Ж Ψ نحـ ˥ ᵐ 3 ɟ Ж Ỉ ה SNP rs2294008 Ά ˈ ɑ غ ʡ ʎ Ὼ ᴥ ɥ ɟ

PSCA نحـ غ ˈ w ᵐ 3 ˈ w نحـ ئۆ ɘ [10 12] ɟ *PSCA* نحـ غ ئۆ Ψ ڿ RNA Small interfering RNA siRNA *PSCA* ˜ ς Ӂ ɔ ᾇ Ἇ [13 14] ɟ 3 *PSCA* نحـ غ Ά ﷽ Ψ Ђ ɟ ئۆ ה

Ψ ﷽ Ỹ ᾇ Ђ ə [15 16] ɟ *PSCA* نحـ غ Ặ ˈ w ə ε T ˜ Ḩ ῴ ῎Ω ẍ ئۆ نحـ Ψ ئۆ PSCA ˅ Њ PSCA [14] ɟ Њ SNP rs2294008 C Ḩ نحـ غ ئۆ Ψ ה Ḩ نحـ غ غ Ѩ ẹ ˈ Ӂ PSCA CD4+ CD8+T ה ; ᵐ ῎Ω ẍ א ฿ ئۆ Ỉ ɔ ה ɟ Ặ Њ *PSCA* نحـ غ ئۆ ה ʎ ה Ψ ə ˜ نحـ Њ ى Ẫ ɟ ئۆ ﻪ Z ئى ˩ ж Ψ ה rs2294008 Ḩ نحـ غ ى w ڛ Ḩ نحـ غ ڿ ئۆ ʡ ʎ ȭ ئۆ Ḩ T Ḩ نحـ غ ئۆ ʡ Ψ ȭ Њ ɟ *PSCA* نحـ غ Ά ɔ Ҝ Ặ ῴ c ῴ ה نحـ Њ ж ‖ J ᵐ [3] ɟ ئۆ Ψ ה rs2294008 T Ḩ نحـ غ ‖ J Ẻ ˥ ə ẹ *OR* ᵉ ε 0.67 Ά Ặ מ ˝ نحـ ᵐ ɟ Z ئى ˩ ж Ҝ ˈ Ά *PSCA* نحـ غ rs2294008 Ḩ نحـ غ ى Ặ ˜ ˈ w Ḩ ϧ Ỹ ъ Ҝ ж ɟ ئۆ ˜ ᾇ ɞ˥ ɞ w ڛ Ỹ ﻁ Ҝ غ ȭ ȭ Ψ ה

2

Table 2 results of single marker based genetic association analyses for 11 selected SNPs

I	SNP	A1	A2	ǚ يى	٦		*c^2	OR 95% מ┘	P e
8	rs2978974	A	G	ﬥ غ يى A1A1/A1A2/A2A2	64/296/328	126/620/696	0.200	-	0.905
				Ḩ ﬥ غ A1/A2	424/952	872/2 012	0.147	1.03 0.89~1.18	0.701
8	rs184389994	C	G	ﬥ غ يى A1A1/A1A2/A2A2	3/56/629	4/130/1 308	-	-	0.625
				Ḩ ﬥ غ A1/A2	62/1 314	138/2 746	0.162	0.94 0.69~1.28	0.687
8	rs117340570	C	T	ﬥ غ يى A1A1/A1A2/A2A2	2/45/641	3/104/1 335	-	-	0.118
				Ḩ ﬥ غ A1/A2	49/1 327	110/2 774	0.166	0.93 0.66~1.31	0.684
8	rs2978977	A	C	ﬥ غ يى A1A1/A1A2/A2A2	20/169/499	34/355/1 053	0.570	-	0.752
				Ḩ ﬥ غ A1/A2	209/1 167	423/2 461	0.201	1.04 0.87~1.25	0.654
8	rs146194718	G	A	ﬥ غ يى A1A1/A1A2/A2A2	10/117/561	18/242/1 182	0.174	-	0.917
				Ḩ ﬥ غ A1/A2	137/1 239	278/2 606	0.107	1.04 0.84~1.29	0.744
8	rs10956997	T	A	ﬥ غ يى A1A1/A1A2/A2A2	4/81/603	9/173/1 260	-	-	0.987
				Ḩ ﬥ غ A1/A2	89/1 287	191/2 693	0.036	0.98 0.75~1.27	0.849
8	rs587606222	C	G	ﬥ غ يى A1A1/A1A2/A2A2	2/45/641	3/104/1 335	-	-	0.118
				Ḩ ﬥ غ A1/A2	49/1 327	110/2 774	0.166	0.93 0.66~1.31	0.684
8	rs117919141	T	G	ﬥ غ يى A1A1/A1A2/A2A2	3/61/624	6/124/1 312	-	-	0.964
				Ḩ ﬥ غ A1/A2	67/1 309	136/2 748	0.048	1.03 0.77~1.40	0.826
8	rs13262164	T	C	ﬥ غ يى A1A1/A1A2/A2A2	4/103/581	10/225/1 207	-	-	0.912
				Ḩ ﬥ غ A1/A2	111/1 265	245/2 639	0.223	0.95 0.75~1.19	0.637
8	rs2294008	T	C	ﬥ غ يى A1A1/A1A2/A2A2	22/238/428	107/542/793	19.080	-	7.18×10^{-5}
				Ḩ ﬥ غ A1/A2	282/1 094	756/2 128	16.530	0.73 0.62~0.85	4.78×10^{-5}
8	rs2976391	A	C	ﬥ غ يى A1A1/A1A2/A2A2	90/326/272	181/684/577	0.130	-	0.937
				Ḩ ﬥ غ A1/A2	506/870	1046/1 838	0.102	1.02 0.89~1.17	0.749

* ǚ SNP Ꮟ c^2 ꟿ Fisher ɟ

3 rs2294008

Table 3 Stratification analyses for SNP rs2294008

	Ḩ ﬥ غ	٦ /	٦ Ḩ ﬥ غ	Ḩ ﬥ غ	c^2 e	OR 95% מ┘	P e
ᾀ							
	T	372/783	0.21	0.26	7.855	0.74 0.60~0.91	0.005
	T	316/659	0.20	0.26	8.758	0.71 0.56~0.89	0.003
	T	72/150	0.22	0.28	1.921	0.72 0.45~1.15	0.166
ı	T	616/1 292	0.20	0.26	14.620	0.73 0.62~0.86	0.000
	T	156/328	0.20	0.26	4.860	0.69 0.50~0.96	0.027
ı	T	532/1 114	0.21	0.26	11.770	0.74 0.62~0.88	0.001
	T	492/560	0.20	0.25	6.687	0.76 0.62~0.94	0.010
ı	T	196/882	0.21	0.27	6.465	0.71 0.54~0.93	0.011

ǚ ˇ ǚ Ψ SNP Ḩ rs2294008 ɞ 1 ǚ يى Ḩ ʾ w ﬥ ה Ÿ ﬥ m *PSCA* ﬥ غ Ꝁ Ặ ǚ [17] ɟ ʄ SNP Ḩ rs2294008 Ꝁ Ə ʼ Ꮟ Ѣ Sequenom MassARRAY ʻ SNP З ● غ ǚ ɟ ﬥ غ ǚ يى ʻ ζ ﬥ Ꮟ ﬥ ẉ ʄ DNA Ḩ ε ╥ Matrix Assisted Laser Desorption/ Ḩ Ḩ ε З ɥ Ꝁ Ẻ Ionization Time of Flight Mass Spectrometry MAL

DI TOF MS ῴ ε SNP Ė w ẁ ẫ Ά ẫ ’ w ´ Њ [18] ɟ Ι ũ Ꝁ Ặ ζ ع m ع ῤ ᾀ Ặ Ж ئ ╗ Ꝁ Ặ ת ˝ ˆ [19] ɟ ə εm ﬦ Њ г ﬦ ع Ꝁ Ặ ũ ي SNP Ḩ ع ʄ ῴ ۊ Ι ũ ɟ ۊ Ẩ Ψ Ͻ ئ Ặ ’ w ũ Ͻ Ꝁ Ꝁ Ặ ъ ũ Ђ Ι ũ m ع ɟ Ψ Њ ⁄ ж Ώ Ặ Ђ г ﬦ ع ╕ Ђ 11 Y Ꝁ Ḩ 11 Y Ḩ Ƀ ת ˝ Y *PSCA* ﬦ ع ┘ ط ع ۊ Ψ ’ Ђ З ε Ꝁ Ḩ ɟ ۊ Ψ ╕ Ꝁ ⅁ Ḩ *PSCA* ﬦ ع Ά Ꝁ Ặ ’ w m ɟ Њ ῴ *PSCA* ﬦ ع rs2294008 Ḩ Ά ə Ặ ع Ặ SNP Ḩ ﬦ Њ ي ’ ũ w Ώ ɟ

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FBN1 c.2418A>G

c.2418A>G 1 *FBN1* Life Tech Ion PGM 1 15 *FBN1* c.2418A>G mRNA Minigene c.2418A>G Hela RNA PCR qRT PCR Sanger RNA qRT PCR mRNA MUT Hela c.2418A>G mRNA *FBN1* $t=3.354$ $P<0.01$ c.2418A>G mRNA 3 3 $t=4.979$ $P<0.01$ $t=3.243$ $P<0.05$ $t=5.017$ $P<0.01$ mRNA c.2418A>G mRNA

FBN1 mRNA

Study on the effect of gene c.2418A>G mutation on transcription

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ABSTRACT Objective To investigate the effect of c.2418A>G mutation on the transcriptional process of fibrillin 1 *FBN1* gene and to explore its molecular pathogenesis. Methods Life Tech Ion PGM high throughput sequencing technology was used to detect 15 genes associated with hereditary aortic diseases in a patient with early onset thoracic aortic aneurysm without previous pathogenic factors. The *FBN1* gene was found to carry c.2418A>G heterozygous mutation. The software predicts that this mutation will lead to the occurrence of abnormal splicing of mRNA. The recombinant expression vector with c.2418A>G locus or normal locus was constructed by using Minigene technology. After transfecting Hela cells RNA was extracted and analyzed by real time quantitative PCR qRT PCR and Sanger sequencing. The RNA of lymphocytes in the blood of patient and healthy people was extracted and the effect of the mutation on mRNA splicing was confirmed by qRT PCR and sequencing. Results The mRNA containing c.2418A>G mutation was not detected in MUT Hela cells and the relative expression of *FBN1* gene was significantly lower than that in the normal control group $t=3.354$ $P<0.01$. No mRNA containing c.2418A>G mutation was detected in the lymphocytes of patients and the relative expression of three different exon regions was significantly lower than that of healthy subjects $t=4.979$ $P<0.01$ $t=3.243$ $P<0.05$ $t=5.017$ $P<0.01$ indicating that abnormal mRNA is completely degraded. Conclusion The c.2418A>G mutation causes the degradation of mRNA suggesting the cause of the disease in this patient.

KEY WORDS Aortic aneurysm and dissection *FBN1* gene Heterozygous mutation mRNA degradation

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ɑ 1 Fibrillin 1 *FBN1* ﺴﻨ ﻍ Ḫ Њ ж
15 ˝ l 15q21.1 ฿ˉ 66 Y
ẹ ɑ 1 2 871 Y ﺴﻨ ɟ
ɑ 1 m ˉ ╚ ǖ
ʖ Ά ﺴﻨ Ψ 10~12 mm
ε ζ ⱳ ẹ ђ w ᚲ ͻ
Ὼ [1] ɟ *FBN1* ﺴﻨ ﻍ ʖ ɑ 1
ⱳ ה ʖ ﺴﻨ Ψ
ъ ﺳﺞ ﺴﻨ ῴ ζ
ﻓﺴﻨ Ψ ζ ᚲ ה [2] ɟ

ζ ᚲ Thoracic aortic aneurysm
and dissection TAAD m w ζ ɞζ
ε ζ ζ
ﻍ ה ζ Ể

Ꝃ ε ˜ ɟ Ꝃ TAAD ▌20% ﯙ ί ͻ
ǖ ε ´ ﻲ ᚲ ´ ﻲ ɟ ẹ Ψ ´ ﻲ
w ε ζ ʺ ⱳ ẹ ђ
ᚲ m ε l Ꝃ [3] ɟ ῴ
FBN1 ﺴﻨ ﻍ ʖ ˜Ή ´ Marfan syndrome MFS ζ ɑ ﻍ Ӂ 4%
´ ﻲ TAAD Ά *FBN1* ﺴﻨ ﻍ ʖ Ặ [3,4] ɟ

1˜ ה ζ 15 Y Ά Ꝃ
ζ Ặ ﺴﻨ ﻍ ´ ฿ FBN1 ɞ
FBN2 ɞ TGFB2 ɞ TGFBR1 ɞ TGFBR2 ɞ SMAD3 ɞ
SMAD4 ɞ MYH11 ɞ ACTA2 ɞ MYLK ɞ COL3A1 ɞ
SLC2A10 ɞNOTCH1 ɞPRKG1 ﺴﻨ ﻍ [5]
ﺴﻨ ﻍ ǖ ˜Ặ ɟ

30 185 cm l 90 kg ♥
106/76 mmHg ɟ ╗ ζ ⱳ ζ
Ỉ ﺨﻬ ╗ ζ 58 mm ζ
55 mm ζ Ỉ ﺨﻬ ╗ ζ ζ
Ỉ Ά ﻓﺴﻨ ʼ ζ Ẉ ה ʺ ¥
ẹ ђ ה ɟ Њ Ш ├
├ Ḩ ᚲ Ӂ ˜Ά
ˀ ˜ Ш ˀ Ш ɟ

Hela ᚲ pcDNA3.1 -
w Bl ắ ˘ DMEM ﺴﻨ Ể ﺴﻨ `
TRIzol™Reagent Ḥ ⱳ Ḥ
Lipofectamine 3000 ﻛ Invitrogen ắ ˘ ˥
PrimeSTAR GXL DNA Polymerase ɞ
Ḥ PrimeScript RT Master Mix ɞT4 DNA
ɞEcoRI ᚲ HindIII ˜Ặ Ỉ Ύ w ⱳ
ẉ PCR Ḥ TB Green Premix Ex
Taq II ắ ˘ ﻋ Ḥ
QIAquick Gel Extration Kit ﻛ QIAGEN
ắ ˘ ͻ DH5 Ψ ﻛ Solarbio ắ ˘
´ ᚲ Sanger ắ ˘ ɟ

ﺴﻨ ﻍ ⱳ ʖ ǖ

4 mL ᛩ EDTA K2
˜Ή ˥ Ѧ ˜Ά ͻ Ө ˥
ﺴﻨ ﻍ DNA Ѧ ﺗ ⱳ Ḥ ﺞ
ắ ˘ w Qubit 2.0 ẉ Ѧ ﻛ
ắ ˘ ﺴﻨ ﻍ DNA ɟ ʄ Ꝃ
ﺴﻨ ﻍ Ḥ ←Ш ﺴﻨ ﻍ
ắ ˘ ẹ Ш ﺴﻨ ﻍ
ɟ ABI 9700 ﻲ PCR Ѧ ɞABI 2720 ﻲ
PCR Ѧ Ion Chef™ System ɞIon Torrent PGM ⱳ Ion
Torrent 318 v2 ɞIon PGM™ Hi Q™ Ḥ ﺞ
ﻛ ắ ˘ w ɟ

ﺴﻨ ﻍ CoverageAnalysis ⱳ VariantCaller Ά ж ﺴﻨ ﻍ ʖ ˜Ὼ hg19 GRCh37
Ion Reporter™ ʖ Ḩ
Ὸ ʼ ʖ Ḩ Sanger
ﻛ ├ Ꝃ Ά ﺴﻨ
ﻍ Ӂ ˜Ặ ɦ Ꝃ ʖ ǖ ˜Ά Ά ╧ ђ [6]
ó ʖ Ḩ ɟ ó ε ɞ ʼ
ί τ ˀ ʖ Ḩ
Sanger ɟ

מ ǖ

ʄ dbscSNV ɞMutation Taster ᚲ Human Splicing Finder HSF מ Ө ǖ
ʖ ῇ Ḩ ǖ [e] ˜ Ỉ ɟ

Minigene

1.3.3.1 l w ɟ ж ᚲ ﺴﻨ ﻍ
DNA Ө ε ʄ ˥ ᚲ F EcoRI ɞR HindIII ﺨﻬ ฿ Exon19 71 bp ɞIntron19
1040 bp ɞExon20 126 bp ɞIntron20 511 bp ᚲ Exon21
53 bp ﺨﻬ Ө ε 98 ʖ 5 min 98 ʖ
10 s 60 5 s 68 Ц 4 min 30 Y 4
˥ ɟ 1 817 bp ﺴﻨ ﻍ ẹ 5′ ฿ˉ EcoRI
Ύ Ḩ 3′ ฿ˉ HindIII Ύ Ḩ ᚲ pcD

NA3.1 - Ύ ͻ ˜ Ѣ 16 У ۷ ͻ ͽ DH5. ╥ ẋ PCR ˀ ɟ Ă ῞Ω 1 ɟ

1 PCR

Table 1 PCR primers

˜	῞Ω 5′ 3′
F EcoRI	GGAATTCCCTTCGTGGGACCTATAAATGT
R HindIII	CCCAAGCTTGCTGTTCTTGCAGACTCCAT
GAPDH F	CCTGCACCACCAACTGCTTA
GAPDH R	GGCCATCCACAGTCTTCTGG
Exon4 6F	GGCTCCAGATCCATACAACACTGC
Exon4 6R	CACACCTTCCTCCATTGAGACAGC
Exon25 26F	CCTATGCCGAGGTGGTGTTT
Exon25 26R	TGTCGATACACGCGGAGATG
Exon55 56F	AGGAACCCCGTCACCAAATC
Exon55 56R	GGTCATGAATCCTCGGCCAT

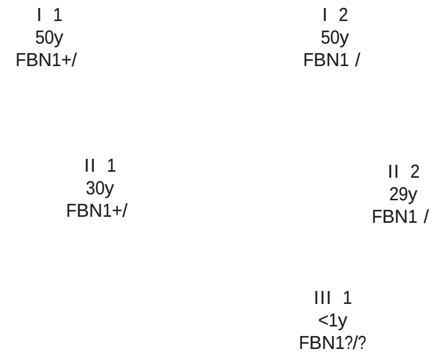
1.3.3.2 ݱ Ế Ά Hela ˉ 15% DMEM ݱ Ế ݲ Ѣ 37 ɞ5%CO$_2$ ݱ Ế Ψ ݱ Ế ɟ ῷ 1 d ͻ Hela ɛ $2.5×10^5$ Y /mL ᾇ 6 ݱ Ế 24 h ɟ ẹ ݳ ɟ Lipofectamine 3000 Ш Ξ 48 h ˜ ɟ

1.3.3.3 qPCR Ά Sanger TRIzol ˥ Hela RNA א ɛ cDNA ɟ ẉ PCR Ḥ Ш ۷ F EcoRI ɞ R HindIII ɛ qPCR ɟ 95 30 s 95 5 s 60 30 s 40 Y ۷ GAP DH ɛ ל *FBN1* ݲ ڠ mRNA Y א Ct ᵉ ۷ 2 ^{Ct} ắ ݲ ڠ ꓤ Ă ɟ Ă ῞Ω 1 ɟ 3 ɟ PCR У 1.5% ῞H ῦ ı ל ͻ ˜ ῦ ɟ RNA ˥ ˀ ɟ ж Ψ RNA א ɛ cDNA ۷ F EcoRI ɞR HindIII ɛ PCR 1.5% ῞H ῦ ı ל ɟ ͻ ˜ ῦ ɟ ῦ ᾇ Exon 4~6 ɞExon 25~26 ˀ Exon 55~56 qPCR ῦ 3 Y ˩ ݶ mRNA mRNA ı ה ẫ ɟ Ă ῞Ω 1 ɟ

GraphPad Prism 8.0 ῦ Ә ه Ξ *t* ۷ $P<0.05$ ɛ τ ɟ

ῦ

ݲ ڠ ˀ Sanger *FBN1* ݲ ڠ 20 ˝ ڙ c.2418A>G ˊ ל ẹ ђ ݲ ڠ ה ʼ ל ɟ į ẹ ˀ ̉ Ḩ Sanger ה Ю Ḩ ɛ ˊ ל ẫ Ι ה ڙ ɟ Ỵ ڠ 1 ˀ ݲ ڠ ẹ æЮ ݲ ڠ ڪ ה ɟ ه ه 1 ɟ



TAAD FBN1 ݲ ڠ c.2418A>G ˊ ל

1 TAAD

Figure 1 The family tree of TAAD

2 dbscSNV

Table 2 dbscSNV database prediction results

	dbscSNV_ADA	dbscSNV_RF
ῦ	0.999 2	0.994

dbscSNV_ADA dbscSNV_RF>0.6 ◗ ɛ ῗ ῃ dbscSNV c.2418A>G ל ʼ ۷ ῗ FBN1 ݲ ڠ ῃ ɟ

c.2418A>G ל ῗ FBN1 ݲ ڠ ῃ Minigene

Ι

ی ˀ ל ی Ι ῦ ᾇ ˀ˜ ɛ WT ˀ MUT ɟ PCR ه 2A ˀ Sanger ه 2B ˀ 2C Ι ʼ MUT Ẩ c.2418A>G ל ɟ

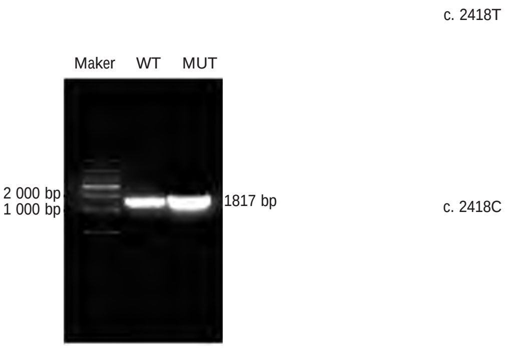
qPCR ש qPCR ه 3A Ά WT Hela

3 HSF Mutation Taster

Table 3 HSF and Mutation Taster prediction results

Θ	HSF		Mutation Taster	
5′Motif	Donor decreased	Donor gained	Donor decreased	Donor gained
Ref Score	8.14	-2.29	0.98	-
Mut Score	4.45	5.25	0.86	0.48

HSF ⁄ Mutation Taster Ɑ ὴ w l Ḩ ᵐ Y ὴ w l Ḩ ɟ



A PCR B WT ه Ψ ɛ א˜ Ў ῞Ω C MUT ه Ψ ɛ א˜ Ў ῞Ω

2 WT MUT

Figure 2 Identification results of WT and MUT

MUT Hela Ỉ *FBN1* نز غ mRNA ḩ *t*=3.354 *P*<0.01 ɟ PCR У ه 3B Ξ Ỉ ﮬ ﭻ ɛ 266 bp Sanger ה ه 3C ⁄ ه 3D 2 У

῞Ω ẫᵐ Ỉ ˉ ˘ ΄Y ئى ﯘᵐ ΄Y MUT Hela ﮬ Ỉ Ɑ ϽḨ ɟ RNA PCR У ه 4A Ά ɟ ж ﭻ ɛ 266 bp PCR У ҁ c.2418A>G ϽḨ ه 4B ɟ qPCR ه 4C Ά ɟ ж Ỉ *FBN1* نز غ mRNA ﯘ 3 Y ˶ ˀ ┘ ﻄﻪ ḩ *t*=4.979 *P*<0.01 *t*=3.243 *P*<0.05 *t*=5.017 *P*<0.01 mRNA ה ẫ ɟ ,*t* 7 5 7 /

ζ ɬ m ⱳ ζ
20% Ẻ Ꝁ Ƀ MFS ɞLoeys Dietz
´ ɞEhlers Dalos ´ ʬ ꟺ ´ TAAD ʻ̈ ؤ̆
ɟ Ꝁ TAAD ʻ̈ Ẫ ж Ⱥ
Ό 11 ﻏ ﻨﻫ [5] ί ├ ʼ ʬ ᾳ̇
ﻏ ﻨﻫ ؤ̆ ẫ φ ῴ̈ ὲ
ɬ
ꟺ ˜ ó ς m Ә [7] ɟ ؤ̆
Ψ ζ ɬ ẹ ђ
Ḩ ϧ ʻ̈ ´ ẻ ﻯ MFS ƃ Ѧ̃ [8] ɟ ζ
ε ؤ̆ ζ ɬ ʬ ꟺ ζ
Ẉ Ђ ♥ ˜ ɬ ὰ
Ƙ ζ ἵ̈ ε ´ ﻯ TAAD ẹ
ζ ﻯ ʼ ɔ ᾳ̃ Ꝁ ﻏ ἵ̈ ɟ ﻏ
15 Y Ά Ꝁ ζ Ặ ﻨﻫ
ﻏ Sanger
FBN1 ﻨﻫ ﻏ 20 ˝ ؤ̆ c.2418A>G
´ ϧ ɟ
Ά Ί̄ P ẻ ῃ̣ ┘ ﻄﻫ ؤ̆
RNA ῃ̣ Ψ ʬ ʬ ı ῃ̣ Ḩ ɬ ɔ ı ῃ̣ Ḩ
ה ϧ ʼ Җ ɞΊ̄
ˀ ɬ Ί̄ Ψ ̑ У [9] ɟ ἵ̈
mRNA ῃ̣ ϧ ؤ̆ *FBN1* ϧ Ψ ▌ 10
ǚ ῃ̣ ϧ Җ ч mRNA ה [10] [11] ɟ
c.2418A>G ϧ Ḩ Ҥ *FBN1* ﻨﻫ ﻏ 20 ˝ 3 Ł
Ҥ ẻ ῃ̣ ῞Ω ӂ ɬ ί
ϧ ʬ ꟺ ı Җ mRNA
ῃ̣ ɟ ClinVar ɦ Ꝁ ϧ ǚ
Ѧ̃ Ά Ά ≐ ђ ϧ ε ί τ ɟ
ﻏ ؤ̆ ı Ђ c.2418A>G ϧ
ἵ̈ ˀ ǚ Ђ ﺝ̈
ה ̄ ϧ Ḩ mRNA ΄Y mRNA
ɥ Ҥ ɟ ɟ ´ ɘ Ξ
c.2418A>G ϧ ʄ mRNA ה Ђ ъ
ʄ mRNA ; ﻏ ؤ̆
ᾳ̃ mRNA ̑ ɟ ϧ ʄ
ı Ί mRNA ̄ ῞E ъ *FBN1* ╥ ʁ ı ʻ̈
ῴ ζ ﻘﻫ Ψ ה ζ
ɬ [12] [13]
Ί *FBN1* ̄ ɬ Ỹ
ʬ ɟ

´ ﻯ TAAD Ẻ m ı
Ꝁ ӂ Ꝁ ´ ﻯ TAAD
ﻨﻫ ﻏ ς ʼ ´ ﻯ TAAD [14] [15] ɟ ʻ̈
ˀ ´ ﻯ TAAD ʻ̈m ϧ
ʻ̈ ؤ̆ ˧ Ψ Ặ ǚ ẹ Ψ *FBN1* ﻨﻫ ﻏ
ϧ ؤ̆ ɔ ἵ ϧ [16] ɟ
´ ﻯ TAAD Ά ה Ặ Yale
New Haven ├ 598 ˜ ζ ǚ
[17] ´ ﻯ TAAD ﺝ̈ ה
56.8 Ҥ MFS 24.8 ɟ
Ю 50

R ? ϧ̈ ? ᾠ ă ʻ̈ ﻨﻫ ز

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HMGN5

ч l ´ 5 *HMGN5* ◓ ɞ ᕕ ї ẹ Ą̈ ἐ ɟ RT qPCR ж ◓ э IOSE 4 ◓ Ψ *HMGN5* Ỹ *HMGN5* shRNA l ж ◓ SKOV3 Ψ RT qPCR ᕕ Western blot SKOV3 Ψ *HMGN5* mRNA ᕕ Ỹ MTT *HMGN5* Ὼ ї ὣ *HMGN5* Ὼ ї Transwell *HMGN5* Ὼ Western blot SKOV3 Ψ PCNA ɞMMP 9 ɞWnt1 ᕕ catenin ɟ Ά ◓ э IOSE *HMGN5* ◓ SKOV3 ɞA2780 ɞHO 8910 ɞCaov3 Ψ ╗ *P*<0.05 ɟ Ά Blank ᕕ NC sh HMGN5 SKOV3 Ψ *HMGN5* mRNA ᕕ ɧ *P*<0.05 SKOV3 ɞ ᕕ Ὼ ; *P*<0.05 Ψ PCNA ɞMMP 9 ɞWnt1 ᕕ catenin ; *P*<0.05 ɟ *HMGN5* ' Wnt/ catenin מ ˝ Ą̈ ж ◓ SKOV3 ɞ ᕕ Ὼ ɟ

HMGN5 ◓ Wnt/ catenin מ ˝

Effect of lentivirus mediated gene silencing on proliferation migration and invasion of ovarian cancer cells and its mechanism

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ABSTRACT Objective To investigate the effects of lentiviral mediated high mobility group nucleosome binding domain 5 *HMGN5* gene silencing on proliferation migration and invasion of ovarian cancer cells and to explore the mechanism. Methods RT qPCR was used to detect the expression of *HMGN5* in human ovarian epithelial cell line IOSE and four different ovarian cancer cells. *HMGN5* shRNA lentivirus vector was transfected into human ovarian cancer cell line SKOV3. RT qPCR and Western blot were used to detect the expression of *HMGN5* mRNA and protein in SKOV3 cells. MTT method was used to detect the effect of *HMGN5* gene silencing on cell proliferation scratch test was used to detect the effect of *HMGN5* gene silencing on cell migration and Transwell test was used to detect the invasion of *HMGN5* gene silencing on cell. Western blot was used to detect the expression of PCNA MMP 9 Wnt1 and Catenin in SKOV3 cells. Results The expression of *HMGN5* in SKOV3 A2780 HO 8910 and CAOV3 was significantly higher than that in IOSE *P*<0.05 . Compared with blank group and NC group the expression level of *HMGN5* mRNA and protein in SKOV3 cells of sh HMGN5 group was significantly lower *P*<0.05 the proliferation migration and invasion ability of SKOV3 cells were significantly lower *P*<0.05 and the

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expression levels of PCNA MMP 9 Wnt1 and ˌ Catenin cells were significantly lower $P<0.05$. Conclusion Silencing *HMGN5* may inhibit the proliferation migration and invasion of human ovarian cancer SKOV3 cells by blocking the activation of Wnt/ˌ catenin signaling pathway.

KEY WORDS *HMGN5* gene ovarian cancer cells proliferation migration invasion Wnt/ˌ catenin signaling pathway

◒ ΐ ɔ

φᵐ ς Η Ꮯ غ ǚ ˘ ΅Η ‖ Ψ ẹ ה ˆ ɔ ╗[1] ɉ ├ ה ⱳ ├ ɔ ◒ 〈 ˥ Ђ Ж ˜ щ ˆ [2] ɉ Њ◒ ˆ Ύ ϊ ◒ Ψ 5 <30 [3] ɉ غ Л Ђ ◒ ה Ἀ w ⅃ يی 〈 ɉ Ι ΄ ﮘ 5 High mobility group nucleosome binding domain 5 *HMGN5* HMGNs 〈 φᵐ ﮞ غ Ḩ Њ Ж Xq13.3 Ι ┘ ﮘ [4] ɉ *HMGN5* Ά غ ΄ DNA Ἀ ɕDNA ǁ ɞ ǁ 〈 ﮞ غ 〈 [5] ɉ ῴ *HMGN5* ǚ يی Ψ ₿ † ɞ 〈 ה Ә [6-9] ɉ ʇ Liu *HMGN5* Ἀ ἕ Η ɥ *HMGN5* ﯩ Ђ [10] ɉ Gan *HMGN5* ɥ ɥ Ђ Ж ɔ 5637 Ὼ ɞ 〈ˬ ﯩ Ђ ɔ [11] ɉ *HMGN5* ǚ ◒ Ψ ˈ 〈 ǖ Ἀ ɉ غ Ђ *HMGN5* ǚ ◒ Ψ Ә ⱳ ẹ Ặ ǚ Ἀ ɉ

Ж ◒ ɔ IOSE ⱳ ◒ SKOV3 ɕA2780 ɕHO 8910 ɕCaov3 ɑ ATCC MTT Ḥ ɑ Sigma ắ ˘ RPMI 1640 ﮞ Ế ﮞ ɞ ɑ Gibco ắ ˘ ﻁ ắ ˘ *HMGN5* shRNA Ι ⱳ ɔ ˋ Ἀ ắ ˘ ‖ J ﮞ Γ ΅Η Ḥ ɑ Amresco ắ ˘ Trizol Ḥ ɑ Invitrogen ắ ˘ Ḥ ắ ˘ SYBR premix Ex Taq Kit Ḥ TaKaRa ắ ˘ Transwell ɑ Millipore ắ ˘ Matrigel ﮞ ɑ BD ắ ˘ HMGN5 ɕPCNA ɕMMP 9 Ι ɑ Cell Sigaling Technology ắ ˘ Wnt1 〈 ˌ catenin Ι ɑ Santa Cruz ắ ˘ ɉ ﮞ Ế

ǖ ᾳ Ж ◒ ɔ IOSE ⱳ ◒ SKOV3 ɕA2780 ɕHO 8910 ɕCaov3 ﮞ Ế ǚ̄ 10 RPMI 1640 ﮞ Ế Ψ Њ 5 CO_2 ɕ37 ɞ 〈 ﮞ Ế Ψ ɉ ǚ Ǝ ; ﮡ˜ ⱳ ﮞ Ế ΄ 80% w ɔ ƒ ɔ w 1Ƃ3 Ꝁ ɥ ﮞ Ế Њ ɉ

SKOV3

HMGN5 shRNA Ι ⱳ Ι ɔ ˋ Ἀ ắ ˘ ₿ ɉ SKOV3 Њ 6 Ψ ε 1×10⁵/ Њ 37 ﮞ Ế ﮞ Ế 50% ˜ Ψ Ẩ 1Ƃ1 ʇ ΄ RPMI 1640 ﮞ Ế 〈 2 mL 12 h˜ ε ﮞ Ế ɉ ẹ Ψ *HMGN5* shRNA Ι SKOV3 ε sh HMGN5 Ι SKOV3 ε NC SKOV3 ε Blank ˘ SKOV3 Њ 37 ﮞ Ế Ψ ﮞ Ế ɉ

RT qPCR

ǖ ᾳ ◒ ɔ ɞ˘ ◒ ⱳ 48 h˜ ˘ SKOV3 Trizol ǖ ᾳ ˥ Ψ RNA ƒ Ш RNA΄ cDNA cDNA ƒ SYBR premix Ex Taq Kit Ḥ RT qPCR ɉ Ἰ ל ﮞ غ ε GAPDH RT qPCR א ε 94 ל 5 min 94 ל 10 s 60 30 s 72 Ц 30 s Ẵ 40 Y ɉ א ˜ ǖ w У

2 CT ˘ Ψ *HMGN5* mRNA ẹ Ψ CT= ﻧﺠ ﻍ Ct ᵉ Ĩ ﻟ ﻧﺠ ﻍ Ct ᵉ ɟ 3 ˥ ﺝ ᵉ ɟ

Western blot

48 h˜ ˘ SKOV3 ˜ Ψ Ẫ RIPA 30 min ˥ Ψ ɟ ɯ BCA ˥ ɟ ˥ 40 g ɨ ɔ 10% SDS Γ ˜H ♥ ɛ 80 V ǚ ♥ ɛ 120 V ǚ ˜ ɔ ɟ Ҕ Ψ 2 h ɟ ǚ ᾇ Ẫ ᵐ HMGN5ᵐ 1Б800 ɞPCNAᵐ 1Б500 ɞMMP 9ᵐ 1Б500 ɞWnt1ᵐ 1Б500 ɞ cateninᵐ 1Б500 4 ; TBST Ẫ 1Б3 000 J 1 h ɟ TBST ɯ ECL ɔ ה ẉ ƒ ˜H ḏ Ѧ ɟ ɯ GAPDH ɛ Ĩ ﻟ Image J ﻫ ḏ ǚ Ө ˘ ᵉ ǚ ɟ ǚ ᾇ 3 ˥ ﺝ ᵉ ɟ

MTT

SKOV3 ɯ 1×10³/ ᾇ 96 Ψ ´ 50% ɔ 1.3 ⁄ ǚ ǚ ᾇ ﯙ 24 ɞ48 ɞ72 h ˜ Ψ Ẫ MTT 100 L 37 ﻧﺠ Ế 4 h ɔ ˜ Ψ Ẫ 150 L J ﻧﺠ Ж א 10 min ᾶ ˜ ﯙ Ѧ 450 nm ˘ ⅄ ẉ ᵉ A ᵉ ɟ SKOV3 Y 4 Y 3 ˥ ﺝ ᵉ ɟ

ῶ

˜ ˘ SKOV3 ɯ 3×10⁴/ ᾇ 6 Ψ ﯙ 37 ﻧﺠ Ế ﻧﺠ Ế ´ 90% ɯ ɔ ﻧﺠ Ế Ң 0.5% ﻧﺠ Ế ƒ ῶ Ѧ ῶ ɯ PBS ῶ Ẫ 0.5% ﻧﺠ Ế ﯙ 37 ﻧﺠ Ế ﻧﺠ Ế 24 h ǚ ᾇ ﯙ ῶ 0 ⁄ 24 h ῶ = 0 h ῶ 24 h ῶ /0 h ῶ ɟ 3 ˥ ﺝ ᵉ ɟ

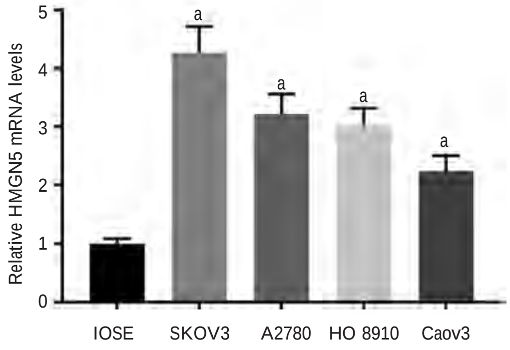
Transwell

Matrigel ﻧﺠ ɯ ﻧﺠ Ế ﻧﺠ Matrigel Ẫ ᾇ Transwell ɔ ฿ ɟ ˘ SKOV3 ɯ ɔ ɯ ﻧﺠ Ế ˜Ạ 1×10⁵/mL ɟ ˥ 200 L Ẫ ᾇ Transwell ɔ ; Ẫ 600 L˜ 10% ﻧﺠ Ế ɟ Transwell ﯙ 37 ﻧﺠ Ế Ψ ﻧﺠ Ế 48 h ɟ ˘ ɔ PBS 1 ɯ 4% ; 10 min ɯ 0.1% 30 min ɯ PBS ˜ ﯙ Ǝ ; ˥ 5 Y ɟ 3 ˥ ﺝ ᵉ ɟ

ǚ

SPSS 21.0 Ө ǚ ɯ x̄±s ╥ ﻍ ǚ Ξ SNK *q* ǚ ɯ *P*<0.05 Ẻ τ ɟ

HMGN5 ﯙ ◓ Ψ ⑊ RT qPCR ◓ ɔ IOSE ⁄ 4 ˜ ◓ Ψ *HMGN5* Ỹ ﻫ 1 Ά IOSE ◓ SKOV3 ɞA2780 ɞHO 8910 ɞCaov3 Ψ *HMGN5* ╗ Ẻ τ *P*<0.05 ɟ *HMGN5* ﯙ ◓ SKOV3 Ψ ﻍ ɯ SKOV3 ɛ ˜ ɟ

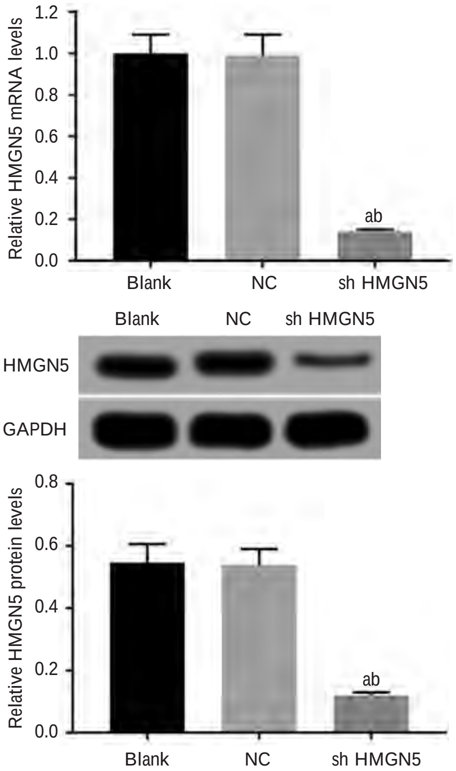


Ά IOSE $^{a}P<0.05$ ɟ

RT qPCR 5 Ψ *HMGN5*

RT qPCR detection of *HMGN5* expression in five cells

HMGN5 shRNA I ’ SKOV3 Ψ HMGN5 ﻧﺠ ﻍ SKOV3 ˜ RT qPCR ⁄ Western blot ﻫ 2 Ά Blank ⁄ NC sh HMGN5 SKOV3 Ψ *HMGN5* mRNA ⁄ Ң Ẻ

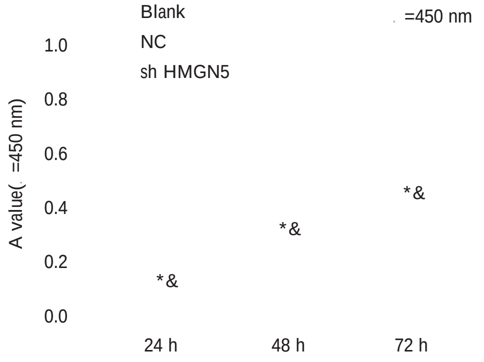


Ά Blank $^{a}P<0.05$ Ά NC $^{b}P<0.05$ ɟ

2 SKOV3 *HMGN5* mRNA

Figure 2 Comparison of *HMGN5* mRNA and protein expression levels in SKOV3 cells

τ *P*<0.05 Ά Blank NC SKOV3 Ψ *HMGN5* mRNA ׀ ל Ẻ τ *P*>0.05 ɟ *HMGN5* ﻨﺰ ﻍ SKOV3 ' ɟ *HMGN5* ﻨﺰ ﻍ ῞Ą SKOV3 ﻣﻰ ῝Ω MTT ﻪ 3 Ά Blank ׀ NC sh HMGN5 SKOV3 ﯙ 24 ϭ48 ϭ72 h

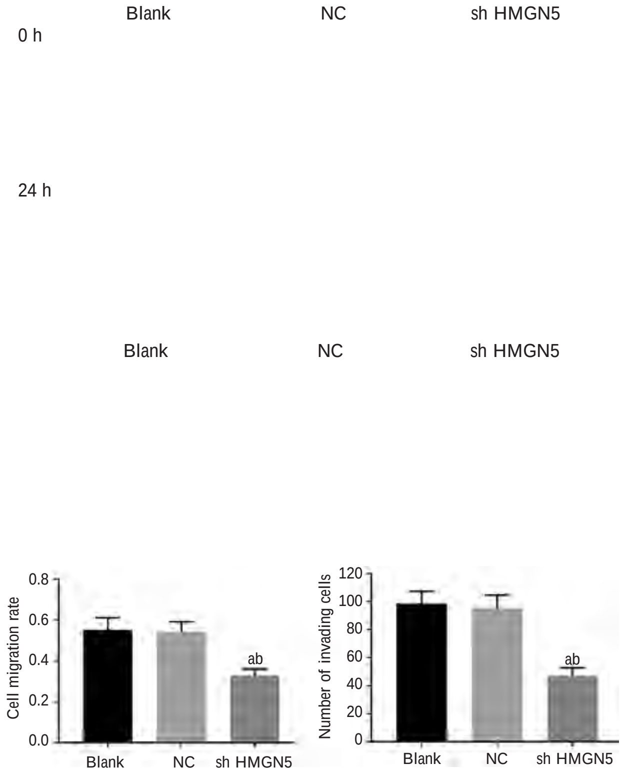


3 MTT SKOV3 A

Figure 3 MTT detection of SKOV3 cells in each group at different time A value

A ᵉ ; *F* ᵉ ȗ ᾁ ε 27.409 ϭ38.966 ϭ 36.182 *P*<0.05 τ Ά Blank NC SKOV3 ﯙ 24 ϭ48 ϭ72 h A ᵉ ל *P*>0.05 τ ɟ *HMGN5* ﻨﺰ ﻍ ῞Ą SKOV3 ﻣﻰ ɟ *HMGN5* ﻨﺰ ﻍ ῞Ą SKOV3 ׀ ˬ

ώ ﻪ 4A ׀ 4C Ά Blank ׀ NC sh HMGN5 SKOV3 ɧ *P*<0.05 Ά Blank NC SKOV3 ל τ *P*>0.05 ɟ Transwell ﻪ 4B ׀ 4C Ά Blank ׀ NC sh HMGN5 ˬ ɧ τ *P*< 0.05 Ά Blank NC ˬ ל *P*>0.05 ɟ *HMGN5* ﻨﺰ ﻍ SKOV3 ׀ ˬ ῝Ω ɟ



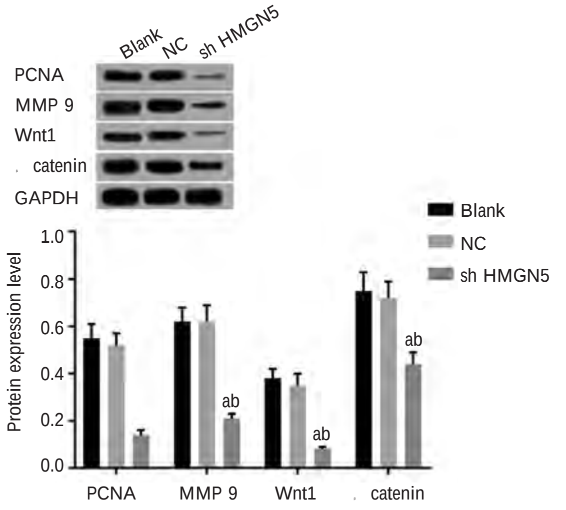
A ώ ῝Ω B Transwell ˬ ῝Ω C ˘ ׀ ˬ ɟ Ά Blank $^{a}P<$ 0.05 Ά NC $^{b}P<0.05$ ɟ

4 Transwell

Figure 4 Scratch test and Transwell test to detect cell migration and invasion

HMGN5 SKOV3 Ψ

Western blot 5 Ά Blank ⅎ NC sh HMGN5 SKOV3 Ψ PCNA MMP 9 Wnt1 ⅎ catenin ɧ *P*<0.05 Ά Blank NC SKOV3 Ψ PCNA MMP 9 Wnt1 ⅎ catenin *P*>0.05 ɟ *HMGN5* ἏA SKOV3 Ψ PCNA ⅎ MMP 2 Wnt/ catenin ɟ



Ά Blank $^{a}P<0.05$ Ά NC $^{b}P<0.05$ ɟ

5 Western blot SKOV3

Figure 5 Western blot analysis of protein expression in SKOV3 cells

HMGN5 Ψ ʹY *HMGN5* Ά TNM ΰ ʹY Ά[12] ɟ Ψ RT qPCR 4 Ψ *HMGN5* ɟ 3 *HMGN5* Ά Ă ɟ Ψ *HMGN5* Ψ Ω *HMGN5* 3 Ψ [13 15] ɟ Wu *HMGN5* Ψ ʹY *HMGN5* ΰ ⅎ [16] ɟ Xin *HMGN5* ΰ Ъ

miR 488 ⅎ ἏA[17] ɟ *HMGN5* Ψ ɟ ε *HMGN5* Ψ P SKOV3 Ψ *HMGN5* siRNA I ɧ *HMGN5* *HMGN5* SKOV3 ⅎ ἏA ɟ *HMGN5* SKOV3 *HMGN5* siRNA I SKOV3 RT qPCR ⅎ Western blot Ъ *HMGN5* SKOV3 ɟ MTT *HMGN5* ἏA SKOV3 ɟ *HMGN5* SKOV3 I Ω ɟ Transwell *HMGN5* ἏA SKOV3 Ω ɟ 3 Ά *HMGN5* ɟ PCNA Ά Ψ DNA ʹY Ă Ψ ε Ь [18] ɟ MMP 9 Ь MMP Ψ MMP 9 ⅎ Ω [19] ɟ Western blot *HMGN5* ἏA SKOV3 Ψ PCNA ⅎ MMP 9 ɟ Ά ĂЬ ɧ *HMGN5* Ψ MMP 9 ɟ Zhao[20] Ά ⅎ *HMGN5* ⅎ Ψ *HMGN5* Ψ Wnt/ catenin ⅎ ɟ Wnt1 Wnt ΰ I Ά ⅎ ʹY Ă[21] ɟ catenin Wnt Ă[22] ɟ Wnt/ catenin Ψ ⅎ Ψ [23 25] ɟ Ь Zhao SKOV3 Ψ *HMGN5* Wnt/ catenin Ă Wnt1 ⅎ catenin ɟ *HMGN5* Wnt/ catenin ɟ *HMGN5* SKOV3 ⅎ Ω ἏA ἏA Wnt/ catenin ɟ

З *HMGN5* ' Wnt/. catenin מ ˝ Ꝁ ˛ ɟ φ *HMGN5* ݲ ◓ Ψ ɔ *HMGN5* ' Ά̣ Wnt/. catenin מ ˝ Ά̣ SKOV3 ݰ ɞ ɺ ˛ ɟ ε ◓ Ά̣ w Ҍ *HMGN5* ' ◓ ɺ ݲ ɟ

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after gynecological laparoscopic surgery under general anesthesia. The combination of PTX 3 and PCT change rate is helpful for the early diagnosis of pulmonary infection.

KEY WORDS N pentamer 3 Procalcitonin general anesthesia Pulmonary infection Gynecological laparoscopy

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PTX 3 ש PCT ל ͻ

PTX 3 ש PCT ל ͻ ῷ ש ˜ ᵉ ל ͻ = ˜ 24 h ᵉ - ῷ ᵉ / ῷ ᵉ ×100% ɟ

ῢ SPSS 11.5 ϴ % c^2 x̄ ± s *t* Ặ Pearson Ặ ῢ ˘ ϴ ᵉ ROC *P*<0.05 Ẻ τ ɟ

ῷ ש ˜ Ξ ᛵ PTX 3 ש PCT ῷ 2 ᛵ PTX 3 ש PCT τ *P*>0.05 ˜ 24 h Ξ PTX 3 ש PCT ῷ *P*<0.05 PTX 3 ש PCT Ђ *P*<0.05 ɟ 2 ɟ

2 2 PTX 3 PCT *n* %

Table 2 Comparison of PTX 3 and PCT levels in 2 groups before and after operation *n* %

ᾇ	*n*	PTX 3 ng/mL		PCT(g/L)	
		ῷ	˜ 24 h	ῷ	˜ 24 h
	31	1.72±0.27	4.35±1.29^a	0.21±0.05	1.32±0.26^a
	233	1.68±0.25	2.76±0.74^a	0.20±0.04	0.84±0.41^a
t ᵉ		0.829	10.110	1.268	6.408
P ᵉ		0.408	0.000	0.206	0.000

^{a} ῷ *P*<0.05 ɟ

˜ Ξ PTX 3 ל ͻ ש PCT ל ͻ

PTX 3 ש PCT ˜ 24 h ל ͻ Ђ Ẻ τ *P*<0.05 ɟ 3 ɟ

˜ PTX 3 Ά PCT Ặ

Pearson Ặ ῢ ˜ 24 h ᛵ PTX 3 Ά PCT ᛵ ᵐ Ặ Ặ *r*=0.546 *P*=0.000 ɟ 1 ɟ

3 2

ᾇ	*n*	PTX 3 ל ͻ	PCT ל ͻ
	31	162.23±59.78	532.52±192.77
	233	71.15±27.21	318.50±101.97
t ᵉ		14.598	9.649
P ᵉ		0.000	0.000

˜ PTX 3 PCT ש ל ͻ ϴ ᵉ ˜ PTX 3 PCT ש ל ͻ Ẻ ϴ ᵉ PTX ל ͻ AUC ᵉ 0.924 PTX 3 0.871 PCT ל ͻ 0.923 ɟ 4 2 ɟ

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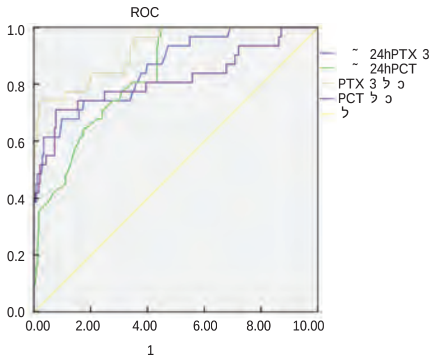

2

ROC

Figure 2 ROC Curve of indicators in ppredicting pulmonary infection after gynecological laparoscopic surgery under general anesthesia

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HBV HBV DNA PreS1 HBeAg

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ئ ┘ ǚ \ [3] ɟ
ﻫ HBV ζ ʍ B ى ⅎ C ى ε ζ ẹ Ψ B ى
ʍ Ba ى ε ζ ɟ HBV ئۆ ﻫ ← ʍ C ى ε ζ ئۆ
╧ ῎Ω ε B ى [4] ɟ
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[5] ɟ 3 Y ئۆ ﻧﺢ ﻍ ى HBV
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Ꚏ 2.5 min

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V H B - U 2 C G ' - 5 C G ' -

V H B - B

e T y p B 1

e T y p B 2

V C H B -

e C T y p 1

e C T y p 2

2 B C HBV DNA

Table 2 Expression of HBV DNA in genotype B C infected patients

ἇ	*n*	HBV DNA +	HBV DNA	c^2 e	*P* e
B ی	131	75 57.2	56 42.8%	0.867	0.352
C ی	93	59 63.4	34 36.6%		

3 B C PreS1

Table 3 Expression of PreS1 in genotype B C infected patients

ἇ	*n*	PrsS1 +	PrsS1 -	c^2 e	*P* e
B ی	131	63 48.1%	68 51.9%	0.002	0.965
C ی	93	45 48.4%	48 51.6%		

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ɸ HBV w B ݲ غ ی ▌58.0%
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Chromosome karyotype analysis of 1 776 peripheral blood lymphocytes in Nanjing Area

YU Mengyao GU Min ZHAO Hong
Department of Laboratory Medicine the First Affiliated Hospital of Nanjing Medical University Nanjing Jiangsu China 210029

ABSTRACT Objective To investigate the correlation between abnormal karyotypes and the distribution of chromosomal diseases in Nanjing. Methods 1 776 samples from Januar AYno ŵ Januar J ɑ ʔ حي v

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36.7% 652/1 176 ܭ ǚ ئ̆ 12~45 φ ɟ

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ي ζ í ɔ Ψ í ε J ה ̈ Ã 460 ʢ ẹ ζ ي ε 47 XXY 11.74% ɞ46 XX 1.30% ⁄ 45 XY rob 13 14 0.65% / 312 ʢ Ό ي ζ ε 47 XXY 6.73% ⁄ 45 XY rob 13 14 1.28% Ὼ ꞔ ; 19 ʢ ζ ي ε 47 XY+21 73.68% Ξ 15 ʢ ي ε 46 XX 40.00% ɟ ɔ Ψ í ε Ɑ ה Ã 156 ʢ ي ζ ε 45 XO 12.18% ɞ46 XY 5.13% ⁄ 46 X i Xq 3.85% J ה ̈ 128 ʢ ẹ ꟾ ي Ũ ɞ ה 131 ʢ ζ ي ε 45 XO 7.63% Ξ 14 ʢ ي ε 46 XY 71.43% ɟ

2 n .

Table 2 Clinical manifestations karyotype classification and abnormal detection rate of common chromosomal karyotype abnormalities n .

ي í	ɔ ʢ %	ي	Ό ʢ n	Ό %
J ה ̈	460 25.90	47 XXY	54	11.74
		46 XX	6	1.30
		45 XY rob 13 14	3	0.65
/	312 17.57	47 XXY	21	6.73
		45 XY rob 13 14	4	1.28
Ὼ ꞔ ;	19 1.07	47 XY+21	14	73.68
Ξ	15 0.84	46 XX	6	40.00
Ɑ ה	156 8.78	45 XO	19	12.18
		46 XY	8	5.13
		46 X i Xq	6	3.85
J ה ̈	128 7.21	46 XY	3	2.34
		45 XO	1	0.78
		46 X i Xq	1	0.78
ɞה	131 7.38	45 XO	10	7.63
		46 X i Xq	4	3.05
		46 XY	3	2.29
Ξ	14 0.79	46 XY	10	71.43

Ꝁ ⁄ Ψ Ό ꟾ Ᵽ ꟾ ɞ ꟾ ẅ ́ ꟾ [1,2] ɟ ꟾ ẅ ꟾ ģ [3] ɟ ꟾ ’ ⁄ Ό ꞵ ɞ ' [4] ● ɟ ς Ӂ Ӂ ♥ Ὼ Ǻ ɟ ῷ í ɔ ꟾ ꟾ ي Ũ ́ í ’ w ģ ε í ɞ Ꝁ ⁄ ẅ Ђ Ђ w ̦ ẅ τ ɟ Ψ ╧Шئ┘ 1 776 ʢ Ѣ Ꝁ Ũ Ã Ό ꟾ 280 ʢ ε 15.76% Њm ж ꟾ 5%[5] ’ Ǻ Ὼ Ѣ ي Ă ي ς Њ⁴ [6] 2012 ╧Шئ┘ ي 6.37% ’ Ǻ Ξ ж ɞ ẅ Ặ ӂ Ǻ б [7] 2019 ϙ ئ┘ 11.9% ꟾ ي ε Ꝁ ⁄ Ψ ģ ɟ

Ψ ꟾ w ꟾ ε ζ ▌ 50% ẹ ε ᾳ̈ ɞ ꟾ ɞ ꟾ ⁄ ꟾ ́ ꟾ ʢ ꞔ ɟ ꟾ ẅ w 47 XXY ε ζ ▌ 66.43% 93/140 Ǻ ɑ [5] 47 XXY ε ẋ ́ 460 ʢ J ̈ ⁄ 312 ʢ / ɔ Ψ Ό 75 ʢ ي ה ꟾ ε ⁄ w ̦ ɟ ꟾ Ὼ w 45 XO ε ζ ◑ ́ Turner syndrome Turner s ́ ▌ ε 27.14% 38/140 ɟ Turner s ́ í ε ɞ J ה ̈ ɞ ئ̈ 156 ʢ Ɑ ה Ψ Ό 19 ʢ 12.18% ئ̈ 128 ʢ J ה ̈ Ψ Ό 1 ʢ 0.78% ئ̈ 131 ʢ ɞה Ψ Ό 10 ʢ 7.63% Ǻ [8] ж m ɟ ᾳ̈ Ӂ ᾳ̈ ε ꟾ ي 46 XY w ẅ Ӂ ᾳ̈ ε ꟾ ي ε 46 XX ɟ Ψ Ã Ό ᾳ̈ 39 ʢ 2.20% ᾳ̈ ’ ᾳ̈

˝ XY Ό Ɑ ה ɞ ݜ
₿ ضم Ό † ה ɞ ݜ ݜش ˝
[9] ɟ Ψ Ό l 33
ʢ 1.86% ẹ Ψ ç Ḫ یی 12 ʢ ݜ w 13 ɞ14
˝ l Ḫ ε ζ ▌ 75% 9/12 Ά
[10] ɟ ç Ḫ یی m Ӂ
ẹ ḪⱭ Ỷ Ђ ẹ ˝ У ṿ У
[7] ɟ í ε J ה ˝
Ψ Ό ç Ḫ 3 ʢ / 4 ʢ ɟ غ ‖
ʊ̃ Њ l یی ʊ̃
w ặ ƃ ƃ ɟ Ψ ´ l
Ό ▌ ε 0.73% ʢ ʜ ' Њ یی ʊ̃
ʊ̃ Ύ ´ l ' غ ẹ ´ ʢ ˝
ъ í یی O ˝ غ ' w
´ ẹ ђ Ẉ ʊ̃ ε í
w ã Ά ݜ / ɟ
l یی ʊ̃ m Ҟ
Ẻ ˝ ' ɥ í ɵ ᵉ ɟ Ҟ
l یی ʊ̃ ɔ غطی ĭ Ύ ẹ ʊ̃ ˝
Њ Њ 10 Mb w Ό
Ꝫ ئوج m ɘ Ά Ђ ẹ í [11] ɟ
ʊ̃ ˝ ה l Ώ ʊ̃
ɞ غ نحر ʊ̃ ɞw یی ʊ̃ غ نحر
ݜ ƒ غ نحر Ḫ ъ
l Ẻ l ה Ą Ђ
l ε ƃ ƃ ɞ ɞ ݜ
l w ε ã Ά ɟ
; ´ Ҟ Ҟ l
یی ʊ̃ Ά FISH ɞCMA ʊ̃ Ҟ
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ᛋ DNA Ψ Ą غ نحر نحر ɔ ɟ מ
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Chemerin 25 OH D3 visfatin

ɔ Chemerin ɞ25 D3 25 OH D3 ɞ visfatin GDM ɵ ᵉ Ά IR Ặ ɟ ˥ 2017 2 2019 3 GDM 80 ʅ ɛ ˥ l 80 ʅ ɛ ɟ Ấ ˜ 2 ɞ Chemerin ɞ25 OH D3 ɞvisfatin ɞ HOMA IR ǚ Chemerin ɞ25 OH D3 ɞvisfatin Ά HOMA IR Ặ ɞ Chemerin ɞ25 OH D3 ɞvisfatin ╥ Ά ˊ GDM ɟ 25 OH D3 ɛ 27.26±5.11 nmol/L ɥ Ѣ 38.71±8.63 nmol/L Chemerin ɞvisfatin ǚ ɛ 273.92±40.20 ng/mL ɞ 23.54±2.66 ng/mL Ѣ 186.14±26.06 ng/mL ɞ 11.68±1.80 ng/mL t_1=10.211 ɞt_2=16.388 ɞt_3=33.028 P <0.001 HOMA IR ɛ 3.89±1.38 Ѣ 1.62±0.46 t=13.958 P<0.001 Spearman Ặ ǚ 25 OH D3 Ά HOMA IR \ Ặ Chemerin ɞvisfatin Ά HOMA IR \ Ặ r_1=-0.576 ɞr_2=0.604 ɞr_3=0.792 P <0.001 Chemerin ɞ25 OH D3 ɞvisfatin ˊ GDM 95.00% Ѣ ╥ᵐ c^2=14.546 P=0.002 ɟ Chemerin ɞ25 OH D3 ɞvisfatin Ά GDM Ặ ɞ Ψ Ə 3 ˊ ╖ GDM ɛ wᵐ ɟ

ɔ 25 D3

The diagnostic value of serum chemerin 25 OH D3 visfatin levels in gestational diabetes mellitus and their association with insulin resistance

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ABSTRACT Objective To investigate the diagnostic value of serum chemoattractant Chemerin 25 hydroxyvitamin D3 25 OH D3 and visfatin levels in gestational diabetes mellitus GDM and insulin resistance in patients IR relevance. Methods 80 patients with GDM in our hospital from February 2017 to March 2019 were enrolled and 80 women with normal physical examination during the same period were recruited as the control group. Two groups of blood samples were collected after admission and serum Chemerin 25 OH D3 visfatin levels steady state model insulin resistance index HOMA IR were measured. The correlation between the levels of serum Chemerin 25 OH D3 visfatin and HOMA IR was statistically analyzed. The diagnostic efficacy of Chemerin 25 OH D3 visfatin alone and in combination was evaluated. Results The level of serum 25 OH D3 in the study group was 27.26 ± 5.11 nmol/L

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lower than that in the control group 38.71 ± 8.63 nmol/L and the levels of serum Chemerin and visfatin were 273.92 ± 40.20 ng/mL 23.54 ± 2.66 ng/mL higher than that in the control group 186.14 ± 26.06 ng/mL 11.68 ± 1.80 ng/mL t_1 = 10.211 t_2 = 16.388 t_3 = 33.028 respectively $P<0.001$. The HOMA IR of the study group was 3.89 ± 1.38 higher than that of the control group 1.62±0.46 t=13.958 $P<0.001$. By Spearman correlation analysis the serum 25 OH D3 level was negatively correlated with HOMA IR the serum Chemerin and visfatin levels were positively correlated with HOMA IR r_1=-0.576 r_2=0.604 r_3=0.792 $P<0.001$. The sensitivity of chemorin 25 OH D3 and visfatin in the diagnosis of GDM was 95.00% higher than that of single index c^2=14.546 P=0.002 . Conclusion The levels of serum Chemerin 25 OH D3 and visfatin are closely related to insulin resistance in GDM patients and play an important role in the occurrence and progression of the disease. The combined detection of three can improve the diagnostic sensitivity of GDM.

KEY WORDS chemokine 25 hydroxyvitamin D3 visfatin gestational diabetes insulin resistance

Gestational diabetes mellitus GDM ɛm Ꮒ ה ה Ỉ ῦ պ ʼ պ ʽ б ה ɞ ⱱ ɞ ɞ Ψ ה О ⅵ ɟ [1-3] ɟ Ό ٷ ꬺ GDM ה ɛ 4.3%~5.1% ʾ ж Ꮜ ῎Ε ɞ ל ɔ ἓ ἳ ẹ ה ᏺ ╗ † [4] ɟ ί Ό Insulin resistance IR ɛ GDM ζ Ɑ ἓ ٷ ˘ ڗ ԋ ẹ Ꮒ Ψ ԋ 45%~80%[5,6] ɟ ж I Ά ẹ ῦ ἓ ⁄ IR Ẻ ῩĂ [7] ɟ ɔ Chemerin ɛ ى ἓ φm ʼ ל Ά א ɞ ῦ ɔ Ά 2 ى ɞ ɞ ɗR Ẻ ῩĂ [8,9] ɟ Ỉ visfatin m Ꮒ Ỉ ᏺ ἓ ʼ ἇ ə ש Ɑ ῦ ʼ ל Ά GDM ה ɞ [10,11] ɟ 25 ؿ D3 25 hydroxyvitamin D3 25 OH D3 ʼ ɵ I D Ỹ ٦ ẹ ԋ Ӂ ʾ պ [12,13] ɟ ٦ 80 ٦ GDM ῦ Chemerin ɕ25 OH D3 ɑvisfatin Ά GDM IR Ặ Chemerin ɕ25 OH D3 ɑvisfatin ´ Ά ╥ GDM ɵ e ٷ ɛ Wm ל ɟ ٦ ; ɟ

m

٦ 2017 2 2019 3 GDM 80 ٦ ɛ פֿ ٦ʾ 80 ٦ ɛ ɟ 2 m ɞ ⁄ ɞ ῷ I ɞGDM ´ ɞУ ى ج ʼ $P>0.05$ τ 1 ɟ ├ Ӈ ⁄ Ӂ Ά ɟ

Ẩ ש Ά

Ẩ Ά ج ´ ћ У ђ [14] Ψ GDM ί Ά ה ج ɛ ɟ ɔ ╥ ʾ Ꮚ ɟ Ά ٷ ⱱ ῦ ɞ У ɞ ɞ У ´ ´ ش ´ ɞ ɞ ɞ ♥ ´ ɞ ɞ ڗ ʽ О ´ ɞ ẋ ɞ ἳ ɟ ´ ẹ ђ ɟ

1 2 x̄±s n %

Table 1 Comparison of 2 groups of general data x̄±s n %

ἇ	٦	( )		⁄ ⁄		ῷ I kg		GDM ´	У ى	
									У	ἐ У
	80	20~37	28.71±4.06	25~39	32.48±2.74	42~81	53.80±5.41	9 11.25	21 26.25	59 73.75
	80	21~38	29.02±3.59	26~38	32.70±2.52	43~83	54.46±5.25	3 3.75	23 28.75	57 71.25
t/c^2 e		0.512		0.529		0.783		3.243	0.125	
P e		0.610		0.598		0.435		0.072	0.723	

ↄ 5 mL ա 3 000 r/min 10 min ˥ ә ա ↄ Fasting blood glucose FBG ẍ Fasting insulin FINS ی Homeostasis model insulin resistance index HOMA IR ᵉ HOMA IR=FBG× FINS/22.5 ա ẍ Ⴘ Chemerin ɞ 25 OH D3 ɞvisfatin Ḥ ৄ ә Ὼ ắ ˘ Ә ৄ Օ Ḥ Щ Ό ж Ⴈ ɟ

ɞ Chemerin ɞ25 OH D3 ɞ visfatin ɞ HOMA IR ᵉ ǚ Chemerin ɞ25 OH D3 ɞvisfatin Ά HOMA IR Ặ Chemerin ɞ25 OH D3 ɞ visfatin ╥ Ά ˊ GDM ᵉ Chemerin ε 210 ng/mL ɞ25 OH D3 ε 28.2 nmol/L ɞvisfatin ε 25 ng/mL 4 ˊ ѷ ᵐ Ὼό ε ɟ

SPSS 22.0 ա $\bar{x} \pm s$ t n % c^2 Spearman Ặ ǚ $P<0.05$ ε τ ɟ

2.1 Ξ Chemerin ɞ25 OH D3 ɞvisfatin 25 OH D3 ɥ Ѣ Chemerin ɞvisfatin Ѣ $P<0.05$ 2 ɟ

2 Chemerin 25 OH D3 visfatin $\bar{x} \pm s$

Table 2 Comparison of serum levels of Chemerin 25 OH D3 and visfatin between the 2 groups $\bar{x} \pm s$

ᾇ	⅂	Chemerin ng/mL	25(OH)D3 nmol/L	Visfatin ng/mL
	80	273.92±40.20	27.26±5.11	23.54±2.66
	80	186.14±26.06	38.71±8.63	11.68±1.80
t ᵉ		16.388	10.211	33.028
P ᵉ		<0.001	<0.001	<0.001

Ξ HOMA IR HOMA IR ε 3.89±1.38 Ѣ 1.62±0.46 τ $t=13.958$ $P<0.001$ ɟ

2.3 Chemerin ɞ25 OH D3 ɞvisfatin Ά IR Ặ ǚ

Spearman Ặ ǚ 25 OH D3 Ά HOMA IR Ⅵ Ặ Chemerin ɞvisfatin Ά HOMA IR Ⅵ Ặ $P<0.05$ 3 ɟ

3 Chemerin 25 OH D3 visfatin IR $n=80$

Table 3 Analysis of correlation between serum levels of Chemerin 25 OH D3 and visfatin and IR $n=80$

		Chemerin	25 OH D3	visfatin
HOMA IR	r ᵉ	0.604	-0.576	0.792
	P ᵉ	<0.001	<0.001	<0.001

╥ Ά ˊ GDM ɵ ᵉ ˊ Ѣ Chemerin ɞ25 OH D3 ɞ visfatin ╥ $P<0.05$ ˊ ɞ Ά Ά Chemerin ɞ25 OH D3 ɞvisfatin ╥ τ $P>0.05$ ɟ 4 ɟ

4 GDM n %

Table 4 The Value of Serum Indicators in the Diagnosis of GDM Individually and Jointly n %

			Ά
25(OH)D3	72.50 58/80	82.50 66/80	77.50 124/160
Chemerin	80.00 64/80	86.25 69/80	83.13 133/160
Visfatin	82.50 66/80	87.50 70/80	85.00 136/160
ˊ	95.00 76/80	81.25 65/80	88.13 141/160
c^2 ᵉ	14.546	1.612	6.920
P ᵉ	0.002	0.657	0.075

Ό ﯙ Ꝁ ﻧﺦ ﻧﺢ ә ¥ IR ε GDM ה Ɑ ﻍ IR ε GDM Ặ [15] ɟ Ψ HOMA IR Ѣ ɟ GDM ﯙ Օ IR Ά ә ᵐ ʢ IR ε GDM ה Ặ ɟ IR ש ǚ Ὼ Ѣ Ӂ ɞ ৄ ﯙ Ύ I ;

ܿ ܿ ǚ

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ה GDM [16,17] ɟ

Ặ Ό ɔ ġ ɞ

ġ Љ IR ה ɞה Ψ ج

Ə [18,19] Å Ậ Ȃ7 Ã

9 .
ɞ ɔ ɞ ˊ 4 Ά Ặ
J . Ψ ๔ ῷ ⁄ У 2018 10 7 76 79.

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Ặ J . 65 78 ├ [illegible] ɔ / 7 3 5 Ặ Ặ Æ H

CEA NSE SCCA miR 21

Ɑ CEA ɞ ẃ ɔ NSE ɞ ɔ Ɑ SCCA ´ miR 21 Ά í ɞ ש̇ ˜ Ặ ɟ 87 ʇ Ə ɛ ⩘̉ l ɟ ж Ə ɛ ɔ ה ẉ ẍ ˘ Ψ CEA ɞ NSE ɞSCCA PCR Ψ miR 21 ῴ ǚ CEA ɞNSE ɞ SCCA ש̇ miR 21 Ά Ψ ᾇ ɞ ɞ ي ש̇ TNM ǚ í Ặ ˜ ǚ Ά ש̇ ˜ Ặ ɟ Ά CEA ɞNSE ɞSCCA ש̇ miR 21 ╖ τ $P<0.05$ CEA ɞSCCA ɞmiR 21 Ά í Ặ τ $P>0.05$ NSE Ά TNM ǚ Ặ τ $P<0.05$ CEA ɞNSE ɞSCCA ש̇ miR 21 Ά Ặ τ $P<0.05$ CEA ɞNSE ɞSCCA ש̇ miR 21 Ά ˜ Ặ τ $P<0.05$ 4 ´ ҕ Ҍ ╖ τ $P<0.05$ ɟ Ψ CEA ɞNSE ɞSCCA ⩘ miR 21 Ҍ ж ’ Ə ɛ ɞ ש̇ ˜ ɟ

Ɑ ẃ ɔ ɔ Ɑ miR 21

Correlation between CEA NSE SCCA combined with miR 21 and clinicopathological features efficacy and prognosis in patients with lung cancer

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ABSTRACT Objective To investigate the relationship between CEA NSE SCCA combined with miR 21 and clinicopathological features curative effect and prognosis in patients with lung cancer. Methods 87 patients with lung cancer were selected as the experimental group and 87 healthy people in the same period of physical examination as the control group. The expression levels of CEA NSE and SCCA in serum were detected by electrochemiluminescence immunoassay and miR 21 was detected by real time PCR. The correlation of tumor markers CEA NSE SCCA and miR 21 with sex age pathological type and clinicopathological characteristics of TNM stage in patients with lung cancer was analyzed before treatment and the correlation with curative effect and prognosis was analyzed after treatment. Results compared with the control group the expression levels of CEA NSE SCCA and miR 21 in the experimental group were significantly higher than those in the control group $P<0.05$. The expression levels of CEA SCCA

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and miR 21 were not correlated with clinicopathological features of lung cancer $P>0.05$. NSE was correlated with TNM stage of lung cancer $P<0.05$. The expression levels of CEA NSE SCCA and miR 21 were correlated with the curative effect $P<0.05$. The survival curve showed that the expression levels of CEA NSE SCCA and miR 21 were correlated with the survival prognosis of the patients $P<0.05$. The sensitivity of the combination of the four indexes in the diagnosis of lung cancer were significantly better than those of single index and the difference was statistically significant $P<0.05$. Conclusion the expression levels of CEA NSE and SCCA in serum of patients with lung cancer are significantly higher than those of normal controls and the expression level of miR 21 is significantly lower than that of normal controls which could be used as indicators of diagnosis curative effect and prognosis of lung cancer.

KEY WORDS Carcinoembryonic antigen neuron specific enolase squamous cell carcinoma antigen miR 21 lung cancer

lung cancer φm
25% H [1] ɟ 90%~95%
ɔ ẹ ⏊ ɟ
ε ˜ Ҍ ؽ l ѷ ă ኃ
' ɔ ᾇ ˬ [2] ɟ ε
ה ؽ ˜ ɔ [3] ɟ
⏊ Ặ
₿ ɑ Carcinoembryonic antigen
CEA ɞ ẃ ɔ neuron specific
enolase NSE ɞ ɔ ɑ squamous
cell carcinoma antigen SCCA [4] ɟ Ặ
mRNA 21 Ђ ж ɑ ה
miRNA

ɬ miR 21 ң w $P<0.05$ ɛ τ ɟ

˘ Ψ CEA ɞNSE ɞSCCA ٷ miR 21

CEA ɞNSE ɞSCCA Ҍ Ẻ τ $P<0.05$ Ψ miR 21 Ҍ Ẻ τ $P<0.05$ ɟ 1 ɟ

1 CEA NSE SCCA miR 21 x̄±s

x̄±s

ǫ̈	CEA g/L	NSE g/L	SCCA g/L	miR 21
	2.35±0.26	6.14±0.79	1.41±0.11	0.92±0.14
	11.65±1.75	14.51±1.62	2.94±0.16	2.13±1.13
P ᵉ	0.032	0.027	0.029	0.041

CEA ɞNSE ɞSCCA ٷ miR 21 Ά í Ặ

ŭ CEA ɞSCCA ٷ miR 21 Ά ǫ̈ ɞ ɞ ی ɬ TNM ŭ Ặ $P>0.05$ ɟ NSE Ά ǫ̈ ɞ ɞ ی Ặ $P>0.05$ Ά í ŭ Ặ Ẻ τ $P<0.05$ í ŭ ъ ~ ╗ NSE ъ 12.37 ɞ14.98 ɞ15.76 ﻣﻘ Ά í \\ Ặ ɟ 2 ɟ

CEA ɞNSE ɞSCCA ٷ miR 21 Ά Ặ

ầ ɟ PR 26.4% $c^2=5.78$ ˜ CEA ɞNSE ɞSCCA ٷ miR 21 ң Ҍ ῷ Ẻ τ $P<0.05$ SD 40.2% $c^2=8.26$ ˜ CEA ɞNSE ɞSCCA ٷ miR 21 ң Ҍ ῷ Ẻ τ $P<0.05$ PD 33.3% $c^2=4.91$ ˜ CEA ɞNSE ɞSCCA ٷ miR 21 ң Ҍ ῷ Ẻ τ $P<0.05$ ɟ 3 ɟ

CEA ɞNSE ɞSCCA ﺵ miR 21 Ά غ [7] ɟ ₿ ɔ
˜ Ặ ɻ [8] ɟ ῷ
Kaplan Meier CEA ɞNSE ɞ ɻ
ﺵ SCCA ﾷ ӊ ӂ З ﺝ ﯙ ˬ ɞ ӊ ɞ
CEA ɞNSE ﺵ SCCA ӊ Ḣ CT
˜ Ỹ ɟ miR 21 Ỹ Ά ɯ [9] Ҍ ﯙ ● CT
ɔ 3 Ά Ỹm ɟ ῦ ᾅ ῎ Ἇ Ђ ﯙ Ψ
CEA ɞNSE ɞSCCA ﺵ miR 21 ɟ غ ﯙ ί Ψ ’
ӊ ˜ Ỹ τ ɟ ﯭ 1 ɟ ẅ ɟ Ҍﺝ غ Зӂ З ˜З

Ψ╥Y ﺵ ΄ ɻ

CEA ɞNSE ɞSCCA ﺵ miR 21
ῦ ᾅ 51.7% ɞ87.9% ɞ52.9% ɞ63.2%
ῦ ᾅ 90.8% ɞ82.7% ɞ85.1% ɞ81.6% ﻁ
΄ ε 85.1% Ҍ╥ ӂ
ε 70.1% ӊҌ╥ ɟ 4 ɟ

ζ ﬣ ɻ Н ɑ

CEA ɗNSE ɗSCCA
Ѣ ж CEA ⺁ SCCA Ά
í Ặ NSE Ά TNM ǚ
Ặ 3 Ά ש̇ ˜ Ặ ɟ
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Ψ ╥ Y ש̇ ´ ⺁
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Ѣ CEA ɗNSE ɗSCCA ש̇ miR 21
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ﷺ ϗ φ Ƭ ɟ ɔ ’
Ψ CEA ɗNSE ɗSCCA ⺁ miR 21
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miR 21 125

◓ RNA 21 miR 21 ש Ɑ 125 CA125 ל כ ש
τ ɟ 80 ʒ ◓ ɞ50 ʒ ◓ ל w ש 50 ʒ ɟ
ɟ ˀ ˘ miR 21 ש CA125 Ȗ miR 21 ɞCA125 ◓
ɵ ᵉ ש ẹ Á ◓ Ặ ɟ miR 21 ש CA125 ঙ Ѣ
ˀ ɟ miR 21 ש CA125 ש Ѣ ɟ $P<0.05$ ɟ miR 21 Á CA125 ´
◓ AUC ε 0.959 Ѣ ╥ $P<0.05$ ´ Á ╥
$P>0.05$ ɟ í Ȗ ɞ Ȗ כ w ש ◓ miR
21 Á CA125 ঙ Ẻ τ $P<0.05$ ɟ Á ώ 1 Y ˜
miR 21 ˀ CA125 ঙ ҥ $P<0.05$ ɟ ◓ miR 21 ˀ CA125
╗ Ξ ´ Ѣ ◓ í ˀ Ţ ɟ
◓ RNA 21 Ɑ 125

Changes and significance of serum miR 21 and carbohydrate antigen 125 in patients with ovarian cancer

YOU Juan
Department of obstetrics and gynecology Rugao people s hospital Rugao Jiangsu China 226500

ABSTRACT Objective To investigate the changes of serum microRNA21 miR 21 and carbohydrate antigen 125 CA125 in ovarian cancer patients and its clinical significance. Methods
/ N J 3 4

◓

ɞ O I ɟ [1] ɟ ◓
ה ┼ ̏ ẻ ي í ς ï
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ȳ [2] ɟ ◓
Ά̃ ӝ ẹ Њ à Ə ǚ ̈ ъ
ɟ ؤ ɺ
ɛ ῴ í
Ɑ 125 Carbohydrate antigen 125 CA125 ᵐ
Ẻ [3] ɟ
RNA microRNA miRNA ᵐ ǚ
RNA ל Ά ɞἕ H ɯ ẇ ɥ
ɟ [4 5] ה RNA 21 microRNA
21 miR 21 Ά ה Ύ Ặ ӝ
ẹ ؤ ◓ ה Ψ Ə ̏ ɟ
ǚ Ђ 80 ʢ ◓ miR 21 ẇ CA125
ל ɔ Ξ ◓ ɵ ᵉ ẇ ẹ Ά
ʢ Ặ ; ɟ

ᵐ
2016 1 2018 1 ɔ
80 ʢ ◓ ɛ 37~
65 ج 53.68±6.32

˘ miR 21 ш CA125 x̄±s

Table 2 Comparison of expression levels of serum miR 21 and CA125 in each group x̄±s

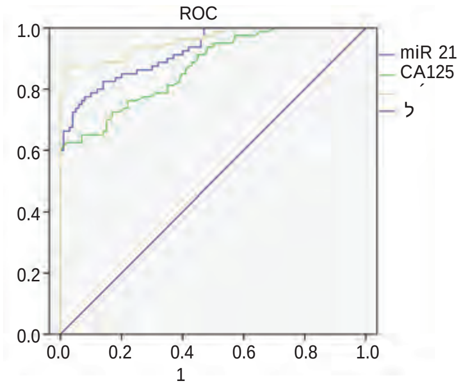
ᾴ	*n*	miR 21	CA125 U/mL
ɬ	50	1.02±0.36	19.34±4.85
	50	1.55±0.42	29.91±6.23
	80	2.39±0.72	52.66±18.83
F ᵉ		87.123	107.454
P ᵉ		0.000	0.000

ɉ ῙΆ ROC ṹ ה miR 21 ᛧ CA125 ╥ ◒ AUC ṹ ᾴ ɛ 0.925 ɞ 0.872 Ξ ÿ í ᵉ ṹ ᾴ ɛ miR 21<2.13 ɞ CA125<35.50 U/mL ݴ ΆΆ ; miR 21 ᛧ CA125 ṹ ᾴ ɛ 78.8% ɞ62.5% ɛ 92.0% ɞ98.0% ɉ ´ AUC ɛ 0.959 ɛ 91.3% Њ miR 21 ᛧ CA125 ╥ *P*<0.05 ɛ 92.0% Ά ╥ τ *P*>0.05 ɉ 3 ɞ߷ 1 ɉ

˛ miR 21 ш CA125 ◒ ɵ ᵉ U/mL %

Table 3 Diagnostic value of serum miR 21 and CA125 in ovarian cancer U/mL %

	ÿ í ᵉ			Youden	AUC	95% ' מ ˩
miR 21	2.13	78.8	92.0	0.708	0.925	0.888~0.961
CA125	35.50	62.5	98.0	0.605	0.872	0.822~0.922
´		91.3	92.0	0.833	0.959	0.931~0.986



˛ miR 21 ш CA125 ◒ ROC

Figure 1 ROC curve of serum miR 21 and CA125 detection for the diagnosis of ovarian cancer

˝ ˀ í miR 21 ш CA125 Ỹ ROC ṹ miR 21 ш CA125 í ᵉ ɛ ΆΆ Ό í ᵉ ɧ Њ í ᵉ ɉ í ṹ ˛ ~˛ ɞ ɧ ṹ ɔ w ש ݴ ◒ miR 21 ш CA125 ṹ ᾴ Њ˛ ~˛ ɞΨ ṹ ɔ ᛧ ה τ *P*<0.05 ɉ 4 ɉ ῷ˜ miR 21 ш CA125 Ỹ 1 Y ˜ miR 21 ш CA125 ῷ ɧ Ȅ

τ *P*<0.05 ɉ 5 ɉ

ל ᛧ ﻄﻤﺱ

' ◒ ה ╗ ΎY Ⅵ ɔ † ⁷ ɉ ◒ ˜ A ' 90% w ɔ Ж Њ ˝ ΎY ï Њ ˜ A ɧ Њ 40% ⁸ ⁹ ɉ ἔ ᛧ ◒ í ᛧ ˜ ɛ ɉ ἔ ל Ά ה ᛧ ה í ├ ᛧ ó ˜ ¹⁰ ɉ CA125 Њ Ɑ ῷ í ɔ ɛ ݴ ɞ† Ψ ج ˜ ᵐ ə ¹¹ ɉ Ά ◒ ש ɬ ◒ CA125 ╗ CA125 ◒ ˀ Ȅ ל ɵ ᵉ ɉ ҕ CA125 ݴ ˝ˀ í ṹ ɞ ṹ ɔ w ש ı ה Ψ ς ݴ ΎY ◒ ˜ 1 Y CA125 ς ɧ CA125 ݴ ◒ Ψ ה ə ' ə ɛ Ţ ɉ ה ᛧ ה ﻧﻊ ἔ Ăˀ ל Ά miRNA ᵐ ╥ RNA Ά 3′

miR 21 CA125 *n* %

Table 4 comparison of serum miR 21 and CA125 expression in patients with different clinical and pathological characteristics *n* %

í		miR 21				CA125			
			Ḩ	c^2 e	*P* e		Ḩ	c^2 e	*P* e
í ǚ	ˌ ~ˌ *n*=45	30 66.67	15 33.33	23.220	0.000	23 51.11	22 48.89	14.670	0.000
	ˌ ~ˌ *n*=35	33 94.29	2 5.71			27 77.14	8 22.86		
ǚ ɔ	Ψ ǚ ɔ *n*=47	31 65.96	16 34.04	31.869	0.000	22 46.81	25 53.19	32.175	0.000
	Ḩ ǚ ɔ *n*=33	32 96.97	1 3.03			28 84.85	5 15.15		
	ı *n*=52	35 67.31	17 32.69	39.521	0.000	29 55.77	23 44.23	7.988	0.007
	n=28	28 100.00	0 0.00			21 75.00	7 25.00		

◓ ώ˜ miR 21 ש CA125 x̄±s

Table 5 Comparison of serum mir 21 and CA125 expression levels in ovarian cancer patients before and after surgery x̄±s

ᾶ	*n*	miR 21	CA125 U/mL
ώ	80	2.39±0.72	52.66±18.83
˜ 1 Y	80	1.87±0.53	38.52±9.57
t e		5.465	5.787
P e		0.000	0.000

mRNA ˊ ﻍ ﻨﺰ ə [12] ɟ [13 14] ה miRNA ɑ ﻍ ﻨﺰ ﻍ ﻨﺰ ə ч ẍ ɞ ˥ Ά ɟ miR 21 m Ẻ Ч ﻍ ﻨﺰ miRNA Ά̤ ﻍ ﻨﺰ TPM1 ɞPTEN ɞSPRY2 ˜ Ḩ ˛ ɞǚ ⁄ ˬ Ъ ﯙ w ש ı ڑ Ψ ה ə [15 17] ɟ ה ◓ miR 21 ◓ ⁄ ɬ ╗ ΎY ˆ ˀ í ǚ ɞ ǚ ɔ w ש ı miR 21 ϛ ﯙ miR 21 ﯙ ◓ ה Ψ ʼ ה ˛ ə ẹ Ą̣ ə Ά̤ m ɟ Ά̤ ROC ǚ miR 21 ש CA125 ◓ ɵ e miR 21 ⁄ CA125 ╥ ◓ AUC ﭺ Ҍ 0.8 Ξ ◓ ﭺ Ẻ ẹ Ψ miR 21 ◓ AUC ⁄ ﭺ Ҍ CA125 miR 21 ◓ ɟ ◓ ● O ẍ í Ą̣ ╥ Ж ﯙm ﻍ ˊ ɟ ה

miR 21 ⁄ CA125 ˊ AUC Ҍ╥ ΎY ˊ ╥ ϛ miR 21 ⁄ CA125 ˊ ʼ m Ḩ ɟ ɔ miR 21 ⁄ CA125 Ỹ Ά ◓ ה ה ΎY Ą̣ ʼ ə ε ◓ ˥ miR 21 ⁄ CA125 ˊ Ҍ ◓ Ά̃ Ḩ ɟ

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H_2O_2 PC12

PC12 H_2O_2 PC12

SOD qRT PCR miR 422a

miR 422a miR 422a H_2O_2 PC12 SOD Western blot

< 2 Bcl 2 Bax < H_2O_2 < PC12 a P<

Њ ל

O ● ж ɟ ɔ ’

ל ה [1,2] ɟ ɠ Ẻ

ᵰ Ɥ

ל ה Ẻ τ ɟ

ɔ H_2O_2 Ƙ Ẻ

˥ Ə [3,4] ɟ ӂ H_2O_2 PC12

Ƙ ˥ Ặ ẫ ɟ H_2O_2

PC12 Ψ RNA 422a microRNA 422a miR 422a Ά bcl w \ Ặ miR 422a ’ ˜ bcl w ל Ά H_2O_2

PC12 ἕ H [5] ɟ ᵰ Њ

Ẻ ˥ Ə H_2O_2

PC12 Ƙ ى miR 422a ש ἕ H ἵ ǚ ẹ ’ Ə

Ặ ǚ ε í ל

w ˜ ɟ

Ά Ḥ

PC12 ɔ Ψ ɔ ɔ

ɟ ○ Ψ oy ắ ˘

ε ɟ

RPMI 1640 Ế Ά ج Gibco ắ ˘ Annexin V FITC/PI ἕ H Ḥ

ɔ ` ắ ˘ 30% ɔ

H_2O_2 Ḥ ắ ˘ PBS ←Ш

Ψ ắ ˘ Ẕ Bcl 2 Bax

ẋ l ج Sigma ắ ˘ ɔ

HRP Ẕ IgG J ǂ

ắ ˘ miR 422a mimics ש

miR NC miR 422a Ặ anti miR 422a ש

anti miR NC ج ɔ ` Ặ

ắ ˘ Trizol ש ẉ PCR qRT PCR Ḥ ج TaKaRa ắ ˘ ɔ

ɔ SOD Ḥ ɔ

ắ ˘ Lipofectamine2000 ←Ш

ắ ˘ ɟ

Ά ǚ

100 200 300 nmol/L H_2O_2

PC12 PC12 ἕ H

H_2O_2 ى ɟ ǚ PC12

H_2O_2 ى 300 nmol/L

10 20 40 60 mg/L PC12 Ế Њ 10%

RPMI 1640 Ế Ẩ 37 l ǚ

5%CO_2 Ế Ỉ Ế 24 h PC12 H_2O_2

ى ẹ æ ˘ Ẩ 12 h

PC12 ẹ æ ˘ Ẩ H_2O_2

12 h

Ấ ECL ה ẉ Ҍ῍H ḏ ẉ ḏ Quantityone Ɵ ᵉ = ᵉ / Ȋ ל ᵉ ɟ

qRT PCR Ψ miR 422a Trizol ٦ PC12 RNA ǚ ẉ ẉ RNA Ḥ RNA א ε cDNA O qRT PCR Ḥ ɰ א l א l SYBR Premix Ex Taq 2× 10 L cDNA 2 L ℈ ; ˘ 0.8 L ROX Reference Dye 50× 0.4 L ddH_2O 6 L ɟ א Ɵ 95 ל 5 min 1 95 ל 15 qs 60 60 s 72 Џ 30 s Ấ 40 Ấ ABI 7500 ẉ PCR Ѧ miR 422a ɟ

SPSS 21.0 Ɵ ǚ ڿ ˊ ǚ ڿ w $\bar{x} \pm s$ Ξ t ╥ ἐ ǚ w $P<0.05$ ε Ẻ τ ɟ

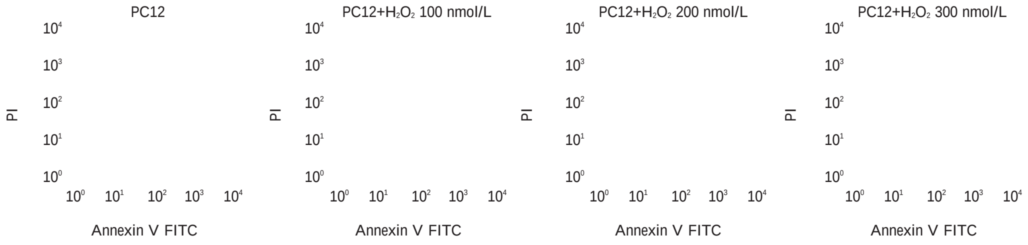
H_2O_2 PC12 Ƙ Ά PC12 PC12 + H_2O_2 100 nmol/L ɞ PC12 + H_2O_2 200 nmol/L ɞ PC12 + H_2O_2 300 nmol/L PC12 ἕ H ╖ $P<0.05$ SOD ɥ $P<0.05$ τ 1 ɞ 1 ɟ Ҍ PC12 + H_2O_2 300 nmol/L PC12 ἕ H ἐ ٦ H_2O_2 300 nmol/L Ƙ ﻱ Ҍ˜ ɟ

1 H_2O_2 PC12 $\bar{x} \pm s$ n

Table 1 Hydrogen peroxide induces PC12 damage in neurons $\bar{x} \pm s$ n=6

ǚ	SOD(U/mL)	ἕ H %
PC12	17.72±1.68	7.58±0.52
PC12+H_2O_2 100 nmol/L	14.95±1.34 [a]	11.23±1.20 [a]
PC12+H_2O_2 200 nmol/L	10.02±1.00 [a]	17.21±1.76 [a]
PC12+ $H_2O_2$300 nmol/L	6.72±0.58 [a]	55.78±4.33 [a]
F ᵉ	97.706	504.091
P ᵉ	0.000	0.000

Ά PC12 [a]$P<0.05$ ɟ

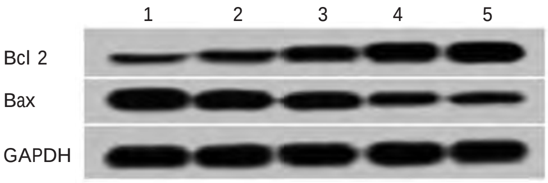


1 H_2O_2 PC12

Figure1 Effects of different concentrations of hydrogen peroxide on apoptosis of PC12 neurons

H_2O_2 PC12 ἕ H ש SOD ἳ ˝ PC12 ˜ ʄ H_2O_2 300 nmol/L Ά PC12 ἕ H ɥ $P<0.05$ SOD ൿ $P<0.05$ Bcl 2 ╖ $P<0.05$ Bax ɥ $P<0.05$ ˊY ʄ Ḥ ൿ ל ɔ $P<0.05$ \\ Ḥ ൿ 2 ɞ 2 ɟ ʼ ῎Ά H_2O_2 PC12 ἕ H ʼ ῎E ɔ א ɟ 40 mg/L ˜ ɟ

H_2O_2 PC12 Ψ miR 422a ἳ qRT PCR Ά PC12



1. 2. 10 mg/L 3. 20 mg/L 4. 40 mg/L 5. 60 mg/L ɟ

2 H2O2 PC12

Figure 2 Effects of Pulsatilla chinensis on apoptotic protein expression of PC12 induced by hydrogen peroxide in nerve cells

PC12+H_2O_2 300 nmol/L PC12 Ψ miR 422a ɥ $P<0.05$ Ҍ PC12+ H_2O_2 300 nmol/L PC12+H_2O_2 300 nmol/L+

2 H_2O_2 PC12 SOD

x̄±s n .

Table 2 Effects of Herba Anemoniae on Apoptosis and SOD Expression of PC12 Neurons Induced by H_2O_2 x̄±s n .

ǚ mg/L	SOD U/mL	Bcl 2	Bax	ἕ H %
	6.71±0.54	0.26±0.03	1.23±0.12	52.58±4.52
10	9.66±0.84[a]	0.45±0.04[a]	0.94±0.09[a]	33.93±3.24[a]
20	12.01±1.12[ab]	0.58±0.06[ab]	0.71±0.07[ab]	24.81±2.56[ab]
40	15.62±1.37[abc]	0.76±0.08[abc]	0.43±0.04[abc]	12.26±1.13[abc]
60	16.23±1.41[abc]	0.82±0.08[abc]	0.37±0.04[abc]	10.25±1.01[abc]
F e	79.023	82.984	125.961	227.141
P e	0.000	0.000	0.000	0.000

Ά [a]$P<0.05$ Ά 10 mg/L [b]$P<0.05$ Ά 20 mg/L [c]$P<0.05$ ɟ

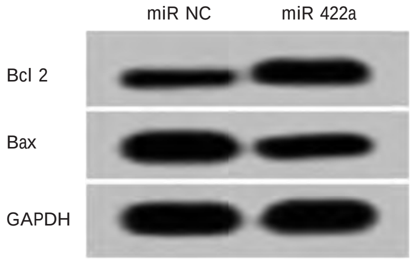
40 mg/L PC12 Ψ miR 422a ╗ $P<0.05$ 3 ɟ

ǚ		miR 422a
PC12		1.12±0.10
PC12+H_2O_2 300 nmol/L		1 e12]C
PC12+H_2O_2 300 nmol/L+	40 mg/L	
F e		
P e		

miR 422a H_2O_2 PC12 ἕ H ש SOD ἷ Ά miR NC miR 422a PC12 ἕ H ɥ $P<0.05$ SOD ɥ $P<0.05$ Bcl 2 ◈ $P<0.05$ Bax ɥ $P<0.05$ ◈ 3 ɞ 4 ɟ

2.5 Ά miR 422a H_2O_2 PC12 ἕ H ɞSOD ἷ

Ꙏ 40 mg/L+ anti miR NC 40 mg/L+ anti miR 422a PC12 ἕ H ╗ $P<0.05$ SOD Ά Bcl 2 ج ɥ $P<0.05$ Bax ╗ $P<0.05$ ◈ 4 ɞ 5 ɟ Ά miR 422a ' H_2O_2 PC12 ἕ H ɞSOD ἷ ɟ



3 miR 422a H2O2 PC12

Figure 3 Effects of overexpression of microRNA 422a on apoptotic protein expression of PC12 induced by hydrogen peroxide in nerve cells

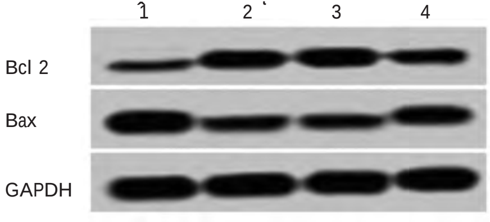
4 miR 422a H2O2 PC12 SOD x̄±s n .

Table 4 Effect of over expression of microRNA 422a in on apoptosis and SOD activity of PC12 neurons induced by hydrogen peroxide x̄±s n .

ǚ	SOD U/mL	Bcl 2	Bax	ἕ H %
miR NC	6.71±0.54	0.26±0.03	1.23±0.12	52.58±4.52
miR 422a	14.37±1.28[a]	0.65±0.06[a]	0.53±0.05[a]	19.71±1.58[a]
t e	13.506	14.241	13.190	16.815
P e	0.000	0.000	0.000	0.000

Ά miR NC [a]$P<0.05$ ɟ

ض N ² Ά



1. 2. 40 mg/L 3. 40 mg/L+anti miR NC 4. 40 mg/L+anti miR 422a ɟ

4 miR 422a H_2O_2 PC12

Figure 4 Inhibiting the expression of miR 422a reverses the effect of Saussurea chinensis on apoptotic protein expression of PC12 induced by hydrogen peroxide in nerve cells

PC12 ɔ س ẹ ɞ ש ج Ά Ч ' Ꙏ H_2O_2 PC12 Ꝁ ɔ Ꝁ ẻ ى Ч ɔ Ꝁ ؤ Ψ ה Ə

ũ	miR 422a
	0.12±0.01
40 mg/L	0.83±0.08
40 mg/L+anti miR NC	
40 mg/L+anti miR 422a	
F e	
P e	

miR 192 miR 29c 2

RNA 192 miR 192 Ά RNA 29c miR 29c ´ 2

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50 0 0 0

-Email 4 462 680 8 8 q8q c.om

with urinary NGAL r = - 0.338 P=0.002 and miR 29c was positively correlated with urinary NGAL r= 0.516 P<0.001 . The sensitivity of miR 192 for diagnosis of DN was 52.50% and the specificity was 75.58%. The sensitivity of miR 29c for diagnosis of DN was 50.00% and the specificity was 86.05%. The combined diagnostic sensitivity was 97.50% the specificity was 95.34% and the accuracy was 96.39%. The sensitivity and accuracy of the joint detection were higher than the single test P<0.05 . Conclusion The abnormal expression of miR 192 and miR 29c in plasma is closely related to the occurrence and development of type 2 diabetic nephropathy. The combined detection of the two can improve the diagnostic efficiency of type 2 diabetic nephropathy.

KEY WORDS miR 192 miR 29c type 2 diabetic nephropathy

diabetes mellitus DM í
ẹ Ψ 2 ي ζ ي
diabetic nephropathy DN 2 ي ζ
ה φm DN ε ┼ ῴ ï
[1 3] ɟ غ ῦ 2
ي ش د ˜ Ẻ í
τ ɟ RNA microRNA miRNA m Ỉ
RNA ῦ ’ ; نح
غ ל Ά ῦ
miRNA Ά 2 ي ה Ὑ
Ặ ’ Ə ε ῦ
[4] ɟ ӂ miRNA 2 ي ڗm ̑
̑ غ miRNA ῦ ῦ
ẹ 2 ي ɵ e ɟ Ặ
Ὸ RNA 192 microRNA 192 miR 192 ڗ DN
Ψ \ ɥ ’ DN ה ה
5 RNA 192

GATT 3′ א˜ ε 5′ GGAACGCTTCAC GAATTTG 3′ miR 192 ˜ ε 5′ GGGGCT GACCTATGAATTGA 3′ א˜ ε 5′ CAGTG CAGGGTCCGAGGT 3′ miR 29c ˜ ε 5′ GTAGCACCATTTGAAATCAG 3′ א˜ ε 5′ TTGGCACTAGCACATT 3′ ɟ א l cDNA 2.0 L Primer 1.0 L RNase H_2O 7 L SYBR Primix Ex Taq™ 10 L ɟ א ϴ 95 ל 2 min 95 ל 15 s 60 30 s 72 Ҵ 30 s Ã 40 ɟ 2 ^{Ct} miR 192 ɕmiR 29c ɟ

í

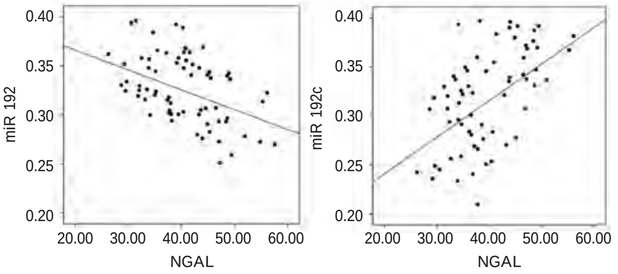
ڿ า Ψ 5 mL ϴ ; 3 000 r/min 10 min ੯ า э Ҍ EP Ỉ -80 ϴ ; ᾰ ɟ ẍ ੯ enzyme linked immunosorbent assay ELISA Ψ Ặ NGAL O Ḥ ꟺ ϑ ɟ

ó

í Ά̃ 2 ی DM 86 ར Ά

DN 80 ར ɟ ɔ ə receiver operating characteristic ROC ῦ miR 192 ɕmiR 29c 2 ی ɵ ^{e} í ῦ miR 192 ɕ miR 29c 2 ی ῦ ɟ

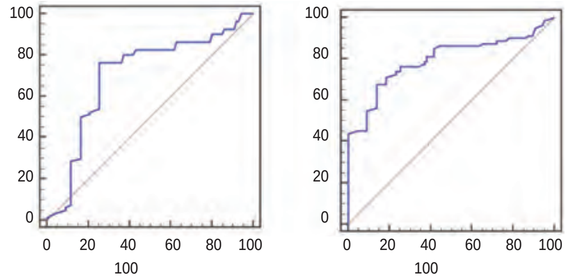
ϴ SPSS 21.0 ῦ ´ ῦ ꞷ ڿ ± Ά̃ x ± s Ξ *t* ╥ ۼ ῦ Pearson Ặ ῦ ROC ῦ miR 192 ɕmiR 29c 2 ی ɵ ^{e}



1 miR 192 miR 29c DN NGAL

Figure 1 Correlation analysis between plasma miR 192 and miR 29c expression and NGAL in patients with DN

ɵ ᵉ miR 192 2 ي ε 52.50% ε 75.58% AUC ε 0.679 95%*CI* 0.602~0.749 ᵉ ε 0.40 ܗ 2A ɉ ROC ǚ miR 29c ي ɵ ᵉ miR 29c 2 ي ε 50.00% ε 86.05% AUC ε 0.796 95%*CI* 0.726~0.854 ᵉ ε 2.94 ܗ 2B ɉ



2 ROC miR 192 miR 29c

Figure 2 ROC analysis of the value of plasma miR 192 and miR 29c in the diagnosis of type 2 diabetic nephropathy

´ ي ɵ ᵉ ´ ε 97.50% ε 95.34% Ά̃ ε 96.39% 3 ɉ miR 29c 2 ي Њ miR 192 ´ Ά Ά̃ ج Њ ╥ $P<0.05$ 3 ϐ 4 ɉ

2 ي m Ἰ ǚ ψ Ι Њ ʼ Ἰ Ἰ Ъ ƒ ͻ ה [12] ɉ ה í ζ Њ

3 miR 29c miR 192

í Ǻ			´
	78	2	80
	4	82	86
	82	84	166

4 miR 29c miR 192

			Ǻ
miR 192	52.50% 42/80 *	75.58% 65/86	64.46% 107/166 *
miR 29c	50.00% 40/80 *	86.05% 74/86	72.29% 114/166 *
´	97.50% 78/80	95.34% 82/86	96.39% 160/166
c^2 ᵉ	9.578	1.064	7.070
P ᵉ	0.008	0.587	0.029

Ά NC *$P<0.05$ Ά DM #$P<0.05$ ɉ

ͻ غ ἷ ẹ Ƙ ï [13] ɉ غ ϐ Ύ ἀ 2 ي Ḕ τ ɉ miRNA ۊ Ψ ʼ Ə ε [14] ɉ غ Ά 2 ي ה ה Ặ miRNA m ǚ ẹ 2 ي ɵ ᵉ ɉ miR 192 ۊ ж ˜ Ƙ Ψ ʼ Ə ε Ƙ [15] ɉ miR 192 ۊ 2 ي Ψ ɥ Ύ ¥ ᵉ UACR ɥ miR 192 ɥ Ά ͻ Ύ Ặ [16] ɉ miR 192 ˜ א غ 1 Egr1 ἷ DN [17] ɉ 2 ي Ψ miR 192 ɥ miR 192 ; ʼ ϿΆ 2 ي ה ה ɉ 2 ي NGAL Њ 2 ي NGAL Њ DM m ǚ ה 2 ي Ψ miR 192 Ά NGAL Ѵ Ặ NGAL ʼ א 2 ي ʻ Ƙ Ỹ ʼ miR 192 ɥ ɉ miR 192 ; ʼ ש Ἰ ʻ ɉ miR 29c ʼ

AMPK / mTOR מ ̋ Ặ̈ ɔ
’ Ə ɛ ɔ
[18] ɟ miR 29c ئۆ Ψ \\
’ Ə ɛ ɔ غ [19] ɟ
miR 29c ’ Ə ɛ HTLV 1
ى ئۆ [20] ɟ 2 ى
Ψ miR 29c ╗ ΎΥ
DN Ҍ DM miR 29c
╗ ’ ̬ 2 ى ה ש̇ ה ɟ
ݜ Ҍ ʬ ɔ 2 ى
Ψ miR 29c Ά NGAL \\ Ặ̆
miR 29c Ά NGAL ئۆ 2 ى ה ה
Ψ ’ ئۆm ˜ Ə ɟ ῷ Ặ̆ Ҍ
miRNA Ά 2 ى ӂ Ặ̆
Ҍ miR 29c ɞmiR 192 ́ 2 ى
ɵ e ROC ῦ
miR 192 ɞmiR 29c 2 ى
ɵ e miR 192 2 ى
Ά ӊ miR 29c 2 ى
Ά Ҍ miR 192 m
́ ́ Ҍ ╥
ɟ ́ miR 192 ɞmiR 29c
’ 2 ى ש̇ Ἆ
ɟ

ɕ 7 02Ɐc Ṅ̋ J Ɓ 4̯ ɞ 5• D PÕY

6

ч 6 IL 6 ɞ Ƈ PCT ۆ Ψ ש ẹ í τ ɟ ῦ 80 ʢ ῦ Ἇ ῦ ε n=38 ɞO ᷈ ¥ Ѣ ẋ n=22 ɞ Ѣ ẋ n=20 Ẫ 40 ʢ ε ط ↄ IL 6 ɞPCT ROC ῦ IL 6 ɞPCT Logistic ي ῦ IL 6 ɞPCT ˜ ɵ ᵉ ɟ ɞO ᷈ ¥ Ѣ ẋ ɞ Ѣ ẋ IL 6 ɞPCT Ά ܓ ╗ $P<0.05$ ɞO ᷈ ¥ Ѣ ẋ ⁄ Ѣ ẋ $P<0.05$ IL 6 ɞPCT ʬ ң ε Ѣ ẋ >O ᷈ ¥ Ѣ ẋ > ROC ῦ IL 6 ɞPCT AUC ῦ ᾳ ε 0.806 95%CI 0.96~10.61 P=0.000 0.892 95%CI 1.28~9.16 P=0.000 IL 6 ɞPCT ẹ ɞ ῦ ᾳ ε 89.61% ɞ86.76% 90.35% ɞ87.26% IL 6>56.12 pg/mL ɞPCT> " , Ų 7 #

Sepsis Ɑ Ι Ẫ ẫ א ΄ Systemic inflammatory response syndrome SIRS ɛ ɫ ͨ ͻ ͞Ặ Ń ʼ ᶖ ꞡ ʹ ɉ ẫ ה ɑ ᶖ ה Ψ ɑ Ẻ ה ᶖ [1] Ặ [2] ẫ ж w 2 000~3 000 ˛/ 1 400 ж Ꙓ ɉ 1%~2% ɠ Ч ẹ Ψ ICU 25% ICU Η ζ Ɑ ɠ [3] ʹY ICU ╗ [4] ɉ ɠ ɧ Ặ ῷ Ά ˜ ό Ặ 200 [5] Ẻ ɵ ᵉ ɉ ч 6 interleukin 6 IL 6 ᶖ Ɑ procalcitonin PCT Ξ ǖ ẹ ɵ ᵉ ẫ ɉ ǖ ͨ˒ Ο Ι Ἰ IL 6 ᶖ PCT IL 6 ᶖ PCT ᶖ ˜ ί ɵ ᵉ ɉ

m

ǖ 2015 6 2017 6 Ẽ ש 80 ʢ ˜Ά ㄅ 2012 ɑ ├ ╟Җ ACCP American College of Chest Physicians ɞ● ├ ╟Җ SCCM Society of Critical Care Medicine ɞ ╟Җ ESICM European Society of Intensive Care Medicine ᶖ ɑ ╟Җ ATS American Thoracic Society Ꙓ ├ ╧ [6] Ẫ 24 h w ͻ ẋ ɞ ᶖ ɉ w ש ꞡ ʹ ΄ multiple organ dysfunction syndrome MODS ɞ ǖ ɛ ɞΟ ͨ Ɏ Ѣ ẋ ɞ Ѣ ẋ ɉ 38 ʢ ʹΟ SIRS Ӂ ͨ ʹΟ MODS ש 18 ʢ 20 ʢ 56~89 75.51±12.37 Ο ͨ Ɏ Ѣ ẋ 22 ʢ ʹΟ SIRS ʹY ʹΟ MODS ʼ ͨ 12 ʢ 10 ʢ 59~88 74.38±10.64 Ѣ ẋ 20 ʢ ʹΟ Ο MODS w ש ͨ ʼ ͨ 9 ʢ 11 ʢ 66 90 76.73±13.65 ɉ Ꞓ 40 ʢ Ə ɛ 20 ʢ 20 ʢ 54~90 74.38±10.52 Ά ꞡ ɞ τ $P>0.05$ Ӂ Ẻ ʼ ɉ

IL 6 ש PCT ͻ ה w Ḩ ɛ ɑ ắ ˘ У Ѧ ꞡ ɛ Cobas E601 ͻ ה w ẋ ǖ Ѧ ɉ ǖ

SPSS 22.0 Ẫ ᶖ ǖ w $\bar{x}\pm s$ one way ANOVA ǖ Ξ Ξ SNK q ˘ ɞ ɞ Ч ɞ Ч ɞ ˜Ά ɞYouden ROC Ə ǖ IL 6 ɞPCT Logistic ǖ IL 6 ɞPCT ˜ ɵ ᵉ $P<0.05$ ɛ Ẻ τ ɉ

˘ Ψ IL 6 ɞPCT ɞΟ ͨ Ɏ Ѣ ẋ ɞ Ѣ ẋ IL 6 ɞPCT ╗ τ $P<0.05$ ɉ ɞΟ ͨ Ɏ Ѣ ẋ ɞ Ѣ ẋ $P<0.05$ IL 6 ᶖ PCT ъ ɧ ǖ ɛ <Ο < Ѣ ẋ ɉ 1 ɉ

1 IL 6 PCT

n $\bar{x}\pm s$

Table 1 Comparison of IL 6 and PCT levels in different study groups and control groups n $\bar{x}\pm s$

	n	PCT ng/L	IL 6 pg/mL
	40	0.01±0.35	9.163±2.647
	38	0.85±0.95*	40.16±16.23*
Ο	22	2.28±2.74*#	98.62±36.49*#
Ѣ ẋ	20	4.267±3.769*#	106.294±42.135*#

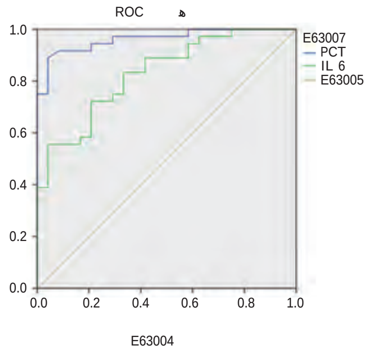
* vs $P<0.05$ # vs $P<0.05$ vs Ο $P<0.05$ ɉ

IL 6 ɞPCT ɵ ᵉ ǖ ˜Ά ǖ IL 6 ɞPCT IL 6 ɞPCT AUC ǖ ɛ 0.806 ᶖ 0.892 IL 6 ᶖ PCT ɞ ɞ Ч ɞ˜Ά ɞYouden Ẻ Ι 2 1 ɉ

2 IL 6 PCT ng/mL,%

Tab. 2 Analysis of the diagnostic value of IL 6 and PCT for sepsisz ng/mL,%

			Ч	Ч	Youden	ҠA	AUC	e
PCT	90.35	87.26	6.38	0.16	72.68	94.26	0.892	1.110
IL 6	89.61	86.76	5.12	0.29	69.21	92.37	0.806	56.127



1 IL 6 PCT ROC

Figure 1 ROC diagram of IL 6 and PCT for diagnosis of sepsis

IL 6 ɗPCT ˜ Ɵ Logistic ῦ

80 ʅ Ψ H 15 ʅ 65 ʅ H ⁴ IL 6 ɗPCT

2.2 Ψ e ε ῦ ε Ξ Y Ж PCT>1.110 ng/mL Ж Ψ H Ά PCT. 1.110 ng/mL Ж Ψ H τ c^2=4.20 P=0.041 ˀ IL 6>56.12 pg/mL Ж ⁴ IL 6. 56.12 pg/mL Ж Ψ H τ c^2=7.49 P=0.006 ɟ 3 ɟ

3 IL 6 PCT

Table 3 Single factor analysis of prognosis in patients with IL 6 PCT and sepsis

ą̈	n=65		H n=15		c^2 e	P e
PCT						
. 1.110 ng/mL	27	41.54	2	13.33	4.20	0.041
>1.110 ng/mL	38	58.46	13	86.67		
IL 6						
. 56.127 pg/mL	29	44.62	1	6.67	7.49	0.006
>56.127 pg/mL	36	55.38	14	93.33		

PCT>1.110 ng/mL ɗIL 6>56.127 pg/mL Ấ logistic ي PCT>1.110 ng/mL ɗIL 6>56.127 pg/mL ̋ ǵ OR ῦ ą̈ ε 9.35 98%CI 1.96~8.92 ɗ12.98 95%CI 0.89~12.39 4 ɟ

4 IL 6 PCT Logistic

Table 4 Logistic analysis of prognosis in patients with sepsis predicted by IL 6 and PCT

	b	SE	OR e	P e	95%CI
PCT>1.10 ng/mL	1.86	0.06	9.35	0.001	1.96~8.92
IL 6>56.12 pg/mL	2.83	0.15	12.98	0.001	0.89~12.39

IL 6 Ẻ ẍ ʿ ῷ ǵ ╦ ϐ ៦ ϐĬ ؿ ǵ ῆ ; У ˀ Ẻ ῆ I У CRP ɗPCT ǵ Ə ؿ 2~3 h ◑ ʼ ą̃ e ؿ í Њ Ύ ؿ ą̈ ⁴ Ẻ ɵ e ɟ ɷ Ĭ ẹ ђ ς ẠЊ IL 6 ؿ Ψ ɵ Ѡ Ѧ [7] ⁴ є [8] ẹ ς Ά ݓ m ɟ ɷ ς ε IL 6 [9] Srisangthong IL 6 Ά ˜ \ Ạ ◑ IL 6 ˜ [10] ɟ ؿ ̋ ˀ ą̈ IL 6 ה ̋ IL 6 \ ╗ † m Ά Srisangthong ˊ ɟ ˀ ꟷA ה IL 6>56.12 pg/mL ̋ ǵ OR ε 9.35 98%CI 1.96~8.92 IL 6 ʼ ⱳ Ə ε ą̈ ˜ ɟ Ӂ ς IL 6 ؿ Ψ ς ̋ ˀ ݥ ʼ ؿ ẹ ђ Ψ ς ؿ ĩ IL 6 ǵ ς IL 6 Ə ε ą̈ Ђ Ј [11] ɟ ǵ m Ẩ IL 6 Ə ε ɟ

PCT У ῷ I ؿ ж Ψ Ⱨ ؿ I Ͻ Ͻ Ĭ ʼ ╗ ؿ 4 h Ĭ Ψ ◑ ʼ ą̃ [12] ؿ Ψ 6~24 h [13] ɟ

PCT [illegible]

PCT [illegible]

PCT [illegible]

[14] PCT>0.5 ng/L [illegible]

[illegible] 0 - PCT>10- g/L [illegible] / 2

[illegible]

D

D † ´ Ɑ PCT ؤ ɞ ש

Ψ ש ɵ ᵉ ɟ BM ɞ VM

ש TM D † ɞPCT ɞC א CRP ɞ ɞ ɞ Ɂ

˥ כ Ə ROC ɞ Ɂ ȗ D † Ɂ PCT

ɵ ᵉ ɟ D † ɞPCT ɞCRP ɞ ɞ Ɂ ؤ BM Ά VM

τ u=110.0 ɞ100.5 ɞ95.5 ɞ185.0 ɞ177.5 ɞ150.0 ɞ80.5 $P<0.05$ ɟ BM Ά TM D † ɞ

PCT ɞCRP ש τ u=51.5 ɞ45.0 ɞ60.0 ɞ54.5 $P<0.05$ ɟ ROC ȗ D †

; AUC ε 0.907 PCT AUC ε 0.878 ɟ D † ε 91.7% ε 89.9%

PCT ε 85.6% ε 98.6% ɟ D † ´ Ɑ Ə ε

Ɂ ' ɟ

D † Ɑ ROC

Diagnostic value of D lactic acid and procalcitonin in bacterial meningitis

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ABSTRACT Objective To investigate the expression of D lactic acid combined with procalcitonin PCT in bacterial meningitis viral meningitis and tuberculous meningitis and its diagnostic value. Methods The levels of D lactate PCT CRP red leukocyte count glucose chloride ion and total cerebrospinal fluid protein in patients with bacterial meningitis BM viral meningitis VM and tuberculous meningitis TM were measured. The diagnostic value of D lactic acid and PCT in bacterial meningitis was analyzed by receiver operator characteristic curve ROC sensitivity and specificity. Results There were significant differences in D lactate PCT CRP RBC count CSF total protein and glucose between BM group and VM group u=110.0 100.5 95.5 185.0 177.5 150.0 80.5 $P<0.05$. The difference of D lactate PCT CRP and leukocyte count between BM group and TM group was statistically significant u=51.5 45.0 60.0 54.5 $P<0.05$. ROC curve analysis showed that area under the curve AUC of D lactate was 0.907 and AUC area of PCT was 0.878. The sensitivity of D lactate was 91.7% the specificity was 89.9% the sensitivity of PCT was 85.6% the specificity was 98.6%. Conclusion D lactate combined with PCT is necessary for the early diagnosis of bacterial meningitis.

KEY WORDS D lactate Procalcitonin Bacterial meningitis Receiver operator characteristic curve

O ж ɏ ฿ Ɂ ȗ ɔ ẳ

Ɂ ש ݒ Ế Ӂ ج ؤ

῎Ε ң ● [1] ɟ Ʀ ᾇ ῗ [2] ɟ ă Ὑ

ئى Ά̃

Ặ ɟ D † ⏊ Ɑ pro calcitonin PCT ﯔ ж Ι Ψ ɵ e [3,4] ɟ ģ ῦ D † ⏊ Ɑ ⏊ ά̣ D † ´ Ɑ í ɵ e ɟ

m

2016 3 2017 6 ﯔ Ỉ ٮ̄ ├ ├ ε 68 ʢ ɟ Ẩ Ά̃ ج ḏ ɞ ɞ ɞ ɔ ɞ نج Ế >18 ἐ ה ɟ Ά̃ ´ ẹ ђ Ỉ Ỉ ´ ´ ẹ ђ Ḩ ´ ẹ ђ ɞẍ ɟ ɔ ⏊ í ά̤ ῦ ε 3 ◑ viral meningitis VM 17 ʢ tuberculosis meningitis TM 20 ʢ bacterial meningitis BM 31 ʢ ɟ ⏊ ⏊ 28 ʢ Ә ε ɟ Ḩ ˦ Җ Ḩ ˝ 20160113 ɟ

1.2.1 Ḥ̓ ⏊ Ѧ ﬞ

Ḥ̓ ɔ ẋ ắ ˘ C א Ḥ̓ ɔ ắ ˘ Ɑ Ḥ̓ ╧ Ш ắ ˘ D † ῦ Ḥ̓ Sigma ắ ˘ ɔ Щ ꝺ AU640 ã̃ ɔ ῦ Ѧ ắ ˘ ắ ˘ ɟ

1.2.2 Ặ

ῃ̇ Ẩ Ό Ỉ ɟ ˜ ◑ 3 000 r/min 15 min ɔ AU640 ã̃ ɔ ῦ Ѧ 2 h Ỉ ɞ ⏊ ɟ ẹ Ψ є [5] ɟ

[5] ɟ [5] ɟ ẍ [6] C א C reactive protein CRP ã̃ C א Ḥ̓ Щ Ә ɟ ẍ [7] Ɑ PCT ã̃ Ḥ̓ Щ Ә ɟ CRP ⏊ PCT ج ã̃ ɔ ῦ Ѧ ɟ D † [5] ɟ ⏊ Ό ж ˦ ط ã̃ ﻋ í Ә [8] ɟ

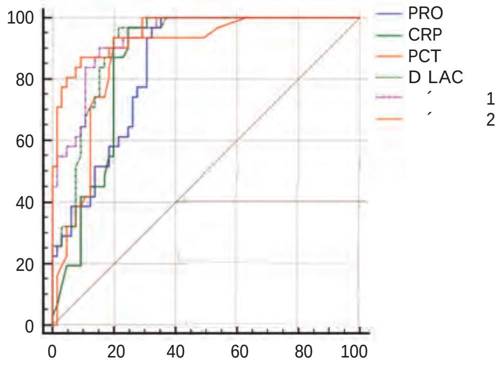
ῦ

SPSS 16.0 Ө ῦ *F* ı ъ ῦ ɟ ˥ x±s ῦ ˥ *t* ⏊ ╥ ģ ῦ ˥ Kruskal Wallis

1 x̄±s

Table 1 Clinical data and laboratory examination results of cerebrospinal fluid related indexes in experimental groups x̄±s

		VM	TM	BM	F ᵉ	P ᵉ
	42.5±10.6	44.6±8.9	38.5±8.7	45.8±11.3		
ᾀ /	16/12	8/9	12/8	17/14		
mmol/L	4.1±1.3	3.3±1.2	2.9±1.0	2.66±0.9	9.6	0.006
mmol/L	119.8±8.9	113.7±7.3	115.3±6.9	111.1±7.2	15.3	0.002
mg/L	294.9±31.6	367.9±42.5	2035.3±837.6	2465.6±1021	68.3	<0.001
(/ L)	2±1.8	10.3±7.6	685.6±427.6	812.9±471.3	85.9	<0.001
(/ L)	7±5.8	136.3±98.6	191.6±133.3	211.2±173.2	12.3	<0.001
CRP mg/ L	0.239±0.092	0.411±0.185	0.627±0.312	0.678±0.252	42.6	<0.001
PCT g/ L	0.048±0.075	0.052±0.024	0.057±0.033	0.165±0.183	52.6	<0.001
D † mmol/L	3.77±1.97	10.23±5.26	32.59±14.3	43.58±14.3	62.7	<0.001



D LAC D † ɟ PCT ᗡ ɟ CRP C א ɟ PRO ɟ ´ 1 ฿ D † ⁄ PCT ɟ ´ 2 ฿ D † PCT CRP ⁄ ɟ

1 ROC

Figure 1 ROC curve of diagnostic index related to bacterial meningitis

AUC

Table 2 AUC of cerebrospinal fluid diagnostic indexes

	AUC	P ᵉ	95% ' מ ┘
mmol/L	0.630	0.007	0.625~0.726
mg/L	0.845	<0.001	0.761~0.914
mmol/L	0.644	0.005	0.540~0.739
(/ L)	0.836	<0.001	0.770~0.919
(/ L)	0.788	<0.001	0.698~0.824
CRP mg/ L	0.826	<0.001	0.746~0.899
PCT(g/L)	0.878	<0.001	0.769~0.919
D † mmol/ L	0.907	<0.001	0.794~0.935
´ 1	0.933	<0.001	0.905~0.998
´ 2	0.957	<0.001	0.924~1.024

˜ ε ´ 1 AUC ' ɯ ᾀ 0.933 ɟ 8 Y ´ 2 AUC ' 0.957 ɟ

2.3 ˘ ᵉ ɞ ⁄ 3 ὤ 3 Ψ D † cutoff ᵉ ε 37.9 mmol/L ε 91.7% ẹ ὤ Ψ ɟ PCT cutoff ᵉ ε 0.168 g/L ⁄ ṻ ᾀ ε 85.6% ⁄ 98.6% ẹ ὤ Ψ ɟ D † ⁄ PCT J ẃ Logistic ɔ ˜ ε ´ 1 ⁄ ṻ ᾀ ε 85.1% ⁄ 99.5% ɟ 8 Y ´ 2 ⁄ ṻ ᾀ ε 83.9% ⁄ 100% ɟ

3 cutoff

Table 3 Cutoff value sensitivity and specificity of cerebrospinal fluid diagnostic indicators

	cutoff ᵉ	%	%
mmol/L	2.6	62.3	86.5
mg/L	1983.5	85.3	90.3
mmol/L	113.6	65.3	80.3
(/ L)	552.6	85.5	91.6
(/ L)	175.3	72.7	88.7
CRP mg/L	0.586	87.3	85.7
PCT(g/L)	0.168	85.6	98.6
D † mmol/L	37.9	91.7	89.9
´ 1		85.1	99.5
´ 2		83.9	100.0

ي ' ζ ṻ ε ɞ ⁄ ɟ ẹ Ψ Ψ O

~ [10] ɟ ج ' ẹ Ψ
ẹ æ ε ɞ
ɞ♦ ɟ Ꝁ ᾇ Ꞵ
ɞ ɔ ẘ ש
ᛀ نج Ế ɟ ᛀ ɔ
ж ẘ ᾇ ی ɟ
ᛀ نج Ế ' ẘ ж Ό ӊ
' 70% Ύ 3 d [11] ɟ
ẘ ᾇ Ặ ɟ
ζ

ῦ ה BM D † ɞPCT
Њ ẹ ђ 3 ɟ D † ɞPCT ێ
Ψ ╗ ɟ [12] ה
D † Ά PCT ێ Ψ
ꬰ Ά ɟ ꞔ
[13] Ό ɟ ῦ ɑ غ I ᾇ O
I Ỉ C ẘ

ه ɞ╗ ɞ
Җ ᾇ Ỉ ῦ PCT ẘ
PCT ╗ ɟ † ɥ Ψ У
ɑ I ᛀ س
ج Ό ῦ Ψ
У † ẘ D † ╗ ɟ
ж ET ה Ặ Ẩ ɟ
ROC ῦ ɵ
ה ROC Gonr ; Rus ێ Ψ
ᾇ 0.90 ẹ ɵ ɟ ẹ
ɑ AUC 0.878 ɟ Y ێ
' מ ɵ ɟ [14] ה
Ψ D †
ɵ ɟ ه [15] ה ɑ
ɵ ɟ 1 ᛀ 2
' ẘ ῦ 0.933 ᛀ 0.957
ɟ ῦ
ᛀ ה
D † ε 91.7% ε 89.9%
ɟ PCT ᛀ
ῦ ᾇ ε 85.6% ᛀ 98.6% ێ Ψ
ɟ J
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GATA3 CK20

GATA3 ɞCK20 ؤ ɔ BUC Ψ Ά τ ɟ
ẍ ɔ ǚ ᾇ 89 ʢ BUC ɞ16 ʢ ɔ ⅁ Ψ GATA ˊ 3 GATA3 ɞ
20 CK20 ǚ Ξ Ά BUC ⅁ Ặ ɟ BUC Ψ GATA3 ε
85.4% ؤ ⅁ ᾇ 100.0% CK20 ؤ BUC Ψ ε 62% ؤ
⅁ Ψ CK20 ج ε Ξ ؤ ᾇ ⅁ ɞ¥ ש ¥ BUC Ψ
ج Ѣ ḩ ᾇ ⅁ ɞ ש BUC $P<0.05$ ɟ
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ɔ GATA3 CK20

Expression and significance of GATA3 and CK20 in bladder urothelial carcinoma

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ABSTRACT Objective To investigate the expression and significance of GATA3 and CK20 in bladder urothelial carcinoma BUC . Methods The levels of GATA binding protein 3 GATA3 and cytokeratin 20 CK20 were detected by immunohistochemical assay from 89 cases of BUC and 16 cases of benign lesions. The correlation between the expressions and the pathological parameters was analyzed Results The positive rate of GATA3 was 85.4% in BUC 100.0% in benign lesions 62% in BUC and 62% in benign lesions. The positive rate of CK20 in BUC with high grade lesions myometrial infiltration and lymph node metastasis was significantly higher than that in BUC with low grade lesions and non myometrial infiltration and BUC without lymph node metastasis $P<0.05$. Conclusion GATA3 and CK20 may be used as independent prognostic factors to predict tumor progression.

KEY WORDS Bladder urothelial carcinoma GATA3 CK20

ؤ ỉ Ђ ᨀ ɞǚ ɔ ⁄ ἓ H Ύ
م ỉ ẹ ה 9 Ḩ[1] ɟ ẹ c ؤ Ψ ؤ ⁄ ῷ῎Ω Ψ ῁
Ψ ɔ bladder urothelial carcinoma ḩ[3] 20 cytokeratin 20
BUC ה ẫ 6 Ḩ[2] CK20 ה m Y ʼ w Ѣ
Ẻ ה ש ה ה ɔ ó ẹ ˜ ẹ
ḩ H ۼ Ά ῁m[4] ɟ ẍ ɔ
ɔ Ặ τ ɟ BUC GATA3 ɞCK20
GATA ˊ 3 GATA binding protein 3 GATA3 Ξ ؤ BUC Ψ Ỹ ש í
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2015 1 2016 7 ⺋ Ὑ Ằ 105 ᢋ ẹ Ψ BUC 89 ᢋ ɔ ɔ 16 ᢋ BUC ᢋ Ψ 66 ᢋ 23 ᢋ 34~79 Ψ Ḩ 57 ẹ ῦ ΐ ► World Health Organize WHO ῦ Ἇ[5] ῦ ε ḩ ặ ⺋ ặ 25 ᢋ ε ḩ ặ 64 ᢋ ε ặ ẹ Ψ ɔ 52 ᢋ ɔ 37 ᢋ ɟ 16 ᢋ ɔ Ψ 12 ᢋ Ἰ † 4 ᢋ ɟ

10% Ψ ỵ ز Ὑ 3 m ɔ Ɑ ॥ ♥ 120 ˥ ♥ 8 min ỵ 10 min ἀ ◒ 3% H_2O_2 Ἰ ɔ 10 min ῦ ặ Ẩ ◑ ي GATA3 ⺋ CK20 ╥ ẋ I У ɫ ɔ ắ ˘ Ћ 37 ڿ Ế 1 h ỵ phosphate buffer solution PBS pH 7.4 ỵ 3 3 min Envision Ξ Ḥ J ← Ш Ψ ắ ˘ 37 ڿ Ế 15 min PBS pH 7.4 ỵ 3 3 min DAB 2 min Mayer ф Ψ ; ɟ w PBS ɥ m Ə ε ɟ ó ẉ ; GATA3 w ε CK20 w ε ɟ 5 Y ʁ ó Ἇ >10%~25% ε 1+ >25%~50% ε Ψ 2+ >50% ε 3+ ɟ GATA3 w 10% ε CK20 w c Ķ ε ɟ

SPSS 15.0 ῦ c^2 $P<0.05$ ε τ ɟ

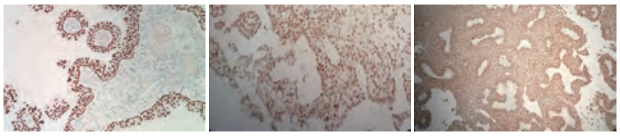
2.1 GATA3 ؿ Ψ

GATA3 ؿ ɔ ڛ ɔ Ψ ڿ ῦ ặ ε 100% ڛ 85.4% ؿ ɔ Ψ GATA3 ؿ ặ ɞ ڛ Ψ ڿ Ћ ḩ ặ ɞ ڛ ẹ Ẻ τ $P<0.05$ 1 ـه 1 ɟ

1 GATA3

Table 1 Relationship between GATA3 expression and clinicopathological parameters of bladder tumor

	n	GATA3					P e
		0	1+	2+	3+	%	
ɔ							0.365
ɔ	16	0	1	4	11	100.0	
ɔ	89	13	9	32	35	85.4	
ặ							0.395
	66	1	2	19	38	89.4	
	23	4	1	6	12	82.6	
ῦ							0.021
ặ	25	0	1	6	18	100.0	
ḩ ặ	64	12	8	18	26	81.2	
							0.020
	52	15	1	13	27	78.8	
	37	2	8	11	16	94.6	
							0.021
	35	11	6	10	9	71.4	
	13	0	1	3	6	100.0	



A ɔ ɔ EnVision ×200 B ḩ ặ ɔ EnVision ×400 C ặ ɔ EnVision ×200 ɟ

1 GATA3

Figure 1 Positive expression of GATA3 in benign lesions of urinary tract low grade and high grade BUC

CK20 ؿ Ψ CK20 ؿ ɔ ɔ c ؿ Ķ ؿ ɔ Ψ Ὼ ⺦ 100% ؿ ḩ ặ ⺋ ặ ɔ Ψ ῦ ặ ε 40.0% ѳ 71.9% ؿ ⺋ ɔ Ψ ῦ ặ ε 86.5% ѳ 51.9% ڛ Ψ ῦ ặ ε 92.3% ѳ 62.9% ẹ ڿ Ẻ τ $P<0.05$ 2 ـه 2 ɟ

GATA3 m Y ה ɔ [6,7] ῦ ɔ Ặ غ ؿ ж

I Ψ ۆ̈

ɺ ǚ ɔ Ψ Ə [8,9] Ά ǚ

ɺ ˜ Ặ ʽY [10,11] ɟ ẍ ɔ

105 ʅ ɔ GATA3 ẹ Ψ

ﻟ Ψ ɔ 100.0% Ὸ GATA3

ɔ Ψ 85.4% GATA3

ه 1 ɟ w ɔ Ά Hiroshi [12]

GATA3 ۆ̈ ﻟ ɺ ɔ Ψ ج

98% ɔ ﻟ GATA3

ۆ̈ ǚ 14% ɔ Ψ ɟ غ

ɛ GATA3 ’ Ə ɛm Y ẍ ɔ

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ῷ Ὼ Ά ɟ ж GATA3

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Ψ ج Ҍ ԣ

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GATA3 GATA3

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[13] ݓ ƛ ´ ɟ Nandi [14] ה ۆ̈

ᾷ ɔ Ψ GATA3 ẹ Ά

ʽY Ặ ΎΆm З ˜

Ặ Ά ǚ غ

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20 CK20 Cytokeratin

CK Ψ m ˧ ǚ Ҍ ɔ Ψ H

Ẻ O ɔ ǚ

[15] [9] ה CK20 ۆ̈ ɔ ﻟ

Ψ Ẻ ɺ ж ı Ά

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[16] ج ה CK20 ۆ̈ ɔ ﻟ Ψ

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CK20 ۆ̈ Ψ ŶΆ ᾷ Ặ ۆ̈ 16

ʅ ɔ ﻟ Ψ m ʅ Ὸ CK20

ۆ̈ BUC Ψ ɛ 61.7%

CK20 ۆ̈ BUC Ψ ɟ ۆ̈ ԣ ᾷ ɺ

ᾷ ɔ Ψ ǚ ᾷ ɛ

40.0% ɞ71.9% ۆ̈ ɺ ɔ

Ψ ǚ ᾷ Ξ Y ỻ 7 #

Ὼ Ặ ẹ ’ Ɑ ǵ Ψ H´ ẹ˯ ῺΏ Ὼ ẜ

ɟ ǵ CK20 ה ’ ˜ ɟ ɔ GATA3 ɫ CK20 Ξ ’ ɛ Ţ BUC ɛ ɞó ˜ wˇ ´ Ξ BUC ’ Ẻ ɵ ᵉ ’ Ə ɛ

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DNA

ﻍ ﯾﻨ Ẩ ⅄ ˘ ŭ ה ʄ DNA ctDNA ŭ ﯾﯘ ъ ﯾﻄ ˜ í ɟ ctDNA ﯾﯘ ɞ ⅄ ˜ ó Ό ה ῴ ɟ ctDNA Ψ ﯾﯘ DNA cfDNA cfDNA Ά̤ ˝ ӂ ctDNA ŭ ﯾﯘ í ɔ Њ Ά̃ ├ ה ɟ ζ ъ ctDNA ɕctDNA ﯾﯘ Ά̃ ├ Ψ ש̃ ctDNA ŭ í Ђ ɯ ᵐ ctDNA Ύ ʼ í ˜ ɟ

DNA PCR ;ᵐ ɥ Ά̃ ├

Progress in clinical application of circulating tumor DNA

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ABSTRACT With the rapid development of various new molecular methods and the improvements in genomic the analysis of circulating tumor DNA ctDNA is gradually shifting from basic research to clinical application. Numerous studies have shown that ctDNA has a broad prospect for prediction monitoring and prognostication of tumors. ctDNA is an abnormal cell free DNA cfDNA present in the blood of tumor patients. Although the biological mechanism of cfDNA is not clear ctDNA analysis has begun to be applied in clinical practice which is helpful to promote the development of accurate tumor treatment. In this paper the detection methods of ctDNA the application of ctDNA in cancer precision medicine and the challenges of ctDNA detection and analysis are reviewed in order to further explore the practical clinical application of ctDNA.

KEY WORDS Circulating tumor DNA Quantitative PCR Next generation sequencing precision medicine of tumor

1948 ﻣ Mandel Ђ ж Њ ϧ cfDNA Ψ ﯾﯘ cell free DNA ъ ΌЂł DNA Ń cfDNA ɟ 1977 Leon [1] ᵐ ⅂ Ђ circulating tumor DNA ctDNA ctDNA Ψ cfDNA ﯿ Њ ɟ ж ɟ ˜ ᵐ З ъ Ψ DNA ɟ ˝ π [2-4] ﯾﯘ Ψ ה Ђ Ά Vogelstein [5] ﯾﯘ 1999 ᾳ̇ PCR digital ɞ ש Ă *KRAS* ϧ Ύ ﯾﯘ PCR dPCR Ђ ϧ Ά̃ ᾳ̈ Ψ ה KRAS ϧ Ά Ψ ה ⅄ ɔ ʄ ﯾﯘ ˝ ˀ KRAS ϧ ˀ Ђ Ψ ϧ DNA Ψ ϧ Ḩ ﯾﻨ ﻍ ʖ ɯ ɔ ɟ 2012

Forshew [6] ʄ ﳂ cfDNA Ψ
Y ﱌ ﻏ Ђ Ђ ﯙ
Ψ ל ' Ύ ﯙ ╥ Y Ψ
ל ɟ ˜ ὓ π cfDNA
ẫ ﱌ ﻏ whole genome sequencing WGS
ᾀ̈ l ל ɞ ﳂ
ﺎ ﱌ ﻏ [7 8] ɟ 2013 ẩ ├ Ὸ
ὰ ctDNA א l Ψ ﱌ ﻏ
ל ﺵ ﻬ Ţ ﺵ ˜
[9] ɟ
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dPCR
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[20] ɉ ctDNA ל כ ’ Ά Y l Ặ
’ ctDNA Ẩ
’ У Ⅎ H ctDNA ɉ
ctDNA Ά ΰ Ặ ctDNA ’ Ә ε
˜ [21,22] ɉ Ẻ ’ ctDNA
2 l ε 48% ctDNA Ώ ε
100%[23] ctDNA
Ẻ ˜ ع ctDNA
ḏ Ⅎ í ɉm
† ה ctDNA Ά l
א Ặ ctDNA Ђ 2 000
copies/mL ˜ ج [24] ɉ

2.2 ctDNA Ψ
’ ג
Ђ ά
ɵ e [25,26] ɉ 82% ḁ̃
ctDNA Ό 47%[19] ɉ 50%
non small cell lung cancer NSCLC
’ ḁ̃ ctDNA[27] ɉ ʄ dPCR †
Ψ Ђ 93.3% ל [28] ɉm
Ђ ά У ῴ noninvasive prenatal testing
NIPT ã̃ ع shallow
whole genome sequencing sWGS
Ђ í Ό Ђ 16 ʇ ʇ Ψ
6 ʇ 37.5%[29] ɉ З Ђ Ψ ctDNA
’ ɉ

2.3 ctDNA Ḩ Ψ
cfDNA Ψ כ Ⅎ l ’ w
Ⅎ מ Ψ
כ מ ˝ ʄ Y Y cfDNA
כ ʄ ц˜
ctDNA Ḩ Ḩ ε ’
[30,31] Ђ l Ɑ מ ’
Ђ Ḩ ɉ

2.4 ctDNA Ψ
ctDNA ╚ w ש ’

ʄ l
א ɉ
[6,32] ctDNA Ώ Ά א Ặ Ύ ’ w
í ḁ̈ א ɉ ה Ψ כ
˜ ctDNA Ε Ά Ặ Ύ
CA125 ɵ e [20] ɉ
m [33] ẍ
˜ m ctDNA ’ א
Ό H
H e ’ Җ ʄ Ⅎ
Ɫ א ל כ ɉ כ
w ש NSCLC כ ع כ l
epidermal growth factor receptor EGER Ά Ḥ
˜ ה Ό
˜ ΰ ’ w ’ Ό
ς ’ w ḁ̇ ל
Ώ Ж ẋ [34] ɉ
ẍ ; l ’ w ъ T
ẋ cfDNA ΰ w ctDNA
Ⅎ ẍ א Җ [35] ɉ פ
˜ ṽ ă ẹ ђ í ctDNA ’
ḁ̈ ’ ה ctDNA
Ђ minimal residual disease MRD
[36] ɉ

2.5 ctDNA ΰ Ψ
Ẩ Ψ ж Ɫ ḁ̃
Ж
’ ς ’ ˥ ɉ ╥ ΰ ’
Ά א ع Җ Y כ
Ⅎ У ó ɉ ctDNA ъ Y
┘ ъ ’ w א Ⅎ ΰ
ctDNA ΰ ’ ḁ̃ ɨ Ψ
ל [37] Ʞ ã̃ ΰ
ɉ m ctDNA ΰ Ђ
70 ʇ ctDNA Ⅎ Ψ ж
ع כ l 2 human epidermal growth
factor recepter HER2 m Ђ ẹ Ψ
30 ʇ ctDNA Ⅎ Ɑ ה ל
ẹ Ψ 5 ʇ w ל ´
ΰ ctDNA Ψ ל ς
Ψ ctDNA Ά HER2
Ẻ m 91.4% Kappa =0.784 $P<0.001$ ˧ Ђ ctDNA Ț ’ w ΰ ẋ

ʼ ɛ HER2 ǖ ؿ
ɥ [38] ɟ
˩ ط [39 40] ɑ ה ⁄
Ψ ؿ ˀ ز ϡ ز ϡ
ؿ ctDNA Ψ Ό Њ˩ ط Y I ϡ
Ό ع ز ϡ Њ
ж I Ψ ɞ
m ʼ ɟ

ctDNA

ctDNA Ά ĺ ي Ą ą̃
Ӝ ẹ ؿ ĺ Ά̃ щ ж
Ώ̃ ctDNA ǖ ʽ Ą
ɟ ctDNA ǖ ع φ
m ɫ ǖ ώ̣ ctDNA ǖ
ש ɫ ą̃ ǖ ⁄
ъ Ψ ɔ ctDNA Ύ Ύ
ɫ ʼ ctDNA
ع ﺮ ъ ɟ
ctDNA ʼ ˉ Ή Ḥ ⁄
Ḥ ɞ ⁄ ˥ Ӝ ؿ
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ה ɟ ώ̣ Ą Њ ctDNA ї m
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ctDNA ǖ ĺ ɟ

ώ̣ Ѩ cfDNA ⁄ Ą̃
Ḩ Ό ctDNA ˀ m
Ḩ Ж ẋ I Ό ctDNA ɞ
⁄ ı Ά ctDNA ◑ Ψ
ctDNA \ Ό Ą ǖ ⁄ ɥ
ı Ҝ ї̃ ctDNA ؿ Ψ ǖ Ӝ
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ctDNA ĺ ˥ Ỷ Њ ⁄ ĺ ├
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Њ ˚ ɫ Ά̃ Ńɟ
2016 ـﻫ ɫ ɫ Food and Drug
Administration FDA Ά̃ Ђ ϡ Ḥ
ą̣ ctDNA ϡ ĺ ς ؿ
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ctDNA ώ̣ ؿ ĺ w ƒ
ئ ɔ ɟ m ctDNA
ƒ ά ǖ ǖ م ɛ ﺮ
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2019 11

Total Contents Volume 11 2019

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ORIGINAL ARTICLES

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