分子诊断与治疗杂志

JOURNAL OF MOLECULAR DIAGNOSTICS AND THERAPY

2019 11 11 6 64 Bimonthly Volume 11 Number 6 November 2019

Ψ
ℏΨ ₐ ├ ŋ ắ ˘
Ψ ₹ ɛ oy ắ ˘

Ὼ
ℏ ỗ Ἀ ŋ
ả Ὼ
ℏΨ ₐ ├ ŋ ắ ˘

┘ ← 179 ˝ ϖ 10~11 510620
020 32290789-206 32290789-201
jmdt vip.163.com
ISSN 1674-6929
CN 44-1656/R
46-283
Z ˜Y ₪
ₐ Ỉ ắ ₪
440100190057
oy ắ ˘
2019 11 18
RMB 15.00 ẃ

Responsible Institution *Sun Yat sen University*
Sponsor *China Family Doctors Magazine Publisher Co. Ltd.*
Organizer *DaAn Gene Co., Ltd. of Sun Yat sen University*
Consultant *SHEN Ziyu*
Editor in Chief *LI Ming*
Managing Director *JIANG Xiwen*
Associate Editor *LIU Yue*
Editorial Office *<JOURNAL OF MOLECULAR DIAGNOSTICS AND THERAPY> Editorial Office*
Editors *LI Xiaolan LI Caizhen LIU Qingqun*
Editing *China Family Doctors Magazine Publisher Co. Ltd.*

Add *10~11 Fl., Xianglong Building, 179# Tian he bei Lu, Guangzhou, China 510620*
Tel *020 32290789−206 32290789−201*
E mail *jmdt@vip.163.com*
CSSN *ISSN 1674−6929*
CN 44−1656/R
Printing *TianYi Yofus Technology Co., Ltd.*
Publish Date *2019.11.18*
Price *RMB 15.00*

扫码订阅《分子诊断与治疗杂志》

分子诊断与治疗杂志

……

……

Snail

……

……

……

……

……

PSCA ……

FBN1 ……

HMGN5

……

……

……

……

……

……

分子诊断与治疗杂志

CONTENTS

……

……

……

……

……

……

……

DAN

……

……

……

JOURNAL OF MOLECULAR DIAGNOSTICS AND THERAPY

CONTENTS

COMMENTS

YANG Yinrong WANG Qian 441

ORIGINAL ARTICLES

YU Xuegao DENG Jiankai CHEN Yaoming CHEN Peisong HE Xiaohong ZHONG Liangying HUANG Bin 446

DU Yandan NIU Yiqing ZHENG Haijun WANG Xiaoyan JIANG Yuhai CHEN Haiqiu LI Yinyan SUN Gang
LI Chunyu LU Desheng SUN Hui 451

SONG Ying LI Shipeng JIAO Weiwei 457

WANGYanli LI Jiming LUO Jinguang 462

LIU Xiaoyun WU Xiaoyan SHAO Qiong LONG Yakang WANG Haiyun DENG Ling 468

PSCA

MO Dachao LI Junjiu PENG Liang LIU Zhiyuan WANG Jieyun YUAN Jiru 474

FBN1

ZHANG Yue LI Jinjie DIAO Yanjun YANG Liu HAO Xiaoke 479

HMGN5

SUN Juan XIN Yanping ZHANG Guomei TIAN Xiaona LIU Huimin 485

YANG Bingrong WANG Jin 491

JOURNAL OF MOLECULAR DIAGNOSTICS AND THERAPY

CONTENTS

WU Pai WU Kunhe LIU Haiyan ZHANG Douxing HU Anqun 495

YU Mengyao GU Min ZHAO Hong 499

LI Maoxiang LI Yu LYU Yuanyuan CAO Yongxian 503

TANG Qiaoya GONG Junming MA Yanling 508

YOU Juan ..... (513)

YU Guangzhou TAN Wengang ZHANG Hao ..... (517)

HE Shuang HUANG Ping FAN Mingjuan ..... (522)

LIU Leilei HUA Wei JU Peihong ZHANG Wenyan HUANG Kan LI Zheng ..... (527)

FAN Wenbing ZHOU Yinsuo ..... (531)

ZENG Yumei DU Juan CHEN Yingzhi CHU Bing ..... (535)

REVIEWS

CHENG Xiaowen..... (539)

ў Ə ɟ HCV Ấ ~ ɔ
ĭ ч З ў Ə Ặ ŏ
ɟ Ъ HCV ы ч Ά ц
φ Ặ ' ε ת w
ɟ ΄Y Ə Ậ ‴ Ə Њ
ė ' ң ″H ' Ė
Б † ɟ

m ˉ ɻ ɞ
ɕ Ǐ ɤ ɞ ɞ ɻ
ɟ Ψ ‴ l
ę ŏ ε † chylomicrons
CM ɞ ң very lowdensity lipopro
teins VLDL ɞΨ intermediate density
lipoproteins IDL ɞң low density li
poproteins LDL ɤ high density li
poproteins HDL ɟ CM ɤ VLDL ˉ ɕ
LDL ɤ HDL ˉ Ÿ ɻ ɟ
CM ң HDL
ɟ
Apo Ξ Ю
ğ ŏ ğ ´ ę m
ğ ц Ψ Ė ˛
ɔ
l ´ ' ɟ ζ ŏ ε Apo A ɞApo B ɞ
Apo C ɞApo D ɞApo E A ɟ ɤ
Ş ' ŏ ε ' P ɤ ‴ ' P
ɟ Apo B100 m Ά LDL ɤ VLDL ´
‴ ' P ę ђ ″Ω ' P
ɟ Apo A ɤ Apo A HDL ζ
Apo C ч CM ɤ VLDL ‴ '
Lipoprotein lipase LPL ″Ң
Apo C ″Ω Ė ″Ậ LPL ' ɟ З ' P
ζ ğ Њ CM ɞVLDL ɤ HDL ɟ
ц Ƀ ɤ Ǐ ɟ
ц ζ ъ 1 3 ɟ

HCV

HCV Ъm Y Ά ζ ў Ə
ш ę ″Ậ ɟ Ά ę ђ ╥
RNA Ч HCV Ẉ ч Њ
ɔ l ɔ l ~ HCV Ƀ

E1 ɤ E2 ч Ấ ɟ HCV ƒ m
Ƀ ɤ ɔ l ğ Ǐ Ấ ė ´
Ά HCV ч ẇ Ấ Ặ Ƀ clau
din 1 ɞB ɔ l l ىي scavenger receptor class
B type 1 SRB1 ɞң ɔ l low density li
poprotein receptor VLDLR ɞCD81 ɤ ﻅ 4
ė З Ấ ı ת í
ğ e ɟ
HCV ∌ ė ‖ Ƀ
C ɞƁ E1 ɤ E2 ш ẇ p7 4
ɤ NS2 ɞNS3 ɞNS4A ɞNS4B ɞNS5A ɞNS5B 6
ɟ ğ HCV Ψ
' ш ╟ HCV ″Ậ
ת Θ 5 HCV ″Ậ Њ II Ǐ
◑ NS4B 5

~ Ψ Ƀ E1 ۱ E2 ´ ặ
ɔ [10] NS2 ч
Ά E1/E2 Ў Ə ğ Ψ ה
Ъ Ă Ə [11] ɟ ğ
Ψ Ά ậ VLDLs ´ [12] ɟ LDs
Ψ Apo B100 ɷApo E ۱ APO C ƚ Ά
Lipid Virus Particles LVPs
HCV ۱ VLDL ´ ɟ Apo B100 ‴ HCV
LVPs ה Θ א Apo C ۱ Apo E
HCV LVPs Ỉ У ‴ ’ ġ
[13] ɟ w HCV ۱ ŏ
Ά VLDL ۱ ŏ ğ Ъm ɟ
l ɕ microsomal triglyceride transfer protein MTP У VLDL Ă ŏ ; MTP Ậ Ъ Γ یی Ậ [14] ɟ ğ
Ψ ط Ά GFP
Apo Em Ş Ъ m [15] ɟ ´
HCV LVPs w VLDL ˚ ъ
Ψ ɟ Ъ Apo E ۱ VLDL ğ
Ψ Ə [16] ɟ

HCV

HCV m Y ẹ ң
΄Y م ۱ ۷ ‴ ڃ
m HCV ε 1.03~1.10 g/mL ɟ m Ά Ч [17] ɟ ˘
VLDL ɗLDL ɗDL ۱ HDL φ P ۱
l Ъ Apo B ẹ ђ ’
ئی ´ ğ ậ ğ Ψ ’
w Ў ΄Y ɟ̌ ئی Ά ẹ ђ ‴̌
HCV م ‴ c ΄Y ɔ l
Ỉ Ψ ɕ ۱ ζ ĭ [18]
۱ LVPs φ ğ Ψ ’ w Ў
[18] ζ ц Ά Ă
΄Y ɟ نح Ế HCV ’ ۱ ж
´ l ж ъ З ŏ ΄O HCV نح
Ế Ψ HCV ΄O ң ۱ Ж
[19] ɟ̌ ئی HCV Ψ ŏ ΄O
HCV Ş Ъ ң ۱
ŏ ΄O ğ Ψ نح Ế
Ж Ķ [20] ң HCV ’ ¯ Ά
Ă ŏ ɟ Ж HCV

Ң 1.07 g/mL Ά LDL VLDL ẹ
ڃ ε 73 nm Ά VLDL [17] ɟ ġ
HCV Ά VLDL Ч ɟ
HCV Ė Apo E ۱ ApoC Apo C ğ
ɔ VLDL [21] ɟ ה
ƒ HCV Ң
l l [22] Ъ
Γ ‴m ğ 40~140 nm م
Ỉ Γ ‴ Ė ẹ Ƀ
ẹ ɟ
ğ ה Ψ ´ ζ
m [23] ɟ HCV Ъ 3
m ‴Ω ´ ğ Ƀ Ỉ
ƚ Ά Ấ ۱ ٦ ӧ ɔ l
ч Ψ ۱ Ə [24] ɟ ӧ ɔ
Ş Ъ HCV m ŏ
[25] ɟ ğ HCV Ψ ´
Apo B ɷApo A ɷApo C ɷApo C ɷApo C
۱ Apo E [24] ɟ نح ġ ה ƒ
ğ HCV У Ψ Ə Ъ Apo E
HCV ה Ă ġ ɔ Ά
Apo E Ă ’ P w ẇ Apo B II
HCV У [26] ɟ ĭ HCV ۷
‴ نح ‴Ω ◑
Apo E אЮ Y Ş Apo B Ψ
Apo B HCV Ă Ỷ ġ [26] ɟ
Ỷ Ъ Apo E ğ HCV Ψ Ə
ğ Ψ Apo E ɔ Ậ
[27] ğ Apo E Ỹ ; Ƀ Ậ ğ
Ỉ ۱ ڃ ‴ У Ж Apo
E Ң Ƀ ~ ‴ ’ [28] ɟ
ğ 293t Apo E Ң
[28] ğ Apo
B/Apo E אنح ġ huh 7 LDs [26] ɟ
φ Apo E ƚ Ά Ъ HCV Є ẹ ɟ

HCV

HCV l نج Ế w ẇ ‴
Ấ ж Ѧ ה m w Ỉ
в в ۱ ŏ ƚ Ά HCV
۷ ğ Ậ Ψ ה Ə ɟ Ō m
HCV Ş Ỉ ц ¯

Ǐ ﻁ w ̣ Ь Ӈ ζ ɥ
У ǐ ɟ
HCV Ӈ ɥ ǐ ζ ε
HCV ң ẇ
Ψ ﻉ ƛ ɟ ң LDL
HCV [29 30] ɟ ɥ ƛ
ˀ Ў Ь ζ 3 Interferon 3 IFN 3 ﻧ
ﻍ ۱ HCV ﻧ ﻍ ﻱ rs12979860 CC ﻱ
IFN 3 ζ ﻧ ﻍ ﻱ ´ HCV ﻧ ﻍ ﻱ 1 G1
ε LDL ӂ HCV ﻧ ﻍ ﻱ 3 G3 ﻧ
ﻍ ﻱ 4 G4 LDL Ω Ą
[31] ɟ m HCV Ъ
ң ה HCV ~ ϭ ̣
[32] ɟ
HCV ɣ ۱ ƛ ’
ɔ ɥ ϭ ǐ ɟ HCV
ﻧ ﻍ ה ƛ Ъ
HCV Ά ƛ ה ‘Y Ą [33] ɟ
HCV ﻧ Ē ~ Ь
ɥ ϭ HCV Ė Ъ Ω [34] ɟ
Ά ф ﻱ Chronic hepatitis B CHB
ƛ Ά Γ ﻱ Chronic hepatitis C CHC
Ą ε ‘Y [35] ɟ CHC ƛ ’
~ ۱ ɔ ɟ
Apo A ƛ Ά HCV RNA Ӈ ۱
У [36] Apo A Ά HCV
C ﻁ ´ [37] ɟ Apo A Ά HCV
HOMA IR Ţ ۱
Ą [38] ﻍ Apo A ’ ƛ Ά HCV
ƛ ɟ Ş ǧ HCV
G1b Ψ Apo A Ч ω ╗ I
ф ﻩ [39] ɟ ﻍ Apo A ǧ
HCV Ψ τ m ɟ Ψ Apo
B 100 Ά HCV ۱
Ą [40] ɟ Ά ę ђ Apo C Ά
Ą ɔ
; [41] Apo C ’ Ά HCV
Ą ɔ Ą ǧ ɟ
Apo E HCV ۱ Ả Ą ŏ ф
m ɟ Apo E 3 Y Ң ﻧ ﻍ E2 ϭ E3 ۱ E4 ę Ψ E3
ɟ E2 Ά ɔ I ´ ̃ ʃ
HCV ң 3~5 ʁ [42] ɟ E4 ’ HCV
O Ж Ӆ Ə [43] ɟ ǧ Apo
E3/E3 ´ Ψ Γ ﻱ
ɔ ’ [44] ɟ ﻍ Apo E Ж ﻱ ’
HCV ה Ɑ ﻍ ф m ɟ

HCV m ﻯ ̣ ζ
ɥ Ъ ﻩ ﻁ ɥ
HCV ۱ ɥ ǧ HCV ۷ ۱ Y
‘Y Ą ɟ HCV ’ ɥ У ǐ
Ş ’ w HCV Ӈ ɟ ɥ ϭ
Ά в ɔ ۱ ה ‘Y Ą ɟ
ה Ą ’ w ɥ ǧ Γ
в ۱ Ả Ψ Ə [16] ε
Γ ﻱ ۱ Ӈ Ъ
ɟ
Ь m ̃ ˚ в
ẇ ǧ HCV ۱ Ψ Ė I ’ ẇ ę
ŏ Ӈ w ẇ í ɔ CHC ה ה ẇ
Ά Ψ ۱
ɟ Ⱶ м Ả HCV Ά ę ζ ɥ
ф Ў Ə ’ m ζ
HCV Ⱪ Ў ė ۱ ה
‘ w ẇ HCV LVPs
HCV ǧ w HCV
ɟ

1 Schauer C van Rijnsoever M Gane E. Surveillance factors change outcomes in patients with hepatocellular carcinoma due to chronic hepatitis C virus infection in New Zealand J . J Viral Hepat 2019 Epub ahead of print

2 Branche E Conzelmann S Parisot C et al. Hepatitis C Virus Increases Occludin Expression via the Upregulation of Adipose Differentiation Related Protein J . PLoS One 2016 11 1 e0146000.

3 Yoshio Aizawa Nobuyoshi Seki Tomohisa Nagano et al. Chronic hepatitis C virus infection and lipopro

6 Paul D Hoppe S Saher G et al. Morphological and biochemical characterization of the membranous hepatitis C virus replication compartment J . J Virol 2013 87 19 10612 10627.

7 Falcón V Acosta Rivero N González S et al. Ultrastructural and biochemical basis for hepatitis C virus morphogenesis J . Virus Genes 2017 53 2 151 164.

8 Dansako H Hiramoto H Ikeda M et al. Rab18 is required for viral assembly of hepatitis C virus through trafficking of the core protein to lipid droplets J . Virology 2014 462 463 166 174.

9 Boyer A Dreneau J Dumans A et al. Endoplasmic Reticulum Detergent Resistant Membranes Accommodate Hepatitis C Virus Proteins for Viral Assembly J . Cells 2019 8 5 487.

10 Vieyres G Dubuisson J Pietschmann T. Incorporation of hepatitis C virus E1 and E2 glycoproteins the keystones on a peculiar virion J . Viruses 2014 6 3 1149 1187.

11 Shanmugam S Saravanabalaji D Yi M. Detergent resistant membrane association of NS2 and E2 during hepatitis C virus replication J . Virol 2015 89 8 4562 4574.

12 Takacs CN Andreo U Dao Thi VL et al. Differential Regulation of Lipoprotein and Hepatitis C Virus Secretion by Rab1b J . Cell Rep 2017 21 2 431 441.

13 Syed GH Khan M Yang S et al. Hepatitis C Virus Lipoviroparticles Assemble in the Endoplasmic Reticulum ER and Bud off from the ER to the Golgi Compartment in COPII Vesicles J . Virol 2017 91 15 .

14 Mirandola S Bowman D Hussain MM et al. Hepatic steatosis in hepatitis C is a storage disease due to HCV interaction with microsomal triglyceride transfer protein MTP J . Nutr Metab Lond 2010 7 1 13.

15 Coller KE Heaton NS Berger KL et al. Molecular determinants and dynamics of hepatitis c virus secretion J . PLoS Pathog 2012 8 1 e1002466.

16 Lee JY Acosta EG Stoeck IK et al. Apolipoprotein E likely contributes to a maturation step of infectious hepatitis C virus particles and interacts with viral envelope glycoproteins J . J Virol 2014 88 21 12422 12437.

17 Gabrielle Vieyres Thomas Pietschmann. HCV Pit Stop at the Lipid Droplet Refuel Lipids and Put on a Lipoprotein Coat before Exit J . Cells 2019 8 3 233.

18 Felmlee DJ Sheridan DA Bridge SH et al. Intravascular transfer contributes to postprandial increase in numbers of very low density hepatitis c virus particles J . Gastroenterology 2010 139 5 1774 1783.

19 Lindenbach BD Meuleman P Ploss A et al. Cell culture grown hepatitis c virus is infectious in vivo and can be recultured in vitro J . Proc Natl Acad Sci USA 2006 103 10 3805 3809.

20 Podevin P Carpentier A Pene V et al. Production of infectious hepatitis c virus in primary cultures of human adult hepatocytes J . Gastroenterology 2010 139 4 1355 1364.

21 Fukuhara T Wada M Nakamura S et al. Amphipathic helices in apolipoproteins are crucial to the formation of infectious hepatitis C virus particles. J . PLoS Pathog 2014 10 12 e1004534.

22 Piver E Boyer A Gaillard J et al. Ultrastructural organisation of HCV from the bloodstream of infected patients revealed by electron microscopy after specific immunocapture J . Gut 2017 66 8 1487 1495.

23 Knodel MM Targett Adams P Grillo A et al. Advanced Hepatitis C Virus Replication PDE Models within a Realistic Intracellular Geometric Environment J . Int J Environ Res Public Health 2019 16 3 513.

24 Wrensch F Crouchet E Ligat G et al. Hepatitis c virus hcv apolipoprotein interactions and immune evasion and their impact on hcv vaccine design J . Front Immunol 2018 9 1436.

25 Lussignol M Kopp M Molloy K et al. Proteomics of hcv virions reveals an essential role for the nucleoporin nup98 in virus morphogenesis J . Proc Natl Acad Sci USA 2016 113 9 2484 2489.

26 Weller R Hueging K Brown RJP et al. Hepatitis C Virus Strain Dependent Usage of Apolipoprotein E Modulates Assembly Efficiency and Specific Infectivity of Secreted Virions J . J Virol 2017 91 18 e00422 00517.

27 Ganesh S Koser ML Cyr WA et al. Direct Pharmacological Inhibition of Catenin by RNA Interference in Tumors of Diverse Origin J . Mol Cancer Ther 2016 15 9 2143 2154.

28 Hueging K Doepke M Vieyres G et al. Apolipoprotein E codetermines tissue tropism of hepatitis c virus and is crucial for viral cell to cell transmission by contributing to a postenvelopment step of assembly J . Virol 2014 88 3 1433 1446.

29 Steba GS Koekkoek SM Tanck MWT et al. SNP rs688 with the low density lipoprotein receptor @

YMDD

ϕ ىي YMDD ل ;m ų ө ɟ
ך Ψ DNA ϕ ىي P نح غ ـ ~
;m ų DNA ά ɱ ŏ Θ ŏ ğ نح غ
ل ;m ų Ҥ ϕ ىي YMDD ل ɟ ϕ ىي
Ă 229 ٪ ;m ų ۱ Sanger ˚ YMDD نح غ ل ө ;m ų
ɟ ך 5 ٪ نح غ ل Ƀ˘ ˚˚ نح غ ل ىي ğ ⍵ ٪ نح غ ل ;m ų
ө ɟ Ъ ϕ ىي YMDD ل ;m ų
ɟ 229 oy í Ψ 2 ج ğ 74 ٪ 74/229 32.31% YMDD ل ę
Ψ ;m ų ğ 44 ٪ 44/229 19.21% rtM204V ل 25 ٪ 25/229 10.92% rtM204I ل 5 ٪
5/229 2.18% rtM204V/I ´ ل ɟ Sanger ğ 47 ٪ 47/229 20.52% rtM204V ل ⍵25 ٪
25/229 10.92% rtM204I ل ۱ 2 ٪ 2/229 0.87% rtM204V/I ´ ل ɟ ;m ų ğ 3 ٪
Sanger rtM204V/I ´ ل ɟ Ά Sanger ;m ų ө ۱ ٣A
ج ε 100% ل ğ ã ´ ε 95.95% 71/74 ŏ ´ ε 4.05% 3/74 ɟ 2 m .
ε 0.97 *P*<0.01 ɟ ;m ų ج m ´ ε 100% ɟ
ϕ ىي YMDD ل ;m ų ٣A
Sanger ğ ´ ل ε ϕ ىي نح غ w Ъ ɟ
;m ų Sanger YMDD HBV نح غ

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

YU Xuegao DENG Jiankai CHEN Yaoming CHEN Peisong HE Xiaohong ZHONG Liangying HUANG Bin

Department of Laboratory Medicine the First Affiliated Hospital Sun Yat sen University Guangzhou Guangdong China 510080

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

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

ϕ ىي ϕ ىي hepatitis B vi rus HBV ɻ ã 3.6 л HBV 100 ˛ ж Нь Ά HBV Ă [1] ɻ ە Нь HBV ה ˩ HBV 9 300 ˛ ϛ [1] ɻ lamivu dine LAM ᵐ Нь ϕ ىي ۱ [2 3] ɻ ᴛ Ъ ϕ ىي Ὸ 1 ˜ 16%~55% Ὸ Ъ [4 5] ɻ LAM ζ Ά HBV ´ نح غ ũ ũ tyrosine me thionine aspartate aspartate YMDD نح 204 Ң ƙ Ă ɻ ƙ ƙ ε reverse transcriptase methionine 204 valine rtM204V Ъ reverse transcriptase methionine 204 isoleu cine rtM204I [6 7] ɻ Ά Γ ╗ ŭ ă ה ŵ YMDD ƙ Ὸ ˜ 2~28 ۱ ۱ 12~31 ۱ [8 10] ɻ غ LAM ƙ Ẇ Нь ├ ε ϕ Ạ Y ɔ ɻ Ψ Ⱡ Ъ ϕ ىي YMDD ƙ ;ᵐ ɥ ɵ w ε í ϕ ىي نح غ w ɻ

ϒ 2017 1 12 ŵ Ψ ᵐ ├ ɔ LAM ᴛ 3 Y ϕ ىي ŭ ۱

5 mL 3 500 rpm 5 min ŭ Нь í HBV DNA ẉ PCR HBV DNA Нь 100 IU/mL Ấ Ắ 229 ϛ ẹ Ψ 182 ϛ 47 ϛ ɻ 17~77 ҷ 46.1±13.63 ɻ Ấ ặ ң ۷ Eppendort Ỉ ă Нь - 80 ỳ ɻ Ⱥ ƫ Ά Ị̇Ң

ABI 9700 PCR Ⱥ LOT N8050200 ɐ Ὼ LOT 4314 ɐOne Touch2 Ⱥ ƫ LOT 2457881 5247 ɐES Ⱥ ƫ LOT 74200 ɐDA8600 ;ᵐ ɥ Ⱥ LOT sn247560288 ɐABI 3500 DX نح غ ŭ Ⱥ LOT 4406019 ɐQubit 3.0 ẉ Ⱥ LOT 2321609904 Ⱥ ƫ ҷ ە Life Technology ắ ˘ ϒ Ị̇Ң LOT #DA Z14648 Ψ نح غ oy ắ ˘ SanPrep PCR У ɔ Ị̇Ң LOT B518141 0100 ɔ ắ ˘ Ion AmpliSeq™ Library Kit 2.0 LOT 4480441 ɐIon PI™ Hi Q™ OT2 Reagents 200 kit LOT A26434 ɐIon PI™ Hi Q™ Sequencing 200 kit LOT 4488315 ɐQubit dsDNA HS Assay kit LOT 1788710 Ị̇Ң ҷ ە Life Technology ắ ˘ ɻ

1.3.1 ;ᵐ ɥ YMDD ƙ

1.3.1.1 ϒ ϒ Ị̇Ң ϒ Ψ DNA Ị̇Ң Ш Ə ƒ Qubit 3.0 ẉ Ⱥ ɻ ϒ˜ DNA ϒ Нь-20 ɻ

1.3.1.2 DNA ﻩ Ẇ ɔ PCR HBV P ﻍ ﻧ ┘ ﻩ ~ ɟ HBV P ﻍ ﻧ ┘ Ώ 1 PCR ﻩ Θ ; 95 ﻟ 15 min ~ 94 ﻟ 30 s 55 30 s 72 Ţ 45 s Ă 40 Y ~ 72 Ţ 7 min ɟ ɔ ă ˘ SanPrep PCR У ɔ Ḥ ɔ PCR ﻩ У Qubit 3.0 ẉ Ѧ ɔ ~ ɟ ɔ ~ DNA Ҥ-20 ⅂ ɟ

PCR

Table 1 Primer sequence of target region for PCR amplification

ﻃ ┘	Ώ	
ﻕ	1 5′ ACAACCAGCACGGGACC 3′	ﻯ ﻕ
א ~	1 5′ GCGGGATAGGACAACAGAG 3′	P ﻍ ﻧ ┘

1.3.1.3 O Ḥ Ш ɔ ~ PCR У Ậ א א У ɔ ~ У ɔ ŏ ậ У ɔ У ɔ ﻩ Ậ א I ﻩ У ɔ ﻩ У ɔ Qubit 3.0 ẉ Ѧ ɟ Ҥ-20 ⅂ ɟ

1.3.1.4 Ậ ∤ DNA ~ ƒ Ion PI™ Hi Q™ OT2 Reagents 200 kit Life Technologies ă ˘ ﻩ Ḥ ﻭ One Touch2 Ѧ ﻁ Ẇ ES Ѧ ﻁ Life Technologies ă ˘ ﻩ ɜ O Ш Ə Ậ ∤ ɟ

1.3.1.5 DA8600 Ѧ ƒ Ion PI™ Hi Q™ Sequencing 200 kit Life Technologies ă ˘ ﻩ Ḥ O Ш Ə ɜ[m] ậ ´ ;[m] ɥ ɟ

1.3.1.6 ŏ ɱ ŏ variantCaller Θ ﻭ ł Allele Call Ń ; ε ł Absent Ń ł No Call Ń Ώ ⅂ ⅂ ◑ ε YMDD ﻯ ɟ ﻭ ł Allele Call Ń ; ε ł Heterozygous Ń ł Homozygous Ń Ώ ⅂ Ң ⅂ ó ε ⅂ ậ YVDD YIDD 2 ɟ

2 YMDD

Table 2 Mutation category of YMDD

ﻧ ﻍ ~	⅂ ậ	ɔ ⅂ ﻧ
HBV	YVDD	739A>G
HBV	YIDD	741G>T

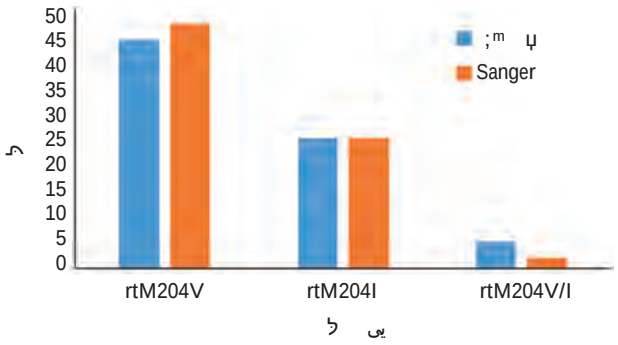
1.3.2 Sanger ƒ Ψ ﻧ ﻍ oy ă ˘ У ﻕ ﻯ ﻧ ﻍ ⅂ Ḥ PCR ﻩ Ẅ 2014 3401444 ˝ O Ḥ Ш ŏ ɟ Ė I Ə ⅂ ƒ ⅂ Ḥ PCR Ḥ Ẅ PCR ﻩ I Ḥ PCR ﻩ ; Ώ Θ ﻩ 50 2 min 95 15 min ﻟ ~ 94 ﻟ 30 s 55 30 s 72 Ţ 45 s Ă 40 Y ~ 72 Ţ 7 min У ɔ У ɔ PCR Ậ PCR I ; Ώ Θ ﻩ 96 1 min ﻟ ~ 96 ﻟ 10 s 50 5 s 60 Ţ 4 min Ă 25 Y ~ 4 ⅂ У ɔ ﻟ BigDye Terminator v3.1 Cycle Sequencing Kit ỏ ɔ ABI 3500 DX ﻧ ﻍ ŏ Ѧ ɜ ŏ SeqScanner ŏ ɟ ŏ SPSS Θ ŏ Kappa *k* [m] ŏ ɟ $P<0.05$ τ ɟ

2.1 ' ;[m] ɥ YMDD ⅂

ﻭ 229 oy ﻕ Ψ ;[m] ɥ ậ 44 ϛ rtM204V ⅂ 25 ϛ rtM204I ⅂ 5 ϛ rtM204V/I ´ ⅂ ɟ

2.2 ;[m] ɥ YMDD ⅂ ɵ ;[m] ɥ ∤ Sanger ﻉ ﻭ 229 oy ﻕ Ψ ậ 74 ϛ YMDD ⅂ ɟ ę Ψ Sanger ậ 47 ϛ rtM204V ⅂ ɑ25 ϛ rtM204I ⅂ ∤ 2 ϛ rtM204V/I ´ ⅂ ɟ ;[m] ɥ 100% 100% Ậ 100% ɟ 2 ậ ﻯ ⅂ ã ´ ε 95.95% 71/

74 ŏ ´ ε 4.05% 3/74 ה ã̃ ʷm
0/74 0% ֹ 1 ɟ ;ᵐ ɥ ǫ̇ 3 ɮ
Sanger rtM204V/I ´ ƙ ɟ



1 Sanger YMDD

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

2.3 ;ᵐ ɥ
Sanger ɮ 5 ɮ ɲ ɹ
ƙ ₿˙ YIDD ƙ 2 ɮ YVDD ƙ
3 ɮ ɷ ɮ ɲ ɹ ƙ ;ᵐ ɥ
ɔ ᵐ
10 ɟ ;ᵐ ɥ

4 Sanger 3
Table 4 Analysis of inconsistent 5 i

˝	ǫ̇	;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

ᶍᵐ ´ ε
100% ɟ
2.4 ;ᵐ ɥ Ä Sanger
;ᵐ ɥ Ä Sanger
3 2 YMDD ƙ ᵐ
Kappa=0.97 P<0.01 ɟ

2.5 3 ɮ ʷm ŏ
;ᵐ ɥ Ä Sanger 3 ɮ
ʷm Sanger ᶍ˜ ʻO YVDD
ƙ ;ᵐ ɥ ʻO YVDD/YIDD ´
ƙ ɟ 3 ɮ ᶍ ε 2 ɮ ɔ 1 ɮ
ᶍ ε HBV DNA ǧ 10^3~
10^5 IU/mL 4 ɟ

ώ HBV ز نح غ ζ m ع

PCR ע ◑ Sanger ϐ ẋ

ϐ نح غ ΦPCR ẉ ۱

P ۔ ۂ ẇ

m Sanger ε DNA ל

ẗA ж Њ ң ΄Ą Ъ ę ع

ۀ ğ ל 20% w ɔ Ό ع

ẋ ' ۂ ΄Ω φ

ʒ Њ ה ´ ж Θ ΄Y

ع نح غ ΦPCR ẉ ۱

P ğ í ɔ ج ƒ m Y Ң

ŏ ά

ל Ң ң ; 18 19 ع

;m ų ğ ₸ א Ψ̓

₸ ˛ DNA ɔ نح ΄Ω DNA

ع Ė ϐ ΄Ω

ع ώ ;m ų ‘ ζ نح Њ ẉ

illumina ‘ ۱ نح Њ Life ╚ ا

‘ ع ; ;m ų

ئى Њ ۱ Ψ 20 21 ع

Ъ ;m ų ф ى

YMDD ל Ъ

ɵ ع ;m ų 229 ʒ í

YMDD نح غ ל ε 32.31% 74/229

ΐ Ж 22 ۱ 23 '

ך ₸ ط ئى ۱

ع Sanger ά 47 ʒ rtM204V

ל ϐ25 ʒ rtM204I ל ۱ 2 ʒ rtM204V/I ´

ל ع ;m ų Ό 44 ʒ rtM204V ל 25

ʒ rtM204I ל ۱ 5 ʒ rtM204V/I ´ ל ع ;m

ų ε 100% 100% ẗA

100% ã ´ ε 95.95% 71/74 ŏ ´

ε 4.05% 3/74 ה ã ۔m 0/74 0% ع

;m ų ´

100% ع 2 m *k* ε 0.97 *P*<0.01 ع

ę Ψ 3 ʒ ğ Sanger ۀ Ό rtM204V

ל ğ ;m ų Ψ Ό rtM204V/I ´

ל ع ;m ų Њ

Sanger ά Sanger

´ ל ع Sanger ۀ ğ ל ʒ Њ 20%

Ό c ų Б † ΄Ω м

ה ל ع ۂ φ ğ Њ Њ

Θ ΄Ą ɣ ẋ m 3 ʒ ۂ

ع

' Ъ ф ى YMDD ל

;m ų ع

ẗA Sanger

ά ´ ל ε ф ى غ نح

w Ъ ۀ ε ę ђ ⊐ ا نح

ġ w Ъ ع ;m ų ה

Θ ɔ

ң ئى Њ í ع

1 Liaw YF Sung JJ Chow WC et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease J . N Engl J Med 2004 351 15 1521 1531.

2 Ong A Wong VW Wong GL et al. Management options for lamivudine resistant chronic hepatitis B patients with suboptimal virological suppression by adefovir J . Aliment Pharmacol Ther 2011 34 8 972 981.

3 Sheng YJ Liu JY Tong SW et al. Lamivudine plus adefovir combination therapy versus entecavir monotherapy for lamivudine resistant chronic hepatitis B a systematic review and meta analysis J . Virol J 2011 8 8 393.

4 Choi YM Lee SY Kim BJ. Naturally occurring hepatitis B virus reverse transcriptase mutations related to potential antiviral drug resistance and liver disease progression J . World J Gastroenterol 2018 24 16 1708 1724.

5 Lee HJ Kim SJ Kweon YO et al. Evaluating the efficacy of switching from lamivudine plus adefovir to tenofovir disoproxil fumarate monotherapy in lamivudine resistant stable hepatitis B patients J . Plos One 2018 13 1 e190581.

6 Masaadeh HA Hayajneh WA Alqudah EA. Hepatitis B virus genotypes and lamivudine resistance mutations in Jordan J . World J Gastroenterol 2008 14 47 7231 7234.

7 θ . ф ى YMDD ל J . Ψ ھ Ά í 2013 13 12 1568 1570.

8 Pas SD de Man RA Fries E et al. The dynamics of mutations in the YMDD motif of the hepatitis B virus polymerase gene during and after lamivudine treatment as determined by reverse hybridisation J . J Clin Virol 2002 25 1 63 71.

9 Sun J Wang Z Ma S et al. Clinical and virological characteristics of lamivudine resistance in chronic hepatitis B patients a single center experience J . J Med Virol 2005 75 3 391 398.

Snail TGF₁ EMT

Snail ɔ ɔ Ä ɔ ɣ 1 TGF₁ 1 ώ″Ω ɔ ɔ EMT Ą ɟ TGF₁ 1 ώ″Ω PC3 ŒDU145 ŒŰ Ẇ EMT ά Western blot ŒRT PCR EMT Ą Marker ɔ EMT Ψ Ỳ ɣ ˘ ɔ KDM4A ώ″Ω Ẇ ɣ Snail ɔ ώ″Ω ɯ Ẇ Gleason ŏ ɓ ɯ ɔ ώ″Ω ˘ 60 ʒ Snail ŒE cadherin ɨ Vimentin ɟ ɔ ŒŰ Ẇ TGF₁ 1 ˜ ώ″Ω PC3 Ė Ẇ Ὼ Western blot ŒRT PCR PC3 EMT E cadherin ; Snail1 ɨ Vimentin ɔ ę Ψ Snail1 ɔ ɛ ɟ ẍ ɔ E cadherin Vimentin ŒSnail E cadherin Vimentin ŒSnail ɟ KDM4A PC3 ɔ ɛ ɔ Snail1 ɔ ɟ *Snail*1 ɔ ɔ Ä TGF₁ 1 ώ″Ω ɔ ɔ Ą ɟ ώ″Ω TGF₁ 1 ɔ ɔ *Snail1* KDM4A

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

DU Yandan[1] NIU Yiqing[2] ZHENG Haijun[1] WANG Xiaoyan[1] JIANG Yuhai[3] CHEN Haiqiu[4] LI Yinyan[1] SUN Gang[1] LI Chunyu[1] LU Desheng[1] SUN Hui[1]

1. Department of Clinical Laboratory Inner Mongolia Forestry General Hospital The Second Clinical Medical School of Inner Mongolia University for Nationalities Yakeshi Inner Mongolia China, 022150 2. Department of Nuclear medicine department Laboratory Inner Mongolia Forestry General Hospital The Second Clinical Medical School of Inner Mongolia University for Nationalities Yakeshi Inner Mongolia China, 022150 3. Department of Department of Anesthesiolog Laboratory Inner Mongolia Forestry General Hospital The Second Clinical Medical School of Inner Mongolia University for Nationalities Yakeshi Inner Mongolia China, 022150 4. Department of Department of endocrinology Laboratory Inner Mongolia Forestry General Hospital The Second Clinical Medical School of Inner Mongolia University for Nationalities Yakeshi Inner Mongolia China, 022150

ABSTRACT Objective To investigate the relationship between the methylation of *Snail* gene and the induction of epithelial mesenchymal transition EMT by TGF₁ 1 in prostate cancer cells. Methods PC3 and DU145 cells were induced by TGF₁ 1. The induced EMT phenotype was screened by cell

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

ώ΄Ω Prostate cancer PCa Ψ m Ψ ہ PCa ה ң Ң ہ ж і ɔ ɯ ٧ ╕ † ɟ PCa Ψ ▌ 95% w ɔ ε PCa[1] ę ‴ m ˬ в в ш ъ ã̃ ƙ ɟ ώ΄Ω ה в Ά ɔ ɔ epithelial mesenchymal transition EMT ‴ ' ŏ [2 3] Ά Ж ıı ŭ ˬ Ψ ה Ə ɟ ﻁﻅΨ ɔ ġ . 1 transforming growth foctor . 1 TGF. 1 נח ŏ ę מ ˝ ; SMAD ɔ ' m ġ ٧ EMT [4] ɟ Ė ġ Snail1 ל Ά EMT ה ɯ ה ɔ Ψ ج Snail1 [5 6] ' Ά Snail1 ġ ٧ ┘ נח ɔ ıı Ă ɟ ɯ נח ɔ ה ŭ H3ˇ Ң [7] ę Ψ H3K9 נח ɔ ١ נח ġ Ж ' w Ą נח ġ ęˇ נח ɔ KDM4A ל Ά ט ɔˇ H3K9 נח ɔ ɟ ŭ KDM4A ל *Snail*1 נח ɔ PC3 ל ɔ ɯ Snail1 ל ɔ ώ΄Ω ה EMT ɟ

ɯ Ң

ж ώ΄Ω PC3 ɗDU145 ATCC ǎ ˘ ɟ ɗ1640 נج Ė в Gibco ǎ ˘ DMSO ہ Sigma ǎ ˘ Trizol ε å̌ Invitrogen ǎ ˘ У ŧ DNA Marker ε TakaRa ǎ ˘ У ŧ ج ɔ ǎ ˘ ´ BCA Ң ہ Thermo ǎ ˘ l ج ε ہ Cell Signaling ǎ ˘ У ẍ ɔ Ң Ң ɔ McLean ǎ ˘ ɟ

90% ´ 24 h 0.5% FBS 1640 נج Ė נח Ψ 10 ١ 20 ng/mL TGF. 1 24 ɗ48 h ʄ w ל ١ ɟ ώ̃ נج Ė ; в נج Ė ɟ ~ נج Ė ɟ 80% ני Ң Ă ώ̃ ‴ ɯ ٦ ھ ŭ ɟ ˬ Transwell Transwell Ǐ 100 L נח ; Ấ 500 L 1640 נج Ė נח Ấ ẍ У ɟ Ǐ ٧ Ấ 100 L 24 в 48 h ز в ~ ŭ ɟ RT PCR ж ώ΄Ω Ψ EMT Ą maker ל ɔ

1.2.1 Trizol ٦ RNA ٦ A260/A280 e Ң 1.8~2.0 φ RNA ٦ 1 g ´ cDNA ɟ w cDNA ΄Ω ε Ǐ ל נח ġ ε GAPDH ﻣﻣ ~ w ; ŭ

2 . Ct=2 Ct ⇗ ⇘ Ct Ĭ ⇗ ⇘ Ct ⇗ ⇘ Ct Ĭ ⇗ ⇘ ɟ

Western Blot ж ѽ ѿ Ψ EMT

Ą maker ㇔ ɔ

ㄱ ѽ ѿ PBS ~

Ấ ㇔ ɞ ɞㄱ ɔ Ấ

loading buffer ɟ ㄱ ɔ 40 g

ѡ . actin Ə ε ㇔ 12% SDS PAGE

~ ɞ ɞ Ɗ ו א ש ה ѡ

ƒ Ä . actin e ˘ ɟ

ẍ ɔ

Ĭ ↳ Δ ├ ѽ ѿ ѩ

ѡ Gleason ŏ 4 ѡ ɔ ѽ ѿ

˘ 60 ʒ ɟ ˚ ɲ

ɞ₿ ת ש ˈY Ң J Ψ ~

Ấ ф ɞ90% ф ѡ 75% ф Ψ

y̰ 1 min Ấ PBS y̰ Ψ ɔ ~

ŏ ɟ

ŏ

ѡ ɔ ҙ ɕ ѡ ɔ $\bar{X} \pm s$

ƒ SPSS 22.0 Ɵ Ψ ŏ

ɟ $P<0.05$ $P<0.01$ ɾ ɟ

TGF. 1 ~ ѽ ѿ ѡ EMT Ą Marker ㇔ ɔ

ת Ế ж ѽ ѿ PC3 ㄴ DU145 TGF. 1 ~ ㇔ ɔ ɞ ㄴ˯ ɟ ṷ Western blot ㄴ RT PCR ה EMT Ą Marker ㇔ ɔ ɟ ѽ ѿ PC3 ㇔ ɔ

1.2.1 ~ ѯ ẇ ;

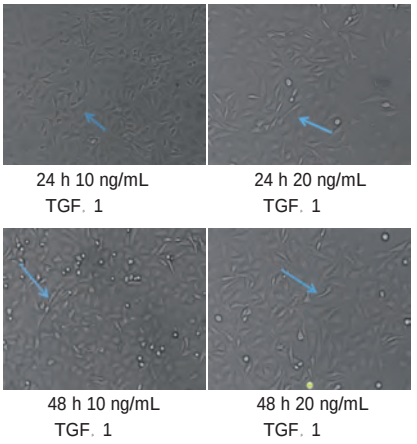
㇔ PC3 ㄴ DU145 ҙ

ۍ ㇔ ε ی ھ 1 A ɞB ę Ψ PC3 ㇔ ɔ Ȯ PC3 ~

ھ 2A ɞB ɟ ѻ ㄴ Transwell˯ TGF. 1 PC3 ~ ㄴ˯ Ѡ ѩ ھ 3 ɞ4 ɟ

2.1.2 RT PCR ㄴ Western blot PC3 EMT Ą Marker ㇔ ɔ

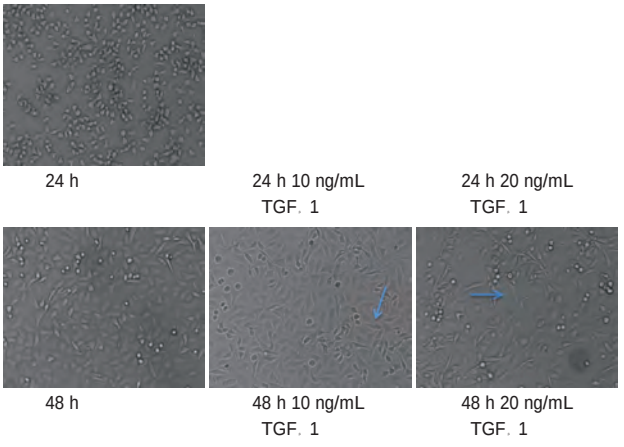
TGF. 1 PC3 EMT Ą Marker ה ㇔ E cadherin ; Snail1 ㄴ Vimentin ɔ ę Ψ Snail1 ㇔ ɔ ھ 5 Western blot ς Snail1 ѩ ɟ ھ 6 ɞ7 ɟ



A DU145 10 ɞ20 ng/mL TGF. 1 24 h~ ѡ ; ㇔ ɔ B 48 h~ ㇔ ɔ ×40 ɟ

1 DU145 TGF. 1 24 48 h

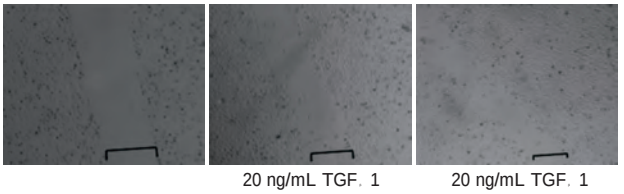
Figure 1 Morphological changes of DU145 cells induced by TGF. 1 for 24 and 48 hours under optical microscope



A PC3 10 ɞ20 ng/mL TGF. 1 24 h~ ѡ ; ㇔ ɔ B 48 h~ ㇔ 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



PC3 Ấ 20 ng/mL TGF. 1 ~ ѻ ㇔ Ѡ ѩ ×10 ɟ

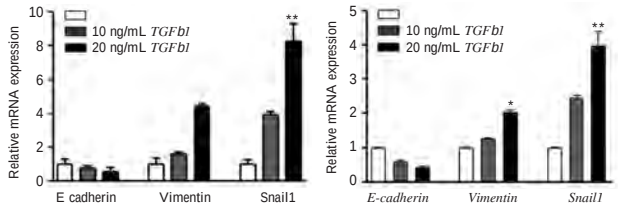
3 PC3 20 ng/mLTGF. 1 24 48 h

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

PC3 Ấ TGF. 1˜ ×10 ɟ

4 PC3 Transwell

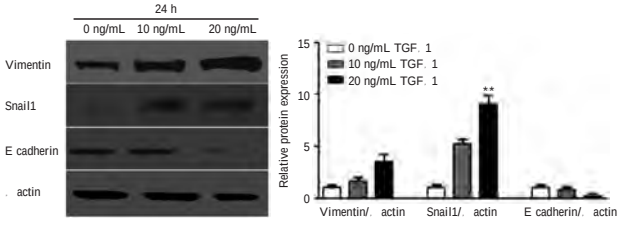
Figure 4 Transwell experiment results of PC3 cells



A ɲ Ế 24 h EMT Ặ Marker mRNA ɔ B ɲ Ế 48 h EMT Ặ Marker mRNA ɔ ɟ

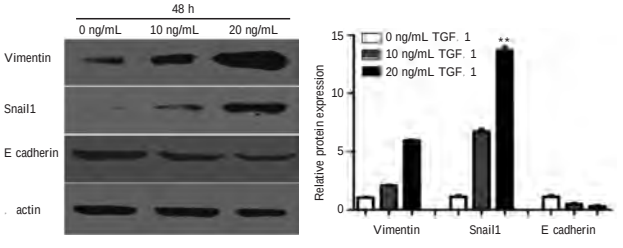
5 TGF. 1 PC3 EMT Marker mRNA

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



6 TGF. 1 PC3 24 h

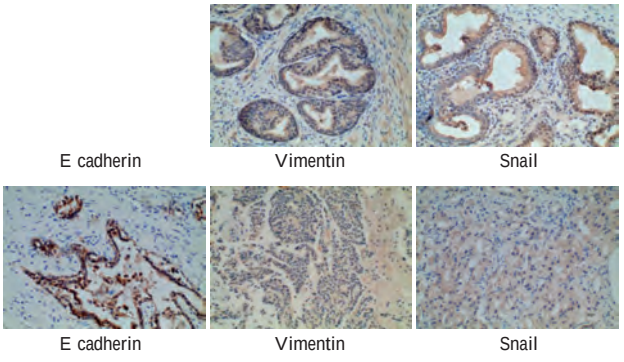
Figure 6 Expression of related proteins in PC3 cells induced by TGF. 1 for 24 hours



7 TGF. 1 PC3 48 h

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

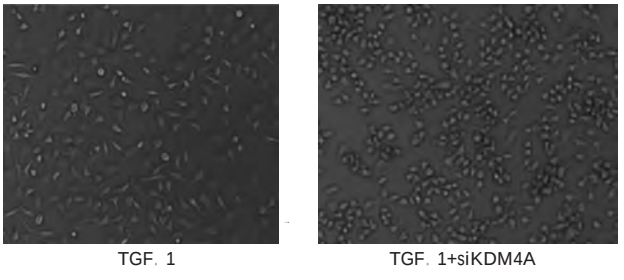
ώ ΅Ω Ẇ ώ ΅Ω Ψ EMT Ặ Marker Ẇ ẍ ɔ Ψ E cadherin ∖ Vimentin ɑSnail ∖ ɸ 8A Ψ E cadherin Vimentin ɑSnail ɸ 8B ɟ ’ Snail ΰ ώ ΅Ω ה ה Ψ ς Ъ Ỳ Ə ɟ KDM4A PC3 Ẇ ɣ Snail1 ɔ ɲ Ế ώ ΅Ω PC3 TGF. 1 Ẇ TGF. 1+siKDM4A ɟ 20 ng/mL TGF. 1 ɲ ɔ KDM4A *Snail1* ɲ ɔ ה ɔ Ặ Ъ EMT ώ ΅Ω PC3 ɔ ε ɔ ɸ 9 marker Snail1 ; ɸ 10 ɟ



A ώ ΅Ω B ώ ΅Ω

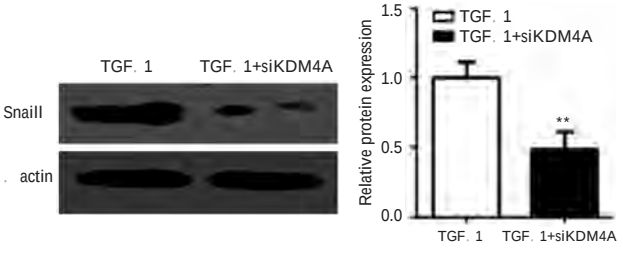
8

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 Ƙ ıı ΄Y Ă ıı

Ƀ ≉ ɔ ɞ ɔ ɞɸ ɔ ۱

≊ [8] ɟ EMT ה ɔ *E-cadherin* ٧

˩ ≁ ≉ ɔ [m] ʒ Ƙ ıı Ï

Θ [9] ’ ה E ɟ[m] Ɜ Ϛ

EMT ẹ Ă ė Snail Slug ٧ ˩ ≁

≉ ɔ Ά ẹ Ă [10] ’ ζ ė EMT Ă

ė Snail ɞZEB1 ۱ ZEB2 [n] HDAC ´

ה EMT ʄ E cadherin [11] ɟ

ѿ ıı Ƙ

≁ [12] ẹ Ψ ѡ H3ˇ Ң ɸ ɔ ≉

ɔ Ѭ ɔ ε ۱ Ƚ [13] ɟ Ă ΄O

H3ˇ Ң ɸ ɔ ıı [m] ڃ ۱

Ă H3 ɸ ɔ ’ ѡ

. ≉ ė ≉ ɔ ıı ΄Ω ٦ ΄Ύ Hь

≉ ė ‘ ’ [14 15] ɟ H3 ≉ ɔ H3

K4 ۱ H3K36 ≉ ɔ ıı ’ ≉ ė H3K9

≉ ɔ ’ ΄Ą ≉

ɟ H3K9ME3 ≉ ė ɔ ۱ ≉

Ά ۱ ≉ ė ۷

Ά e[m] ɟ

ıı ɟ ה

≉ ɔ Ƚ ε Ɵ ΄Ą

≉ ≉ ɔ

[18 19] ɟ K ۱ [m] [20]

9 / 3 ϑ bP % B€ ,!

17 Krivtsov AV Armstrong SA. MLL translocations his tone modifications and leukaemia stem cell develop ment J . NATURE REVIEWS CANCER 2007 7 11 823 833.

18 ΅Ι . Snail1 ↠ ξ ↿ ┘ ıı ϯ ώ΅Ω EMT ח Ψ Θ J . ǻ ├ 2016 50 5 397 402.

19 Mcdonald OG Wu H Timp W et al. Genome scale epigenetic reprogramming during epithelial to mesen chymal transition J . Nature Structural & Molecular Bi ology 2011 18 8 867 874.

20 Gut P Verdin E. The nexus of chromatin regulation and intermediary metabolism J . Nature 2013 502 7472 489 498.

21 Mohan M Herz HM Shilatifard A. Snapshot Chro matin Lysine Methylase Complexes Transcriptional Regulation and Epigenetics J . Cell 2012 149 2 498.

22 Ν . ↠ ↄ Ά J . Z ├ 2018 39 17 2675 2678.

23 . KDM4A AP 1 Ά i ϟ ˜ Ą Ψ ΅Ω A . Ψ ╞ i ├ Ж i ├ ŭ Ж . Ψ ╞ i ├ Ж i ├ ŭ Ж J Ж C . Ψ ╞ i ├ Ж i ├ ŭ Ж Ψ ╞ i ├ Ж 2017 1.

30 Andrade VG Yamashiro FDS Oliveira CV et al. In crease of lipids during HCV treatment virus action or medication J . Arq Gastroenterol. 2018 55 2 184 187.

31 Rojas Á del Campo JA Maraver M et al. Hepatitis C virus infection alters lipid metabolism depending on IL28B polymorphism and viral genotype and modu lates gene expression in vivo and in vitro J . Viral Hepat 2014 21 1 19 24.

32 Miyazaki T Honda A Ikegami T et al. Hepatitis C virus infection causes hypolipidemia regardless of he patic damage or nutritional state An epidemiological survey of a large Japanese cohort J . Hepatol Res 2011 41(6) 530 541.

33 Moriya K Yotsuyanagi H Shintani Y et al. Hepati tis C virus core protein induces hepatic steatosis in transgenic mice J . Gen Virol 1997 78 Pt7 1527 1531.

34 Tanaka N Moriya K Kiyosawa K et al. Hepatitis C virus core protein induces spontaneous and persistent activation of peroxisome proliferator activated receptor alpha in transgenic mice implications for HCV associ ated hepatocarcinogenesis J . Cancer 2008 122 11 124 131.

35 Moro an E Mihailovici MS Giuşcă SE et al. Hepatic steatosis background in chronic hepatitis B and C significance of similarities and differences J . Morphol Embryol 2014 55 3 suppl 1041 1047.

36 Mancone C Steindler C Santangelo L et al. Hepati tis C virus production requires apolipoprotein A I and affects its association with nascent low density lipopro teins J . Gut 2011 60 3 378 386.

37 Sabile A Perlemuter G Bono F et al. Hepatitis C vi rus core protein binds to apolipoprotein AII and its se cretion is modulated by fibrates J . Hepatology 1999 30 4 1064 1076.

38 Petit JM Jooste V Duvillard L et al. Apolipoprotein AII concentrations are associated with liver steatosis in patients with chronic hepatitis C J . Dig Dis Sci 2007 52 12 3431 3434.

39 Seki N Sugita T Aida Y et al. Assessment of the features of serum apolipoprotein profiles in chronic HCV infection difference between HCV genotypes 1b and 2 J . Hepatol Int 2014 8 4 550 559.

40 Yoshizawa K Abe H Aida Y et al. Serum apolipo protein B 100 concentration predicts the virological re sponse to pegylated interferon plus ribavirin combina tion therapy in patients infected with chronic hepatitis C virus genotype 1b J . Med Virol 2013 85 7 1180 1190.

41 Rowell J Thompson AJ Guyton JR et al. Serum apolipoprotein C III is independently associated with chronic hepatitis C infection and advanced fibrosis J . Hepatol Int 2012 6 2 475 481.

42 Silva GF Andrade VG Moreira A et al. Waiting daas list mortality impact in HCV cirrhotic patients J . Arq Gastroenterol 2018 55 4 343 345.

43 Gomaa HE Mahmoud M Saad NE et al. Impact of Apo E gene polymorphism on HCV therapy related out come in a cohort of HCV Egyptian patients J . J Gen et Eng Biotechnol 2018 16 1 47 51.

44 Fabris C Vandelli C Toniutto P et al. Apolipopro tein E genotypes modulate fibrosis progression in pa tients with chronic hepatitis C and persistently normal transaminases J . Gastroenterol Hepatol 2011 26 2 328 333.

HMGB1 TGF 1 GP73

1 HMGB1 ɷ ɔ ɻ 1 TGF 1 ɯ ɜ ɪ
73 GP73 ɲ Ƙ ˜ Ţ ɵ ᵉ ɟ ɳ 86 ɾ ɲ Ƙ ɛ
80 ɾ ╥ ɛ Ξ HMGB1 ɷTGF 1 ɯ GP73 ŏ ĭ
ɲ Ƙ ˜ ● ɻ HMGB1 ɷTGF 1 ɯ GP73 ˜ Ţ ɟ
HMGB1 ɷTGF 1 ɯ GP73 ɷ Ɓ *P*<0.05 Ƙ
HMGB1 ɷTGF 1 ɯ GP73 ς ∖ ɔ ╗ † *P*<0.05 ɟ APACHE ŏ ɷ Ƙ O ɷ
HMGB1 ɷTGF 1 ɯ GP73 ╗ ´ Ƙ ‴ ˜ ● ɻ *P*<0.05 ɟ
HMGB1 ɷTGF 1 ɯ GP73 ˜ Ţ ROC ; ŏ ǫ ɛ 0.805 ɷ0.827 ɷ0.855 ȳ í ᵉ ɛ
HMGB1>33.44 g/L ɷTGF 1>40.63 g/L ɯ GP73>79.84 g/L ɟ HMGB1 ɷTGF 1 ɯ GP73 ´ AUC
ɛ 0.917 ´ Ţ ɛ 72.7% Ά ╥ τ *P*>0.05 ´ ɛ
95.3% Ɓˇ ╥ *P*<0.05 ɟ HMGB1 ɷTGF 1 ɯ GP73 Ά
Ƙ Ỹ ˈY Ặ ɕ ' Ə ɛ Ţ ɲ Ƙ ˜ ㄴ ɟ
Ƙ 1 ɔ ɻ 1 ɜ ɪ 73

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

SONG Ying[1] LI Shipeng[2] JIAO Weiwei[1]
1. The 1st Affiliated Hospital of Henan University of Science and Technology Luoyang Henan China 471000 2. Department of General Surgery Jiaozuo People s Hospital Jiaozuo Henan China 454000

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

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

ε Ɑ ãă א

´ ' ה ჯ '

H Ɑ ė [1] ɟ Ь Ѿ ẇ

' ʄ ę ε ה Ψ

ɔ ჯ φ[m] [2] ɟ [3]

Ҡ ה 30% ˆ ε 60% ɟ

ה Ҡ H • ė ӂ

ѽ Ь Ҡ ה Ѧ [4] ɟ [5 6] ה

1 high mobility group box 1

HMGB1 ɞ ɔ ė . 1 transforming growth factor . 1 TGF. 1 ẇ ≉ l 73 golgi glycoprotein 73 GP73 ė ᵹ

l ∤ Ҡ ෙ Ə

ӂ Ă Ь ה Ҡ ˜ Ţ

ɟ ה Ҡ

HMGB1 ɞTGF. 1 ẇ GP73 ㄴ ɔ ŏ

ę Ά Ҡ ẇ ˜ Ă ε

Ţ ˜ w ㄴ ɟ

m

ך 2017 1 2019 1 ├

86 ϗ ה Ҡ ε

43 ϗ 43 ϗ 36~78 ෙ 57.50± 11.32 Ң 66 ϗ ɞ 20 ϗ

Ҡ 25 ϗ ɞѰ 42 ϗ ɞ 19 ϗ

˜ Ỹ 64 ϗ H 22 ϗ ɟ ך 80 ϗ ╥

ε ę Ψ 46 ϗ 34 ϗ

38~75 ෙ 56.32±10.57 Ң

57 ϗ 23 ϗ ɟ Ξ ѽ ɞ

≉ τ P>0.05

1 ɟ ├ ∤ Ж ɔ

ෙ ɟ

1 n %

Table 1 Comparison of basic data in 2 groups n %

ѽ	n	ѽ /		Ң	
	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	

Ă Ѧ

Ѧ ´ ơ ├ Ж ẇ

├ Ж ´ Ѧ [7] ◑ ᵹ w

; ỽ 2 w ɔ ה 38 w ɔ Ң

36 w ; ∤ Ч 20 /min w ɔ

90 /min w ɔ ╗ 12.0×10[9]/L w ɔ

Ң 4.0×10[9]/L w ; ɟ Ҡ ˟

total bilirubin TBIL ẇ Γ ala nine transaminase ALT [8] ◑ TBIL> 34.1 mol/L ALT> 80 U/L Ҡ ŏ Ѧ

Ҡ ALT ╗ e ɔ 1~3 ʁ TBIL ╗ e ɔ 1~2 ʁ Ψ Ҡ ALT ╗ e ɔ 3~5 ʁ TBIL ╗ e ɔ 2~ 3 ʁ Ҡ ALT ╗ e ɔ 5 ʁ w ɔ TBIL ╗ e ɔ 3 ʁ w ɔ ɟ

Ấ Ѧ ´ w ɔ ẇ Ҡ Ă ŏ Ѧ 36~80 è

├ ف ´ ˟ ъ í

ɟ Ѧ ɞ

ɞ ɞ ɞ ẇ '

Ψ Ҡ ´ '

ɞ ӧ

ˆ † í ˟ ãă Ψ

ɟ

Ấ ậ ɞ ɞ Ң ɞ APACHE. ǔ ɞ ≥ Ỹ Ấ 28 d ˜ ˜ Ỹ Ấ 5 mL 1 000 g 15 min ˥ -80 ˥ ẍ Ɏ HMGB1 ɞTGF. 1 ẇ GP73 Ḥ ɔ Ậ ɬ ç ü 800 ي ã Ⱥ Ⱨ ₳ BIO RAD ắ ˘ ɟ

SPSS19.0 Θ ǔ ẘ x̄±s ´ ǔ *t* ǔ ‴ ´ ǔ ɻ ẘ % c^2 ƾ Logistic ɤ ǔ ǐ ˜ ● ɇ ɔ Θ receiver operating characteristic curve ROC HMGB1 ɞTGF. 1 ẇ GP73 ˜ Ţ *P*<0.05 ɛ ɪ ɟ

Ξ HMGB1 ɞTGF. 1 ẇ GP73 HMGB1 ɞTGF. 1 ẇ GP73 Ⱨ Ė ɪ *P*< 0.05 ɟ 2 ɟ

.Ξ HMGB1 ɞTGF. 1 ẇ GP73 x̄±s g/L

Table 2 Comparison of serum HMGB1 TGF . 1 and GP73 levels between two groups x̄±s g/L

ậ	*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

‴ Ƙ HMGB1 ɞTGF. 1 ẇ GP73

Ƙ HMGB1 ɞTGF. 1 ẇ GP73 ⱨΨ Ƙ ɻ Ƙ Ψ Ƙ ˘ ẇ Ⱨ *P*<0.05 ɟ 3 ɟ

ǐ ´ Ƙ ˜ ╥ ɇ ǔ ╥ ɇ ǔ ɻ Ң ǯ ɞ

3 HMGB1 TGF. 1 GP73 x̄±s , g/L

Table 3 Comparison of HMGB1 TGF. 1 GP73 levels in patients with different degrees of liver injury x̄±s , g/L

	ậ	*n*	HMGB1	TGF . 1	GP73
	Ƙ	25	26.48±5.40	30.16±6.39	57.46±14.95
Ψ	Ƙ	42	30.63±8.16	35.382±8.22	68.39±17.81
	Ƙ	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

ậ ɞ ɪ *P*>0.05 Ң APACHE. ǔ ɞ HMGB1 ɞTGF. 1 ẇ GP73 ᶍ Ⱨ *P*< 0.05 ‴ ˜ Ƙ ɪ *P*<0.05 ɟ 4 ɟ

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

ƾ	*n*=64	Ң *n*=22	*t*/c^2 e	*P* e
	56.59±11.07	60.14±10.95	1.299	0.198
ậ /	31/33	12/10	0.981	0.396
APACHE. ǔ ǔ	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
Ƙ (/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

ɇ Logistic ɤ ǔ ẘ ˜ Ỹ ɛ ɇ ƾ ẘ APACHE. ǔ ɞ Ƙ ɞ HMGB1 ɞTGF. 1 ẇ GP73 Ỹ ɛ ╟ ƾ Logistic ɤ ǔ ɟ APACHE. ǔ ɞ Ƙ O ɞ HMGB1 ɞ TGF. 1 ẇ GP73 ╗ ´ Ƙ ‴ ˜ ● ɇ *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. ǔ	0.375	0.167	5.008	0.025	1.454	1.048~2.019
Ƙ	1.887	0.926	4.153	0.042	6.600	1.075~40.521

2.5 HMGB1 ϐTGF. 1 ϣ GP73

ה Ϗ ˜ Ţ

w H ε w ε

Ά ROC ŏ ˘ ˜ Ţ

ɟ HMGB1 ϐTGF. 1 ϣ GP73 AUC ŏ

ά ε 0.805 ϐ0.827 ϐ0.855 ϵ Ţ ˜ ȳ í

e ŏ ά ε HMGB1>33.44 g/L ϐTGF. 1>40.63 g/L

ϣ GP73>79.84 g/L HMGB1 ϐTGF. 1 ϣ GP73

˜ Ţ ۱

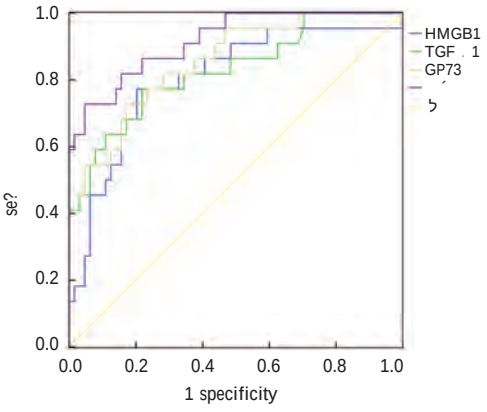
τ *P*>0.05 ɟ HMGB1 ϐTGF. 1 ϣ GP73 ´

AUC ε 0.917 ´ Ţ Ά ₸

τ *P*>0.05 ´

ʔΩ Ь ϵ ₸ *P*<0.05 ɟ

6 ϐ۵ 1 ɟ



1 HMGB1 TGF. 1 GP73 ROC

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

ã ├ Ă ̓ Ą

’ ׀ א ڑ ’

Ϗ [9] ɟ ל Ά ׀ ӧ ϐ ɔ ϐ א w

ϣ ”H Ψ ε

ɔ ڑ [10] ɟ Ϗ ’ ה Ь ỳ

ă Ь ã̃ א

׀ ɔ א

ч ۱ ɔ ע ĭ

Ϗ [11] Ɠ ● ; ׀

Ɠ Ẉ w ϐ ۷ Ὼ ڑ

Ь ң ’ ש

[12] ɟ ѽ í ɔ ɵ Ϗ

Ƀ Γ ۱ ӂ ę

ɔ ę ђ ĭ ɟ

[13] Ψm З ى Ά

Ϗ Ė Ą ’ ε Ϗ

۱ ˜ Ţ w ˜ ɟ HMGB1 m

ገ ŏ Ė ϐ

’ ɟ ׀ ɔ ά̈ ὰ Ϗ ϐ

ή HMGB1 ɔ ӧ

ظ Ά Ą ɔ ׀ ´ ה א

Ϗ HMGB1 ’ ˬ ч 6 ϐ ש

ė ė א ל Ά

۱ ڑ Ϗ [14] ɟ TGF. 1 εm Ė ӧ

Ə ė ۼ Ϣ ϐ

ŏ ɔ ۱ ׀ ӧ Ψ

Ə ɟ [15 16] Ὸ א

Ψ TGF. 1 Ά Ą ɔ ׀ ´ Ą ч

׀ ה א ά̈ Ə

TGF. 1 ₹ Ӂ ή א

ڑ Ϗ ɟ

GP73 نح ׀ ɜm ى ۼ

Ψ ӂ ˘ ė

Ϗ Ǐ GP73 ש Ӂ Ὸ

₹ [17] ɟ ė GP73 ’ Ə εm ى Ϗ

ɟ Xu [18] ה GP73 Ά

Ϗ ʹY Ą ę Ь ф

ӂ ALT Ė ל ϐ

e ɟ ה Ϗ

HMGB1 ϐTGF. 1 ϣ GP73 Ь

ה Ϗ ʹY Ϗ O

ھ HMGB1 ϐTGF. 1 ϣ GP73 ς ۷

ɔ ₹ † HMGB1 ϐTGF. 1 ϣ GP73

Ά Ϗ ۼ Ą

Ψ ӧ ۱ Ά ч

ŏ ھ ڑ Ϗ ɟ

Logistic ع ŏ ´
Ƙ ~ ǐ ɻ ה APACHE. ŏ ɞ
Ƙ O ɞ HMGB1 ɞTGF. 1 ẇ GP73 ╗
H ● ɻ ŏ ę Ɑ ɻ
' . APACHE. ŏ ╗ O
ɔ Ƕ ɳ ɔ '
ʍ . HMGB1 ɞTGF. 1 ╗
א ɔ Ƙ ɻ
~ . GP73 ╗ Ɯ Ƙ O
ɷ ǐ ~ ɟ
ROC ŏ ה HMGB1 ɞTGF. 1 ẇ
GP73 Ţ ´ Ƙ ~ Ė m
ל τ ɟ ה Ƙ m Y
ę ~ Ỹ ɔ ɻ ǐ ╥
ל ǔ ε ɿ ε Ɯ
~ ó מ ´ Ǎ ŏ w
ɿ ε Ɯ ɟ
´ HMGB1 ɞTGF. 1 ẇ GP73 '
ה Ƙ ~ Ţ ɟ
ж ï ˘
ǔm ˚ ɻ ~ Ấ ã
m HMGB1 ɞTGF. 1 ẇ GP73 ǔ
ה Ƙ Ψ Θ Ą ε ó
w ˚ ɟ
ɔ HMGB1 ɞTGF. 1 ẇ GP73
Ά ה Ƙ Ỹ 'Y Ą ´
HMGB1 ɞTGF. 1 ẇ GP73 Ɦ
~ Ţ wm ל ɟ

1 Jensen IJ Sjaastad FV Griffith TS et al. Sepsis Induced T Cell Immunoparalysis The Ins and Outs of Impaired T Cell Immunity J . J Immunol 2018 200 5 1543 1553.

2 Ɯ Ъ . Ą Ƙ í ẇ ~ ŏ J . Ψ ہ Ǐ 2019 39 2 163 167.

3 Ỳ ط ɿ . ǐ ỵ Ƙ ● ɻ J . 2019 24 3 259 262.

4 Woźnica EA Inglot M Woźnica RK et al. Liver dysfunction in sepsis J . Adv Clin Exp Med 2018 27 4 547 551.

5 ╞ . Ƙ J . î ╞ א ɔ 2017 25 30 2681 2688.

6 Cao Z Li Z Wang H et al. Algorithm of Golgi protein 73 and liver stiffness accurately diagnoses significant fibrosis in chronic HBV infection J . Liver Int 2017 37 11 1612 1621.

7 Singer M Deutschman CS Seymour CW et al. The third international consensus definitions for sepsis and septic shock sepsis 3 J . JAMA 2016 315 8 801 810.

8 Kobashi H Toshimori J Yamamoto K. Sepsis associated liver injury incidence classification and the clinical significance J . Hepatol Res 2013 43 3 255 266.

9 Schuler A Wulf DA Lu Y et al. The Impact of Acute Organ Dysfunction on Long Term Survival in Sepsis J . Crit Care Med 2018 46 6 843 849.

10 Sukkummee W Jittisak P Wonganan P et al. The prominent impairment of liver/intestinal cytochrome P450 and intestinal drug transporters in sepsis induced acute kidney injury over acute and chronic renal ischemia a mouse model comparison J . Ren Fail 2019 41 1 314 325.

11 . ỵ Treg/Th17 Ά ã א ɞ ɔ Ƙ Ą J . ┻ ┤ 2017 23 21 2968 2971.

12 Li J Xia K Xiong M et al. Effects of sepsis on the metabolism of sphingomyelin and cholesterol in mice with liver dysfunction J . Exp Ther Med 2017 14 6 5635 5640.

13 Inkaya AC Demir NA Kolgelier S et al. Is serum high mobility group box 1 HMGB 1 level correlated with liver fibrosis in chronic hepatitis B J . Medicine Baltimore 2017 96 36 e7547.

14 Yu Y Yang Y Bian Y et al. Hydrogen Gas Protects Against Intestinal Injury in Wild Type But Not NRF2 Knockout Mice With Severe Sepsis by Regulating HO 1 and HMGB1 Release J . Shock 2017 48 3 364 370.

15 . ɔ ɣ . 1 ی ä ' ǐ J . Ψ ہ ┤ 2018 47 12 1111 1115.

16 Han K Zhang Y Yang Z. Cilostazol protects rats against alcohol induced hepatic fibrosis via suppression of TGF. 1/CTGF activation and the cAMP/Epac1 pathway J . Exp Ther Med 2019 17 3 2381 2388.

17 Ą . ا نح 73 ǔ ϕ ی Ψ w ɔ Ƙ Ψ J . í 2018 34 4 755 759.

18 Xu Z Shen J Pan X et al. Predictive value of serum Golgi protein 73 for prominent hepatic necroinflammation in chronic HBV infection J . J Med Virol 2018 90 6 1053 1062.

miR 137 SETD7

miR 137 Ϟ Ə ϣ ę Ἄ ɟ
H9C2 Ũ ε ɞ ɞ +miR con ɞ +miR 137 ɞ +miR 137+
pcDNA ɞ +miR 137+pcDNA SETD7 ɟ qPCR H9C2 Ψ miR 137 ㇔ SETD7 mRNA
Western blot SETD7 ɑCyclin D1 ɑCleaved Caspase 3 MTT ⍟
LDH ɑMDA Ѧ ĕ H TargetScan ´ Ҳ ẉ ϶ Ũ miR 137 ㇔
SETD7 ˜ Ă ɟ ң H9C2 Ψ miR 137 ɑCyclin D1 ㇔
P<0.05 SETD7 mRNA ϣ ɑLDH ɑMDA ˜ ɑCleaved Caspase 3 ㇔
ĕ H *P*<0.05 ɟ ɔ miR 137 H9C2 Ĩ miR 137 ɑCyclin D1 ㇔
P<0.05 ң SETD7 ɑLDH ɑMDA ɑCleaved Caspase 3 ㇔ ĕ H *P*<0.05 ɟ
miR 137 ˜ SETD7 ɟ SETD7 Ũ miR 137 ϗ H9C2 Ə
ɟ miR 137 ˜ ; SETD7 ⍟ Ἄ ĕ H
ϗ ɔ Ϟ ɟ
miR 137 SETD7 ⍟

Effect of miR 137 targeting down regulation of SETD7 expression on oxidative stress induced by hypoxia reoxygenation in cardiomyocytes

WANG Yanli[1] LI Jiming[2] LUO Jinguang[1]
1. Department of cardiovascular medicine Anyang people s hospital, Anyang Henan China 455000
2. Department of cardiology Dongfang hospital affiliated to Tongji university Shanghai China 200092

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

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

ã ï H ١ ζ ɑ ė

φm ɟ ŵ Ψ Ő ’ w

ﻅ ợ ӂ Ӂ ѣ Ő Isch

emia reperfusion IR Ҡ[1 2] ɟ IR Ҡ ŵ

m ~ w ﻉ ặ

Ҡ[3] ɟ ѽ Ҍ IR Ҡ ŏ

Ạ ã ɟ ė IR Ҡ

١ ŏ Ạ Ҍ ѣ IR Ҡ Ė

τ ɟ RNA MicroRNA miRNA/miR / 2 5 . N ŧ O ę ¡ ɟ - ł

m ٦ RNA[4] miR

NA ל Ά IR Ҡ [5 6] ɟ miR 137 Ά

Ặ[7] ’ w ٦ ẃ ẍ ɔ IR Ҡ[8] ɟ ӂ

Ặ Ҍ miR 137 ŵ IR Ҡ Ψ Ə ɟ

w ṇ H9C2 P Ə Ŏ م نجي ´

Ầ 20 L ε 5 mg/mL MTT 37 ﹢ Ế 4 h ɔ 150 L DMSO 37 ﹢ Ế 10 min Ꮒ Ѧ ˘ ğ 490 nm Ꮍ ẉ e OD e % = OD e / OD e ×100% ɟ

LDH ɷMDA

H9C2 Ꮒ 96 80% LDH Ḥ LDH ɟ PBS H9C2 ʔ ɔ ˚ MDA Ḥ Ш MDA ˜ ɟ

Ѧ ĕ H

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

Ẋ ẉ Ꮍ

TargetScan Θ http //www.targetscan.org/ Ὸ miR 137 Ά SETD7 3′ ⅃ 3′untranslated region 3′UTR Ψ ŏ ﹢ ğ ў ɟ ی SETD7 3′UTR WT SETD7 ۱ ط ی SETD7 3′UTR MUT SETD7 Ꮍ ŏ ą miR con miR 137 48 h˜ Ẋ ẉ ɟ ŏ

SPSS 22.0 Θ Ά ŏ ẉ x̄±s ɟ Ξ *t* ₸ Ė ŏ Ξ Ξ SNK *q* *P*<0.05 ɥ τ ɟ

miR 137 ۱ SETD7 ĭ

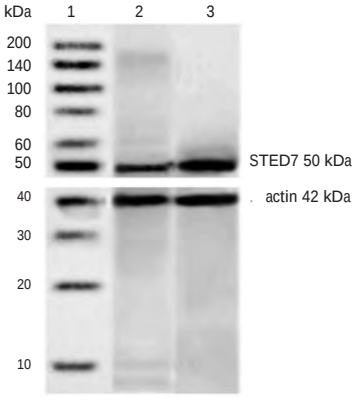
Ά ɳ H9C2 Ψ miR 137 *P*<0.05 1 SETD7 mRNA ۱ *P*<0.05 1 ɷ 1 ɟ

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

ą	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

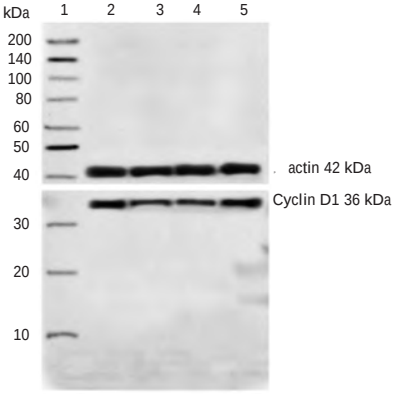


1. Maker 2. 3. ɟ

1 SETD7

Figure 1 SETD7 protein expression in cardiomyocytes

ɔ miR 137 ɷ Ꮒ Ꮒ ɳ H9C2 Ψ miR 137 ɷCyclin D1 ۱ *P*<0.05 2 ɷ ɷ 2 LDH ɷMDA *P*<0.05 2 ɟ Ꮒ +miR con miR 137 H9C2 Ỉ miR 137 ɷCyclin D1 ۱ *P*<0.05 ɷ 2 ɷ 2 ɳ LDH ɷMDA *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 Ἀ ĕ H Ά ɷ H9C2 Ψ Cleaved Caspase 3 ۱ ĕ H *P*<0.05 3 ɷ ɷ 3 ɟ Ά +miR con miR 137 Ἓ H9C2 Ψ Cleaved Caspase 3 ۱ ĕ H *P*<0.05 3 ɷ ɷ 3 ɟ

ϙ

+miR con

ẇ ش

5 miR 137 SETD7 X±S *n*=9 ĕ H *P*<0.05 6 ʚ 5 ɟ H9C2

Table 5 miR 137 targeted SETD7 to regulate its expression Ψ SETD7 miR 137

X±S *n*=9

ą	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 e	251.826
P e	0.000

Á miR con [a]*P*<0.05 Á anti miR con [b]*P*<0.05 ɟ

~ ę Ң *P*<0.05 6 ʚ 5 ɟ

ɔ ʅ ĕ H ğ IR

Ҡ Ψ [9] ɟ ę Ą ɔ ʅ

7 ĕ H ’ ~ ε ş IR ҷ Ҡ ʅ ɟ

ő ! ñ77 T58Dô58D™58,58);58,U58)158.ú8*L3,ɣ

ѽ Ҡ Ə IR Ҡ I

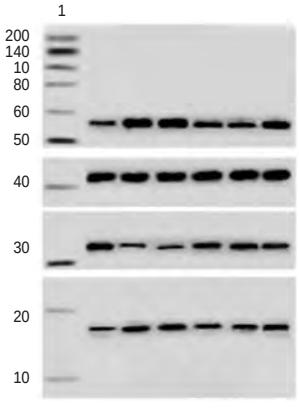
6 SETD7 miR 137 ş Ω Ì563ó56 `³S`™SS` Lś FD...X ĕ ğ ñ Vs÷o38 - ‘ Z ñ Ṕ r

Table 6 Overexpression of SETD7 partially reversed the protective effect of miR 137 on myocardial cells treated with hypoxia and reoxygenation. X±s, n

UÀ! XHÐ Q• ñ•• UÀ W • 7 • Fw˜C$ B P

ą	SETD7	Cyclin D1	Cleaved Caspase 3	LDH U/L	MDA nmol/mL	%	ĕ H %
	0.31±0.07	0.71±0.05	UÀ! ŸÀ @ B 0.27±0.03	Á @UÀ W • 7 • Ð ‰ñ™8 467.27±36.29	D 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 Mt	14.21±1.33[a] ë	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 e	176.638	180.671	77.640 X	84.893	60.712 hu \l V \l	60.796 ɟ ىں	17.625
P e	0.000	0.000	0.000	0.000	0.000	0.000	0.000

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



ñ•AĐ) ! + ð „ $ n O ˜2Ý ð5 [“Q3ó5 `³Q

ἕ H ¨E ROS У ; Keap1 ﾍ
Nrf2 ч ɔ ת ˝ Ƙ ¨E
Ƙ [20] ɟ Ψ ΅Ạ
SETD7 ɔ SETD7 Ъ miR 137
ך Ə ɟ miR 137
˜ SETD7
ך Ə ɟ
ɔ miR 137 ’ ѡ ך
ɔ Ƙ
ﾰ ΅Ạ ἕ H ΅Ạ Ά ˜ SETD7
Ặ ε IR Ƙ wЪ ɟ

1 Hu SY Zhang Y Zhu PJ et al. Liraglutide directly protects cardiomyocytes against reperfusion injury possibly via modulation of intracellular calcium homeostasis J . Journal of geriatric cardiology JGC 2017 14 1 57 66.

2 Zhou H Wang J Zhu P et al. NR4A1 aggravates the cardiac microvascular ischemia reperfusion injury through suppressing FUNDC1 mediated mitophagy and promoting Mff required mitochondrial fission by CK2. J . Basic research in cardiology 2018 113 4 23 42.

3 Joladarashi D Garikipati VNS Thandavarayan RA et al. Enhanced cardiac regenerative ability of stem cells after ischemia reperfusion injury role of human CD34⁺ cells deficient in microRNA 377 J . Journal of the American College of Cardiology 2015 66 20 2214 2226.

4 ¯ . Γ miR 133a/SOX4 HCT116 ﾰ ἕ H ΐ J . ŏ Ά 2019 11 3 238 244.

5 Yang F Li T Dong Z et al. MicroRNA 410 is involved in mitophagy after cardiac ischemia/reperfusion injury by targeting high mobility group box 1 protein J . Journal of cellular biochemistry 2018 119 2 2427 2439.

6 . RNA 98 ﾝ ך Ƙ Ψ Ə ѡ ę ΅Ạ J . Ψ ╞ ├ 2019 25 2 136 141.

7 Li W Zhang X Zhuang H et al. MicroRNA 137 is a novel hypoxia responsive microRNA that inhibits mitophagy via regulation of two mitophagy receptors FUNDC1 and NIX J . Journal of Biological Chemistry 2014 289 15 10691 10701.

8 Shi F Dong Z Li H et al. MicroRNA 137 protects neurons against ischemia/reperfusion injury through regulation of the Notch signaling pathway J . Experimental cell research 2017 352 1 1 8.

9 He Y Li C Ma Q et al. Esculetin inhibits oxidative stress and apoptosis in H9c2 cardiomyocytes following hypoxia/reoxygenation injury J . Biochemical and biophysical research communications 2018 501 1 139 144.

10 Yang Y Ding S Xu G et al. MicroRNA 15a inhibition protects against hypoxia/reoxygenation induced apoptosis of cardiomyocytes by targeting mothers against decapentaplegic homolog 7 J . Molecular medicine reports 2017 15 6 3699 3705.

11 Ong SB Katwadi K Kwek XY et al. Non coding RNAs as therapeutic targets for preventing myocardial ischemia reperfusion injury J . Expert opinion on therapeutic targets 2018 22 3 247 261.

12 Yang HH Chen Y Gao CY et al. Protective effects of MicroRNA 126 on human cardiac microvascular endothelial cells against hypoxia/reoxygenation induced injury and inflammatory response by activating PI3K/Akt/eNOS signaling pathway J . Cellular physiology and biochemistry 2017 42 2 506 518.

13 Guedes EC da Silva IB Lima VM et al. High fat diet reduces the expression of miRNA 29b in heart and increases susceptibility of myocardium to ischemia/reperfusion injury J . Journal of cellular physiology 2019 234 6 9399 9407.

14 Wu QQ Zheng B Weng GB et al. Downregulated NOX4 underlies a novel inhibitory role of microRNA 137 in prostate cancer J . Journal of cellular biochemistry 2019 120 6 10215 10227.

15 Feng Q Wu Q Liu X et al. MicroRNA 137 acts as a tumor suppressor in osteosarcoma by targeting enhancer of zeste homolog 2 J . Experimental and therapeutic medicine 2017 13 6 3167 3174.

16 Ziu M Fletcher L Rana S et al. Temporal Differences in MicroRNA Expression D J . Ex[illegible]

NSCLC ctDNA ≈иЬ ŏ UMI
J µ NGS Ặ ≈и ė ƛ ŏ Ţ ctDNA J µ ~ ɵ
e ẇ ï ɟ Ấ Ъ 163 ɂ 2017 7 2019 7 ğ Ψ ├
NSCLC ≈иЬ ŏ J µ ctDNA Ψ Ά NSCLC Ặ
11 Y ≈и ė ƛ Ỹ ɟ 98 ɂ è è NSCLC Ψ 37.8% 37 ɂ Ό
≈и ė ƛ ɟ ę æ Ψ *TP53* ɷ*EGFR* ɷ*ERBB2* ɷ*RET* ɷ*KRAS* ɷ*ALK* ɷ*PIK3CA* ɷ*BRAF* ɷ*MET* ɷ*ROS1* ɷ*NRAS*
ƛ ŏ ą̈ ε 33% ɷ24% ɷ10% ɷ8% ɷ7% ɷ6% ɷ4% ɷ3% ɷ3% ɷ3% ∧ 1% ɟ 57 ɂ EGFR TKI ~ ~
NSCLC Ψ 21 ɂ 36.8% Ό T790M ƛ 3 ɂ 27.3% ɔ ɕ µ EGFR TKI ~ Ό
T790M Ά T797S ƛ ɟ ę ɦ ƛ Ƀ *KRAS* ≈и ė ƛ 2 ɂ ɷ*MET* ≈и ė ѩ 1 ɂ ẇ
ẇ *PIK3CA* ≈и ė ƛ 8 ɂ ɟ 29 ɂ ARMS PCR *EGFR* ƛ
Ά ctDNA *EGFR* φ m ε 13.8% ɟ ‴ c Ό è è NSCLC
Ψ ~ ƛ ' ẇ ~ ~ ƛ Ỹ ɟ ė ' ≈иЬ ŏ
J µ NSCLC Y ɔ ɟ
J µ ŏ I DNA ƛ

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

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

Î ɔ ה ۱ H ж

ɟ ζ ŏ ε non small cell lung cancer NSCLC ۱ small cell lung cancer SCLC Ξ ę Ψ NSCLC ▌ 85% w ɔ ɟ NSCLC ζ ж NSCLC è Ψ ‴ Hь ‾ w ε ζ [1] ɟ ĂA ├ ۱ ŏ ה ~ ƒ NSCLC ĂA ~ ה [2 4] ɟ ȣHь Ţ I ’ [5 6] ɟ I ŏ

Ƀ DNA cell free DNA cfDNA ɞ ɞ I ɟ

cfDNA ۱ DNA circulating tumor DNA ctDNA cfDNA ۱ ĕ H˜ Ấ ۷ DNA ctDNA ˜ Ấ ۷ DNA ɟ cfDNA ɲ è ’ Hь ą̈ ˜ ɞ Ţ א ɞ ɞ [7 8] ɟ ώ NSCLC ɲ è Ƀ *EGFR* ɞ*ALK* ɞ*ROS1* ɞ*KRAS* ɞ*ERBB2* ɞ*RET* ɞ *PIK3CA* ɞ*BRAF* [9] ɟ Hь ɲ è ƾ NSCLC ˜ ’ w د ɟ è Hь è ’ w ɲ è ƾ Ỹ ĂA ɟ Hь ˜ ƾ Ỹ w ẇ ɟ ctDNA ɲ Hь ŏ J ɥ ŏ NSCLC Ă ɲ è ƾ Ỹ Ţ ctDNA J ɥ Hь ˜ ẇ ˜ í ɵ [e] ɟ

Ψ ├ ├ Ң ۱ Ж Ķ ĂA ˀ ɟ Ă Ấ 163 ʒ 2017 7 2019 7 ŵ Ψ ├ NSCLC ę Ψ 97 ʒ 59.5% 66 ʒ 40.5% ɟ 28~88 ę Ψ 60 w ɔ 90 ʒ 55.2% 60 w ; 73 ʒ 44.8% ɟ ۱ ´ 57 ʒ 35.0% ۱ ´ 42 ʒ 25.8% Ỹ ‴ 64 ʒ 39.2 % ɟ 130 ʒ 79.8% 8 ʒ 5.0% ŏ ‴ 25 ʒ 15.2% ɟ í ŏ ε ~ 33 ʒ 20.2% ~ 101 ʒ 62.0% ŏ ‴ 29 ʒ 17.8 % ɟ

DNA ɳ ẇ Ƀ ת DNA ɳ

10 mL ۱ ɨ EDTA ˀH ɟ ε ẍ cfDNA ɲ è DNA ɳ ۱ ɨ ŵ 4 ; ɳ Hь 2 h Î ŏ ɟ 2 ŏ ɟ Ẉ 10 mL ɨ ŵ 4 1 600 g Θ ; 15 min ɟ ۱ ɳ ɔ ɔ 4 16 000 g Θ ; 15 min ɟ ۱ ɳ ɔ 1.5 mL Ψ ɟ ą̇ ▌ Ţ ɟ QIAamp Circulating Nucleic Acid extract kit Qiagen ɳ ɨ Ψ cfDNA ɟ Ƀ ת DNA ƒ QIAamp DNA FFPE Tissue Kits Qiagen ắ ˘ ɳ ɟ ɳ ˜ ctDNA ẇ DNA ڜ ƒ Qubit dsDNA HS Assay Kit Thermo Fisher Scientific ŏ ɟ

1.3 ARMS PCR

ą̇ ARMS PCR ˀĦ ж

EGFR ز ز ل ٬Ḥ ·

EGFR ل Ỹ ɟ Ə ٬Ḥ

Ə ɟ ٬Ḥ ' ل I DNA ˉ

>1% ɨ ɟ *EGFR*

ز ز 18 ɞ19 ɞ21 ˝ 36 Y EGFR TKI

ل Ḥ 20 ˝ Ƀ T790M ɞS768I ɞ Ấ ل

9 Y ل Ḥ ɟ

ậ / DNA ٬Ḥ

cfDNA ɨ J ɥ ɞ

Ƀ ۱ ┘ ظ ɟ ctDNA Ấ

ε 60 ng ɳ ˝ ɳ Ь 30 ng ɟ ┘ ظ

150 ز ز panel ɟ Ə ٬Ḥ

/ DNA ٬Ḥ

Ə ɟ DNA Ь Illumina

NextSeq500 Ⱥ Illumina ɔ ﻫ

75 bp ɟ ctDNA ε

ﺝ Ь 10 000X Ь

2 000X ɟ

ctDNA J ɥ ŭ

Illumina NextSeq500 Ⱥ Illumina У

; ε bcl Θ ʄ bcl2fastq Conver

sion Software Illumina bcl Θ ε

fastq Θ ɟ ˥ ŭ ↀ ~

Ẉ ậ BWA MEM [10] ″Ω ậ ل ж

ز ز ɔ hg19 Θ ل ε ɟ

Ƀ ậ fgbio Θ ز ز

ɱ ۱ ŭ ɱ Ь˺ m

DNA ″Ω reads ŭ εm m

″Ω consensus reads ɟ ậ BWA MEM

consensus reads ậ ل ж ز ز ɔ ɟ ز

Ь ~ ˘ Y ↀ ;

ɞ ┘ ظ ↀ ɞ ┘ ظ ↀ

ɞ ɔ ɞreads ' ɞ ┘ ظ

95% Ḥ ɱ ɟ ز Ь˘ ~

Ŧ ز ی Ḥ ۱

Ấ / ɟ m ز Ь

Ţ ɞ ж cfDNA ی ɞل

ɞل Ḥ ۏ ″Ω Ψ ٬O Ḥ w ẇ ل

Ḥ ı ‘ Θ

˥ ' ɱ ا ل ɟ '

ɱ ل ˘ ل

Ḥ ɟ

è è NSCLC ctDNA ز ز ل

Ấ Ấ 98 ٪ è è NSCLC ẹ Ψ

73 ٪ 8 ٪ ẹ ℏ 17 ٪ ŭ ی ˝ ɟ 98

٪ è è NSCLC Ψ 11 Y ز ز

ل ɱ ﻫ 2 Ƀ ₸ ز ل ɞ Ấ

ɞ ´ ل ɞ ﺵ 4 ل ی ɟ ﻫ Ψ

″Ω ɥ m ٪ Ψ 11 Y ز ز ل

Ỹ ɥ m Y ز ز ۏ 98 ٪ Ψ ل

Ỹ ﻫ Ψ ς Ъ 98 ٪ ậ ɞ ɞ

ŭ ی ɞí ŭ ɞ٧ ´ Ỹ ɔ ﻫ Ъ

٪ Ψ 11 Y ز ز ل Y ^

ﻫ Ъ Y ز ز ۏ 98 ٪ Ψ ل

ɟ ẹ Ψ 37 ٪ 37.8% ٬O ز

ز ل 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*

ل ŭ ậ ε 33% ɞ24% ɞ10% ɞ8% ɞ7% ɞ6% ɞ

4% ɞ3% ɞ3% ɞ3% ۱ 1% ﻫ 2 ɟ *TP53* ز ز ل

NSCLC Ψ ٬O ẹ ل ۏ ˘ '

ظ ŭ ﻫ 1 ɟ 23 ٪ 24% *EGFR* ل

Ψ ε ε L858R ل 34.8% 8 ٪ ۱ 19

˝ ل 26.1% 6 ٪ 2 ٪ 8.7%

٬O 20 ˝ Ấ ل 1 ٪ 4.3% ٬O *EGFR*

ﺵ 1 ٪ 4.3% ٬O *EGFR* A864V ɞ

E746_A750del ۱ *EGFR* ﺵ 3 ل ẹ

ℏ ل Ƀ K757R ɞA613T ɞA647T ɞT678M ɞ

L833V+H835L 21.7% 5 ٪ *EGFR* ل ɱ

ﻫ 1 ˝ Ƀ *EGFR* ﺵ ɟ 7 ٪

7.0% ٬O *KRAS* ل 1 ٪ ε *KRAS*

ﺵ ẹ ℏ 6 ٪ ٬O 2 ˝ ل 5 ٪ G12V/C/

R ل 1 ٪ G13D ل ɟ *ALK* ´ ۏ 3 ٪

Ψ ﺝ ε *ALK-EML4* ´ ɟ 3 ٪ ٬O

ERBB2 Y772_A775dup ل ɟ ۏ 1 ٪ Ψ ٬O

NRAS G12D ل ɟ

EGFR TKI ~ ~ ل

Ấ 65 ٪ ɔm ɥ ɞJ ɥ ɕ ɥ EGFR TKI

~ NSCLC ẹ Ψ 57 ٪ ɔ

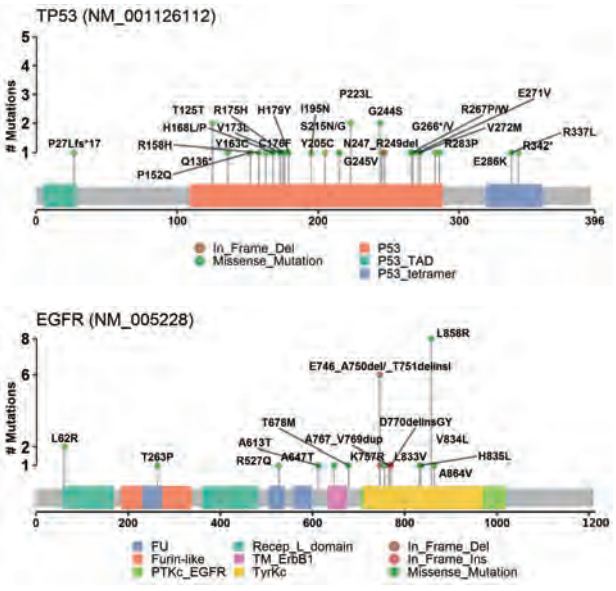
EGFR TKI ~ 8 ٪ ٬O Ấ

~ ل ŭ ɟ 57 ٪ ~ Ψ

42 ٪ ɔm ɥ TKI 1 ٪ ɔ J ɥ TKI

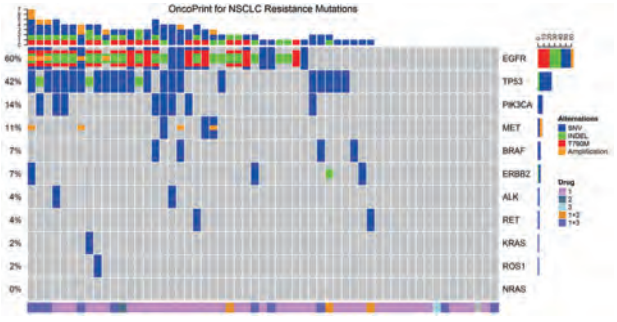
1 ٪ ɔ ɕ ɥ TKI 3 ٪ ɔm ɥ TKI ~

J ɥ TKI 10 ٪ ɔm ɥ TKI ~



1 *TP53* *EGFR*

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



Ώ μ m ϗ ϸ SNV ϒ ϖ ϸ INDEL Ấ T790M *EGFR* ϖ ϳ T790M ϸ Amplification ϰϰ 1 m μ EGFR TKI 2 J μ EGFR TKI 3 ϵ μ EGFR TKI 1+2 m μ EGFR TKI J μ EGFR TKI 1+3 m μ EGFR TKI ϵ μ EGFR TKI ϳ

3 EGFR TKI

Figure 3 Resistance mutation distribution in 57 EGFR TKI relapsed patients

Ώ μ m ϗ ϸ SNV ϒ ϖ ϸ INDEL Ấ Fusion ΄ ϸ Amplification ϰϰ M F Unknown ΅ LUAD LUSC Y Ϥ ΄ N Ϥ ΄ ϳ

2 98 NSCLC

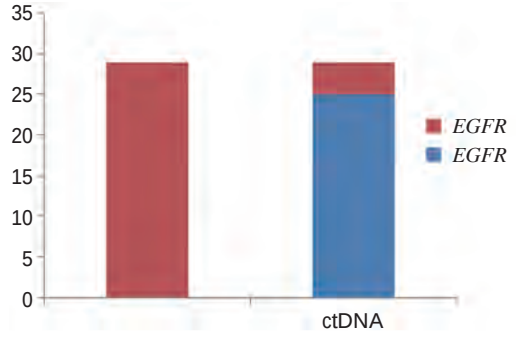
Figure 2 The mutation spectrum of 98 patients with tyrosine kinase inhibitor TKI naïve non small cell lung cancers

ϵ μ TKI ϳ 21 ϗ 36.8% ϗ EGFR TKI ~ Ό T790M ϸ ϳ Ă 11 ϗ ϗ ϵ μ TKI ~ ę Ψ 3 ϗ 27.3% Ό T790M Ά T797S ϸ ϳ ę ħ ϸ Ƥ *KRAS* ϖ ϳ ϸ 2 ϗ *MET* ϖ ϳ ϰϰ 1 ϗ w Ϣ *PIK3CA* ϖ ϳ ϸ 8 ϗ ϳ 11 Y NSCLC ϖ ϳ ϑ EGFR TKI ~ ~ Ψ ϸ ΐ Ỹ ϰ 3 ϳ

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

ϑ Ấ 163 ϗ NSCLC Ψ 29 ϗ ϑ ARMS PCR ϳ 29 ϗ ϫ ε ε *EGFR* ϸ Ƥ 13 ϗ Ό *EGFR* ϖ ϳ 19 ΅ ϸ 15 ϗ Ό *EGFR* ϖ ϳ L858R ϸ 1 ϗ Ό *EGFR* ϖ ϳ 20 ΅ τ ϸ ϳ 29 ϗ NSCLC Ψ 18 ϗ í ΐ ε ~ ~ ε 4 ϗ 7 ϗ í ΐ ε ΅ ϳ 29 ϗ ΐ ϫ ε 3 ϗ >5 cm ę æ ϫ ΅ 3 cm ϳ 4 ϗ ctDNA J μ Ό *EGFR* ϸ 1 ϗ K757R ϭ ϗ L858R ϭ 1 ϗ E746_A750del ϭ ϗ L858R ΄ V834L ϸ ϰ 4 ϳ ctDNA J μ Ό *EGFR* ϸ 4 ϗ Ψ 3 ϗ >5 cm 3 ϗ í ΐ ε ~ 1 ϗ ΐ ε ϳ

Ά ├ ε Ă NSCLC ϖ ϳ Ă ς ϩ Ђ ϳ ğ J μ ƒ NSCLC Ά ɔ ~ ϖ Ђ ϖ ϳ ~ ϸ ϳ *EGFR* ϖ ϳ ϸ NSCLC ϸ φ m ώ *EGFR* ~ Ƥ m μ / J μ / ϵ μ TKI ϳ m μ / J μ 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

~ ΫĄ ε *EGFR* T790M ƛ ɟ ѽ í ɔ ɲ ɠ ƛ Ƶ Ж Њ ʅ ΫĄ ϐ ϐ Њ ~ ~ ɲ ɠ ƛ Ỹ [11 12] ι Ə ε[m] ὰ ϐ ʅ ϐ ά Њ Њ ~ Ė ѽ ɟ Ấ 163 ʒ NSCLC ŏ Ъ NSCLC Ă 11 Y ɲ ɠ ƛ Ỹ ɟ ŏ ctDNA Њ è è NSCLC ɲ ɠ ƛ ɻ NSCLC ~ ƛ Ỹ ’ ğ w ; ɟ Ẉ ctDNA J ɥ NSCLC *EGFR* ƛ Ὸ ε 24% ɳ Њ Ж ж Ψ *EGFR* ƛ [13] ɟ Њ Ấ è è NSCLC Ψ 33 ʒ í ŏ ῀ 17 ʒ Ψ ctDNA ῀ Ά ŏ Ă ƛ Ὸ ɳ Њ Ὸ ɟ ẹ cfDNA Ψ ctDNA ῀ ’ ɳ Њ 1% [14] cfDNA ctDNA Ė Ə ῀ ’ ӧ Ὸ ^ Ỹ ɟ ɠ Њ è è ŏ Ỹ ι ctDNA ɟ ğ ctDNA ε ^ Ỹ щ w Ə ε ˚ ɟ *EGFR* T790M ƛ [m] ɥ / J ɥ EGFR TKI ~ ΫĄ ’ ğ 50% w ɔ Ψ Ὸ ƛ [15 16] ɟ ~ ~ Ψ T790M ƛ Ὸ ε 36.8% ɳ Ψ ƛ ɟ Њ ŏ ğ ctDNA ɲ ɠ ѽ ɔ ɔ ctDNA ƛ ’ ῀ ɥ ŏ ΫĄ ɟ T790M Ὸ ɳ Њ Ψ Ί Ὸ ɟ ɠ ctDNA J ɥ Ỹ Ƶ ɟ ctDNA J ɥ Ỹ Ɓ † ğ Њ Њ cfDNA ɲ ɠ ’ w ẇ Ψ ƛ Ỹ Ύ ὰ ɔ ѫ ɟ ѽ ה Ъ ɳ Ÿ ƛ J ɥ [17 20] w ğ ι Ỉ cfDNA ѧ Ψ 3 ctDNA ƛ ɟ *EGFR* ƛ Ά ctDNA *EGFR* φ [m] c ε 13.8% ɟ 29 ʒ í ŏ ẇ ŏ ה c 4 ʒ ε . ~ . 7 ʒ í ŏ ε ῀ c 3 ʒ >3 cm ẹ æ ҙ ῀ 3 cm ɟ ɻ Њ 3 cm Ψ ɲ ɠ ƛ Ὸ ɳ [21 22] ɟ ɲ ɠ Њ[m] Њ ɲ ɠ ~ ی Щ Ə ε Ƶ ӧ ctDNA Ὸ ^ Ỹ ɟ ɔ Њ NSCLC ɳ J ɥ ctDNA ğ ^ ’ Ж ῀ c Ὸ è è NSCLC Ψ ~ ƛ ’ w ~ ~ ƛ Ỹ ɟ ɲ Њ ŏ J ɥ ΫE Ə ɻ Ψ У Њ ctDNA ɲ ɠ ƛ ’ ƛ Ὸ ɟ ɠ ğ ŏ ɻ ϐ Ỹ w ẇ O Θ ; ’ ɲ Њ ŏ J ɥ NSCLC Y ɔ ɟ

1 Herbst RS Heymach JV Lippman SM. Lung cancer J . New England Journal of Medicine 2008 359 13 1367 1380.

2 Maemondo M Inoue A Kobayashi K et al. Gefi tinib or chemotherapy for non small cell lung cancer with mutated EGFR J . New England Journal of Medi cine 2010 362 25 2380 2388.
3 Waqar SN Morgensztern D Sehn J. MET mutation associated with responsiveness to crizotinib J . Jour nal of Thoracic Oncology 2015 10 5 e29 e31.
4 Solomon BJ Cappuzzo F Felip E et al. Intracranial Efficacy of Crizotinib Versus Chemotherapy in Patients With Advanced ALK Positive Non Small Cell Lung Cancer Results From PROFILE 1014 J . Journal of Clinical Oncology 2016 34 24 2858 2865.
5 Taniguchi K Uchida J Nishino K et al. Quantitative detection of EGFR mutations in circulating tumor DNA derived from lung adenocarcinomas J . Clinical cancer research 2011 17 24 7808 7815.
6 McCoach CE Blakely CM Banks KC et al. Clinical Utility of Cell Free DNA for the Detection of ALK Fu sions and Genomic Mechanisms of ALK Inhibitor Re sistance in Non Small Cell Lung Cancer J . Clinical Cancer Research 2018 24 12 2758 2770.
7 Kobayashi S Boggon TJ Dayaram T et al. EGFR mutation and resistance of non small cell lung cancer to gefitinib J . New England Journal of Medicine 2005 352 8 786 792.
8 Diaz LA Jr Bardelli A. Liquid biopsies genotyping circulating tumor DNA J . Journal of clinical oncolo gy 2014 32 6 579 586.
9 Zhu QG Zhang SM Ding XX et al. Driver genes in non small cell lung cancer Characteristics detection methods and targeted therapies J . Oncotarget 2017 8 34 57680 57692.
10 Li H Durbin R. Fast and accurate short read align ment with Burrows Wheeler transform J . bioinformat ics 2009 25 14 1754 1760.
11 Hiley C de Bruin EC McGranahan N et al. Deci phering intratumor heterogeneity and temporal acquisi tion of driver events to refine precision medicine J . Genome biology 2014 15 8 453.
12 Ignatiadis M Lee M Jeffrey SS. Circulating tumor cells and circulating tumor DNA challenges and op portunities on the path to clinical utility J . Clinical Cancer Research 2015 21 21 4786 4800.
13 Maemondo M Inoue A Kobayashi K et al. Gefi tinib or chemotherapy for non small cell lung cancer with mutated EGFR J . New England Journal of Medi cine 2010 362 25 2380 2388.
14 Diehl F Schmidt K Choti MA et al. Circulating mu tant DNA to assess tumor dynamics J . Nature medi cine 2008 14 9 985.
15 Eberlein CA Stetson D Markovets AA et al. Ac quired resistance to the mutant selective EGFR inhibi tor AZD9291 is associated with increased dependence on RAS signaling in preclinical models J . Cancer re search 2015 75 12 2489 2500.
16 Ohashi K Sequist LV Arcila ME et al. Lung can cers with acquired resistance to EGFR inhibitors occa sionally harbor BRAF gene mutations but lack muta tions in KRAS NRAS or MEK1 J . Proceedings of the National Academy of Sciences 2012 109 31 E2127 E2133.
17 Newman AM Lovejoy AF Klass DM et al. Integrat ed digital error suppression for improved detection of circulating tumor DNA J . Nature biotechnology 2016 34 5 547.
18 Kinde I Wu J Papadopoulos N et al. Detection and quantification of rare mutations with massively parallel sequencing J . Proceedings of the National Academy of Sciences 2011 108 23 9530 9535.
19 Schmitt MW Kennedy SR Salk JJ et al. Detection of ultra rare mutations by next generation sequencing J . Proceedings of the National Academy of Scienc es 2012 109 36 14508 14513.
20 Lou DI Hussmann JA McBee RM et al. High throughput DNA sequencing errors are reduced by or ders of magnitude using circle sequencing J . Proceed ings of the National Academy of Sciences 2013 110 49 19872 19877.
21 Wan JCM Massie C Garcia Corbacho J et al. Liq uid biopsies come of age towards implementation of circulating tumour DNA J . Nature Reviews Cancer 2017 17 4 223 238.
22 Chen Y Han T Zhou Y et al. Comparing the effica cy of targeted next generation sequencing in the identi fication of somatic mutations in circulating tumor DNA from different stages of lung cancer J . Neoplasma 2019 66 4 652 660.

PSCA

PSCA ג ּ ג Ή Ά Ж Ă ֿ ג ּ ג Ж Ă ֿ ג Ъ Ψ ה Ж 688 ˜ w ֱ 1 442 ˜ ֿ כ *PSCA* ג ּ ג 11 Y SNP Ή Ά ğ Ă ֿ ֿ ֿ c^2 ֿ Y SNP Ή Ă ֿ ˝ ŭ ֿ SNP Ή rs2294008 Ά ğ Ă *OR*=0.73 *P*= 4.78×10^{-5} SNP T Ή ג ּ ğ Ψ ŭ Ъ ֿ τ *P*<0.05 ּ Ė m ן Θ ֿ *PSCA* ג ּ ' ג ּ ֿ *PSCA* ג ּ Ж Ψ ה Ж Ă

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

MO Dachao LI Junjiu PENG Liang LIU Zhiyuan WANG Jieyun YUAN Jiru
Department of General surgery Dongguan Tungwah hospital Dongguan China 523110

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*=4.78×10^{-5}. 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

Ъ כ m m ה ג ּ ג Ъ Ж ã ג ּ ג Ă ge Ъ Ж ן Θ ε m ה Ъ כ nome wide association study GWAS Ή Ъ ן ה Y א ה Θ *PSCA* ג ּ ג ɔ ֿ single nucleotide O ĭ Ъ 1 3 ֿ polymorphism SNP Ή rs2294008 Ά כ ğ Ă 7 ֿ Ъ ˝ ˝ Ж ן ظ Ă ˝ Θ Ж ּ ' ן Ж m ğ Ψ ה Ж w ę 39% ה 3 ֿ Ψ ֿ ג ּ ג ֿ ֿ ֿ

ğ Ą ğ Ψ ה א Ψ Ţ ת ע
Ą SNP ה Ỹ ɟ

í

ζ Ƀ ʒ ۱ ɾ
Y I ﺝ Ћ Z Z ⊧ ⊦
ɟ Ξ כ ﺝ ε Z ש ẹ ۱ ﺉ ⌟
Ύ Ą ɟ כ ﺝ כ
ğ ך Ό Ъ Ξ Y ɟ
Ấ Ầ א ɔ
ל
ל ɟ א Ó ŵ ɟ Ξ ┤ ɟ Ξ ñ _

1 11 SNP

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

l	Ң	SNP	Allele1	Allele2	MAF	*P* e	' ┘ 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	Ǐ ˚
8	142674302	rs2978977	A	C	0.148	0.528	Ǐ ˚
8	142674428	rs146194718	G	A	0.097	0.172	Ǐ ˚
8	142676182	rs10956997	T	A	0.066	0.281	Ǐ ˚
8	142677319	rs587606222	C	G	0.037	0.461	Ǐ ˚
8	142677886	rs117919141	T	G	0.048	0.129	Ǐ ˚
8	142679754	rs13262164	T	C	0.084	1.000	Ǐ ˚
8	142680513	rs2294008	T	C	0.244	0.277	Ǐ ˚
8	142681306	rs2976391	A	C	0.364	0.333	Ǐ ˚

1 *PSCA* 11 SNPs

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

Ж Ψ ҷ τ ę *OR* e ŭ ˚ˆ

Ж Ψ ת ך m 3 ɟ Ж

Ǐ ה SNP rs2294008 Á '

Ɑ ġ ʔ ۱

ٰΩ ᵡ Ң ɟ

PSCA ת ġ ' w m З

' w ת ŭ ɔ [10 12] ɟ

PSCA ת ġ ŭ Ψ

ҷ RNA Small interfer

ing RNA siRNA *PSCA* ˜

ς Ж Ɔ ǭ ¨Ą [13 14] ɟ З

PSCA ת ġ Á ھ

Ψ Ъ ɟ ŭ ה

Ψ ھ Ÿ ǭ Ъ Ə [15 16] ɟ

PSCA ת ġ Ă ' w Ə

ε T ˜ Ң ѽ ̋Ω ẍ ŭ

ת Ψ ŭ PSCA ˚ Ң

PSCA [14] ɟ Ң

SNP rs2294008 C Ң ת ġ ŭ Ψ ה

Ң ת ġ ġ Ⱨ ę ' Ж

PSCA CD4+

CD8+T ה ; m ̋Ω ẍ א

Ƀ ŭ Ǐ ɔ ה ɟ

Ă Ң *PSCA* ת ġ ŭ ה ۱ ה Ψ Ə

˜ ת Ң ی Ấ ɟ

ŭ ھ Z ئ ┘ ж Ψ ה

rs2294008 Ң ת ġ ی w ᶙ Ң ת ġ ҷ

ŭ ʔ ۱ ŏ ŭ

Ң T Ң ת ġ ŭ ʔ Ψ

ŏ Ң ɟ *PSCA* ת ġ

Á ɔ Ж Ă

ѽ c ѽ ה ת Ң ж

‖ J m [3] ɟ ŭ Ψ

ה rs2294008 T Ң ת ġ ‖ J Ė

ך Ə ę *OR* e ε 0.67 Á

Ă ɱ ˝ ת m ɟ

Z ئ ┘ ж Ж '

Á *PSCA* ת ġ rs2294008 Ң ת ġ ی Ą ˜

' w Ң ל Ÿ ъ Ж

ŭ ɟ ж

˜ ǭ ϐ ϐ w ᶙ

Ÿ ظ Ж ġ ŏ ŏ Ψ ה

2

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

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

* ŏ SNP Ҥ c^2 Ⱨ Fisher ɟ

3 rs2294008

Table 3 Stratification analyses for SNP rs2294008

	Ӈ نح غ	ٹ / نح غ ٹ	Ӈ نح غ Ӈ	c^2 e	*OR* 95% ⊓ ⌟	*P* e
ǫ̈						
	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

ğǔ ŏ Ψ SNP Ӈ rs2294008 ɷ ɻ ŏ یی Ӈ ’ ѡ نح

ⓝ Ỹ نح m *PSCA* نح غ Ӄ Ă ŏ [17] ɟ ʃ

SNP Ӈ rs2294008 Ӄ Ə ’ Ҥ Ҭ Sequenom MassARRAY ‘ SNP

З • ġ ğǔ ɟ نح غ ŏ یی ‘ ζ نح Ҥ نح ѡ

ʃ DNA Ӈ ε ╥ Matrix Assisted Laser Desorption/

Ӈ Ӈ ε 3 ɥ Ӄ Ḗ Ionization Time of Flight Mass Spectrometry MAL

DI TOF MS ώ ε SNP

Ė w ẇ Ἄ ã̃ ’ w

΄ Ηь [18] ɟ

l ŏ Ж Ă Ζ ė

m ė ΅ ᾰ Ἄ Ж

ﺉ ╕ Ж Ă ת ˝ ˆ [19] ɟ Ə

ε[m] ﻧ Ηь ר ﻧ ė Ж Ă ŏ ﻱ

SNP Ң ė ɮ ώ

ǔ l ŏ ɟ ǔ

Ấ Ψ ↄ ﺉ Ἄ ’

w ŏ ↄ Ж Ж

Ἄ ъ ŏ Ђ l ŏ m ė

ɟ Ψ Ηь ﺍ ж Ὼ Ἄ

Ђ ר ﻧ ė ٦ Ђ 11 Y Ж

Ң 11 Y Ң Ꞵ ת

΅ Y *PSCA* ﻧ ė ┘ ﻁ ė ǔ

Ψ ’ Ђ З ε Ж Ң

ɟ ǔ Ψ ٦ Ж

Ъ Ң *PSCA* ﻧ ė Ά Ж Ă

’ w [m] ɟ

Ηь ώ *PSCA* ﻧ ė rs2294008 Ң Ά

Ə Ἄ ė Ă SNP

Ң ﻧ Ηь ﻱ ˙ ŏ

w Ὼ ɟ

1 Kavitt RT Lipowska AM Anyane Yeboa A et al. Diagnosis and treatment of peptic ulcer disease J . The American journal of medicine 2019 132 4 447 456.

2 . Ά J . ├ 2009 25 6 841 843.

3 ΅ . ΅ˀ í J . Ψ ﻩ ├ ˜Y 2012 14 5 744 745.

4 Salih BA Abasiyanik MF Bayyurt N et al. H pylori infection and other risk factors associated with peptic ulcers in turkish patients a retrospective study J . World J Gastroenterol 2007 13 23 3245 3248.

5 ٦ ╞ ▒ ΐ Й . ɷ‖ J ẇ Ă ė ع ŏ 204 ٯ J . ΐ ╞ ж ↄ 2011 19 6 628 630.

6 ╞ Z . Ậ ΐ ė J . Ψ ﻩ 2012 32 1 132 133.

7 Tanikawa C Urabe Y Matsuo K et al. A genome wide association study identifies two susceptibility loci for duodenal ulcer in the Japanese population J . Nature genetics 2012 44 4 430 434.

8 Purcell S Neale B Todd Brown K et al. PLINK a tool set for whole genome association and population based linkage analyses J . Am J Hum Genet 2007 81 3 559 75.

9 Barrett JC Fry B Maller J et al. Haploview analysis and visualization of LD and haplotype maps J . Bioinformatics 2005 21 2 263 265.

10 Marra E Uva P Viti V et al. Growth delay of human bladder cancer cells by prostate stem cell antigen down regulation is associated with activation of immune signaling pathways J . BMC Cancer 2010 10 7 129.

11 Xia B Wang Y Wang X et al. In utero and lactational exposure of DEHP increases the susceptibility of prostate carcinogenesis in male offspring through PSCA hypomethylation J . Toxicology letters 2018 292 78 84.

12 Heinrich MC Göbel C Kluth M et al. PSCA expression is associated with favorable tumor features and reduced PSA recurrence in operated prostate cancer J . BMC Cancer 2018 18 1 612.

13 Gu Z Yamashiro J Kono E et al. Anti prostate stem cell antigen monoclonal antibody 1G8 induces cell death in vitro and inhibits tumor growth in vivo via a Fc independent mechanism J . Cancer research 2005 65 20 9495 9500.

14 Raff AB Gray A Kast WM. Prostate stem cell antigen a prospective therapeutic and diagnostic target J . Cancer letters 2009 277 2 126 132.

15 Yee EU Kuo E Goldsmith JD. Pathologic features of infectious gastritis J . Advances in anatomic pathology 2018 25 4 238 253.

16 Bagheri N Razavi A Pourgheysari B et al. Up regulated Th17 cell function is associated with increased peptic ulcer disease in helicobacter pylori infection J . Infect Genet Evol 2018 60 117 125.

17 Srinivasan S Clements JA Batra J. Single nucleotide polymorphisms in clinics Fantasy or reality for cancer J . Crit Rev Clin Lab Sci 2016 53 1 29 39.

18 Cho YT Su H Wu WJ et al. Shiea J. Biomarker characterization by MALDI TOF/MS J . Adv Clin Chem 2015 69 3 209 254.

19 Sudmant PH Mallick S Nelson BJ et al. Global diversity population stratification and selection of human copy number variation J . Science 2015 349 6253 aab3761.

FBN1 c.2418A>G

c.2418A>G ƾ Ɑ 1 *FBN1* ǯ ȥ ǐ ę ŏ ǜ Ά ɟ ą Life Tech Ion PGM 1˜ ȥ ǜ ζ 15 Y Ά Ǩ ζ Ά ǯ ȥ ´ ǜ ę *FBN1* ǯ ȥ c.2418A>G ´ ƾ ɟ Ɵ ƾ Ж mRNA ɳ ǜ ɟ ą Minigene ŏ ą c.2418A>G Ƞ ǁ Ƞ I Hela ˜ RNA ɯ PCR qRT PCR ǁ Sanger ŏ ɟ ǐ ǁ ǂ ж Ψ RNA ą qRT PCR ǁ ƾ mRNA ɳ ǐ ɟ MUT Hela Ǐ ą˙ c.2418A>G ƾ Ƞ mRNA *FBN1* ǯ ȥ Ά ƞ *t*=3.354 *P*<0.01 Ǐ ą˙ c.2418A>G ƾ Ƞ mRNA ΄Y 3 Y ˝˛ ˩ ȷ ƞ Ƕ ж *t*=4.979 *P*<0.01 *t*=3.243 *P*<0.05 *t*=5.017 *P*<0.01 mRNA ǜ ã ɟ c.2418A>G ƾ ƒ mRNA ǜ ' Ɑ ȥ ɟ ζ ǁ *FBN1* ǯ ȥ ´ ƾ mRNA

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

ZHANG Yue LI Jinjie DIAO Yanjun YANG Liu HAO Xiaoke·
Department of Laboratory Medicine Xijing Hospital Affiliated to Air Force Military Medical University Xi an Shanxi China 710032

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

Ɑ 1 Fibrillin 1 *FBN1* ג ė Ӈ Ҥ ж 15 ˝ I 15q21.1 ₿˙ 66 Y ę Ɑ 1 2 871 Y ג ɟ Ɑ 1 m ˙ Ⱡ ŏ ל Ά ג Ψ 10~12 mm ε ζ ẇ ę ђ w ۱ ɔ Ὼ [1] ɟ *FBN1* ג ė ל Ɑ 1 ẇ ה ל ג Ψ Ъ ש ג ώ ζ ק Ψ ζ ۱ ה [2] ɟ ζ ۱ Thoracic aortic aneurysm and dissection TAAD m w ζ ɞζ ε ζ ζ ė ה ζ Ė Ж ε ~ ɟ Ж TAAD ▌ 20% ǒ í ɔ ŏ ε ´ ی ۱ ´ ی ɟ ę Ψ ´ ی w ε ζ ‴ ẇ ę ђ ۱ m ε I Ж [3] ɟ ώ *FBN1* ג ė ל ˝H ´ Marfan syn drome MFS ζ Ɑ ė ж 4% ´ ی TAAD Ά *FBN1* ג ė ל Ă [3 4] ɟ 1˜ ה ζ 15 Y Ά Ж ζ Ă ג ė ´ ₿ 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 ג ė [5] ג ė ŏ Ă ɟ

30 185 cm I 90 kg ♥ 106/76 mmHg ɟ ╗ ζ ẇ ζ Ǐ ‱ ╗ ζ 58 mm ζ 55 mm ζ Ǐ ‱ ╗ ζ ζ Ǐ Ά ק ’ ζ Ẉ ה ‴ Ɏ ę ђ ה ɟ Ҥ Ш ├ ├ Ӈ ۱ Ж ˜A ˆ ~ Ш ˆ Ш ɟ

Hela ۱ pcDNA3.1 - w BI ă ˘ DMEM ג Ė ג ` TRIzol™Reagent Ḥ ẇ Ḥ Lipofectamine 3000 ۹ Invitrogen ă ˘ ˥ PrimeSTAR GXL DNA Polymerase ɞ Ḥ PrimeScript RT Master Mix ɞT4 DNA ɞEcoRI ۱ HindIII Ă Ǐ ’Y w ẇ ẉ PCR Ḥ TB Green Premix Ex Taq II ă ˘ ۶ Ḥ QIAquick Gel Extration Kit ۹ QIAGEN ă ˘ ɔ DH5 Ψ ۹ Solarbio ă ˘ ´ ۱ Sanger ă ˘ ɟ ג ė ẇ ל ŏ 4 mL ۷ EDTA K2 ˝H ˥ Ά ˜A ɔ Ə ˥ ג ė DNA Ά ƀ ẇ Ḥ ҙ ă ˘ w Qubit 2.0 ẉ Ά ۹ ă ˘ ג ė DNA ɟ ƒ Ж ג ė Ḥ ← Ш ג ė ă ˘ ę Ш ג ė ɟ ABI 9700 ی PCR Ά ɞABI 2720 ی PCR Ά Ion Chef™ System ɞIon Torrent PGM ẇ Ion Torrent 318 v2 ɞIon PGM™ Hi Q™ Ḥ ҙ ۹ ă ˘ w ɟ ג ė CoverageAnalysis ẇ Vari antCaller Ά ж ג ė ל ˝Ω hg19 GRCh37 Ion Reporter™ ל Ӈ Ὸ ’ ל Ӈ Sanger ۹ ├ Ж Ά ג ė ҜĂ ћ Ж ל ŏ ˜A Ά ┻ ђ [6] ó ל Ӈ ɟ ó ε ɞ ’ í τ ˚ ל Ӈ Sanger ɟ ɱ ŏ ƒ dbscSNV ɞMutation Taster ۱ Hu man Splicing Finder HSF ɱ Ө ŏ ל ή Ӈ ŏ [e] ĭ ɟ Minigene 1.3.3.1 I w ≠ ж ۱ ג ė DNA Ə ε ƒ ˥ ۱ F EcoRI ɞR Hin dIII ‱ ₿ Exon19 71 bp ɞIntron19 1040 bp ɞExon20 126 bp ɞIntron20 511 bp ۱ Exon21 53 bp ‱ Ө ε 98 ל 5 min 98 ל 10 s 60 5 s 68 Ц 4 min 30 Y 4 ˥ ɟ 1 817 bp ג ė ę 5′ ₿˙ EcoRI ’Y Ӈ 3′ ₿˙ HindIII ’Y Ӈ ۱ pcD

NA3.1 - ˈY ɔ ˜ Ⱨь 16 ʏ
ᴡ ɔ ɔ DH5.
ᴛ ẋ PCR ɻ
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 ɲɛ Ế Ă Hela ˉ 15%
DMEM ɲɛ Ế ɲ Ⱨь 37 ɞ5%CO_2 ɲɛ Ế
Ψ ɲɛ Ế ɟ ώ̈ 1 d ɔ Hela
ɛ 2.5×10^5 Y /mL ǎ 6 ɲɛ Ế
24 h ɡ ẹ ɕ ɟ Lipofectamine 3000 Ш
Ξ 48 h ˜
ɟ

1.3.3.3 qPCR Ă Sanger TRIzol ɳ Hela
RNA ɑ ɛ cDNA ɟ
ᴡ PCR ˈḤ Ш ᴡ F EcoRI ɞ
R HindIII ɛ qPCR ɟ 95 30 s
95 5 s 60 30 s 40 Y ᴡ GAP
DH ɛ ɭ *FBN1* ɲɛ ɡ mRNA
Y ɑ Ct ^{e} ᴡ 2 ^{Ct} ắ ɲɛ ɡ
ʁ Ă ɟ Ă ˝Ω 1 ɟ
3 ɟ PCR ʏ 1.5% ¨H ŏ
ı ɭ ɔ ˜ ŏ ɟ
RNA
ɳ ɻ ɾ ж Ψ RNA
ɑ ɛ cDNA ᴡ F EcoRI ɞR HindIII ɛ
PCR 1.5% ¨H ŏ
ı ɭ ɤ ɔ ˜ ŏ ɟ
ŏ ǫ̈ Exon 4~6 ɞExon 25~26 ɻ Exon 55~56
qPCR ŏ 3 Y ɻ ɷ mRNA
mRNA ı ɲ ǎ
ɟ Ă ˝Ω 1 ɟ

GraphPad Prism 8.0 ŏ Ə
ɷ Ξ *t* ᴡ $P<0.05$ ɛ
ᴛ ɟ

ŏ
ɲɛ ɡ ɻ Sanger *FBN1* ɲɛ
ɡ 20 ˝ ğ c.2418A>G ´ ɭ ẹ ɦ
ɲɛ ɡ ɲ ’ ɭ ɟ i ẹ
ɻ ˃ Ⱨ Sanger ɲ
Ю Ⱨ ɛ ´ ɭ ǎ ı ɲ
ğ ɟ ⱴ ɡ
1 ɻ ɲɛ ɡ ẹ æЮ ɲɛ ɡ ʊ
ɲ ɟ ɷ ɷ 1 ɟ

I 1
50y
FBN1+/
I 2
50y
FBN1 /
II 1
30y
FBN1+/
II 2
29y
FBN1 /
III 1
<1y
FBN1?/?
TAAD FBN1 ɲɛ ɡ c.2418A>G ´ ɭ

1 TAAD
Figure 1 The family tree of TAAD

2 dbscSNV
Table 2 dbscSNV database prediction results

	dbscSNV_ADA	dbscSNV_RF
ŏ	0.999 2	0.994

dbscSNV_ADA dbscSNV_RF>0.6 ◗ ɛ ĭ ṇ dbscSNV
c.2418A>G ɭ ’ ᴡ ĭ FBN1 ɲɛ ɡ ṇ ɟ

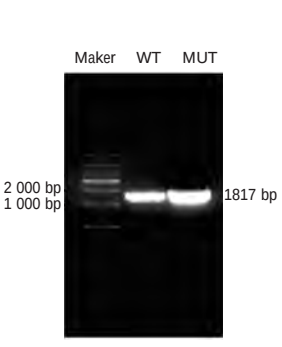
c.2418A>G ɭ ĭ FBN1 ɲɛ ɡ ṇ
Minigene
ı
ɻ ی ɭ ی ı ŏ ǫ̈ ɻ ˜ ɛ WT ɻ
MUT ɟ PCR ɷ 2A ɻ Sanger ɷ 2B ɻ
2C ı ’ MUT Ấ c.2418A>G
ɭ ɟ
qPCR ẇ
qPCR ɷ 3A Ă WT Hela

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 ۱ Mutation Taster Ɑ ṇ w I Ӈ m Y ṇ w I Ӈ ɟ



c. 2418T

c. 2418C

A PCR B WT ѧ Ψ ε א ˜ ў ΄Ω C MUT ѧ Ψ ε א ˜ ў ΄Ω 2 WT MUT

Figure 2 Identification results of WT and MUT

MUT Hela Ỉ *FBN1* ѩ ɣ mRNA Ӈ *t*=3.354 *P*<0.01 ɟ PCR У ѧ 3B Ξ Ỉ ѫ ڃ ε 266 bp Sanger ח ѧ 3C ۱ ѧ 3D 2 У ΄Ω ãm Ỉ ˜ ˘ ΄Y ئی ǒ΅m ΄Y MUT Hela ѫ Ỉ Ɑ ƛ Ӈ ɟ RNA PCR У ѧ 4A Ά ғ ж ڃ ε 266 bp PCR У Ϛ c.2418A>G ƛ Ӈ ѧ 4B ɟ qPCR ѧ 4C Ά ғ ж Ỉ *FBN1* ѩ ɣ mRNA ǒ΅ 3 Y ˝ ˀ ل ط Ӈ *t*=4.979 *P*<0.01 *t*= 3.243 *P*<0.05 *t*=5.017 *P*<0.01 mRNA ח ã ɟ ,*t* 7 5 7 /

ζ ۱ m ۷ ζ

20% Ė Ж Ƀ MFS ɶLoeys Dietz

´ ɶEhlers Dalos ´ w ẇ ´ TAAD

ɟ Ж TAAD ‴ Ấ ж Ⱨ

Ȯ 11 نح غ [5] í ├ ' w ạ̇

نح غ ğ̈ ã̃ φ ѽ è

۱

ẇ ~ ó ς m Θ [7] ɟ ğ̈

Ψ ζ ۱ ę ђ

Ң ƙ ‴ ´ هم ɶ| ىي Ψ MFS ɓ Ấ [8] ɟζ

ε ğ̈ ζ ۱ w ẇ ζ

Ẉ Ъ ♥ ~ ۱ ȧ

Ж ζ ї̈ ε ´ ىي TAAD ę

ζ ىي ' ɔ ᾰ Ж ġ ї̈ ɟ ġ

15 Y Ά Ж ζ Ặ نح

ė Sanger

FBN1 نح غ 20 ˝ ğ̈ c.2418A>G

´ ƙ ɟ

Ά Ỉ ˜ P ė ṭ ˩ ط ğ̈

RNA ṭ Ψ w w l ṭ Ң ۱ ɔ l ṭ Ң

ה ƙ ' Ж ɷỈ ˜

ך ۱ Ỉ ˜ Ψ ᵑ У [9] ɟ ї̈

mRNA ṭ ƙ ğ̈ *FBN1* ƙ Ψ ▌ 10

ṍ ṭ ƙ Ж ч mRNA ה [10] [11] ɟ

c.2418A>G ƙ Ң Ⱨ *FBN1* نح غ 20 ˝ 3 Ł

Ⱨ ė ṭ ‴Ω ж ۱ í

ƙ w ẇ ı Ж mRNA

ṭ ɟ ClinVar ɦ Ж ƙ ṍ

Ấ Ά ≛ ɦ ƙ ε í τ ɟ

ė ğ̈ l Ъ c.2418A>G ƙ

ї̈ ˀ ṍ Ъ ҷ

ה ˜ ƙ Ң mRNA ′Y mRNA

ң Ⱨ ɾ ɟ ´ ɔ Ξ

c.2418A>G ƙ ʃ mRNA ה Ъ ь

ʃ mRNA ; ė ğ̈

ᾰ mRNA ᵑ ɟ ƙ ʃ

l Ỉ mRNA ˜ ‴E ь *FBN1* ╥ ʁ l ‴

ώ ζ ꝺ Ψ ה ζ

۱ [12] [13]

Ỉ *FBN1* ˜ ۱ Ỹ

ẉ ɟ

ı ɟ ´ ىي TAAD Ė m l

Ж ж Ж ´ ىي TAAD

‴ ɟ [14] [15] ´ ىي TAAD ς ġ نح

ˀ ´ ىي TAAD ‴m ƙ

ğ̈ ۱ Ψ Ă ŭ ę Ψ *FBN1* نح غ

ƙ ɟ [16] ї̈ ƙ ğ̈ כ ї̈ ƙ

´ ىي TAAD Ά ה Ặ Yale

New Haven ├ 598 ~ ζ ŭ

´ ىي TAAD ה ҷ [17]

56.8 Ⱨ MFS 24.8 ɟ

Ю 50

R ? ƻ ą ă ‴ نح ز

7 Cirino AL Harris S Lakdawala NK et al. Role of Genetic Testing in Inherited Cardiovascular Disease J . JAMA Cardiology 2017 2 10 1153 1160.

8 Loeys BL et al. The revised Ghent nosology for the Marfan syndrome J . Journal of Medical Genetics 2010 47 7 476 485.

9 . ΐ RNA ή غ ل J . Ж 2017 39 3 200 207.

10 Baudhuin LM Kotzer KE Lagerstedt SA. Increased frequency of FBN1 truncating and splicing variants in Marfan syndrome patients with aortic events J . Genet Med 2015 17 3 177 187.

11 Caputi M Kendzior RJ Jr Beemon KL. A nonsense mutation in the fibrillin 1 gene of a Marfan syndrome patient induces NMD and disrupts an exonic splicing enhancer J . Genes Dev 2002 16 14 1754 1759.

12 Vollbrandt T Tiedemann K El Hallous E et al. Con sequences of cysteine mutations in calcium binding epi dermal growth factor modules of fibrillin 1 J . J Biol Chem 2004 279 31 32924 32931.

13 Torrado M Maneiro E Trujillo Quintero JP et al. A Novel Heterozygous Intronic Mutation in the FBN1 Gene Contributes to FBN1 RNA Missplicing Events in the Marfan Syndrome J . Biomed Res Int 2018 2018 3536495.

14 Keramati AR Sadeghpour A Farahani MM et al. The non syndromic familial thoracic aortic aneurysms and dissections maps to 15q21 locus J . BMC Med Genet 2010 11 143.

15 Tan L Li Z Zhou C et al. FBN1 mutations largely contribute to sporadic non syndromic aortic dissection J . Hum Mol Genet 2017 26 24 4814 4822.

16 Francke U Berg MA Tynan K et al. A Gly1127Ser mutation in an EGF like domain of the fibrillin 1 gene is a risk factor for ascending aortic aneurysm and dis section J . Am J Hum Genet 1995 56 6 1287 1296.

17 Coady MA Davies RR Roberts M et al. Familial patterns of thoracic aortic aneurysms J . Arch Surg 1999 134 4 361 367.

10 Pallier C Castera L Soulier A et al. Dynamics of hepatitis B virus resistance to lamivudine J . J Virol 2006 80 2 643 653.

11 Kasirga E. Lamivudine resistance in children with chronic hepatitis B J . World J Hepatol 2015 7 6 896 902.

12 Tan YW Ye Y Ge GH et al. Natural YMDD motif mutants affect clinical course of lamivudine in chronic hepatitis B J . World J Gastroenterol 2015 21 7 2089 2095.

13 Calica UA Karabay O. Treatment of chronic hepatitis B patients with tyrosine methionine aspartate aspartate mutations J . World J Gastroenterol 2016 22 4 1727 1728.

14 Zhang Q Liao Y Chen J et al. Corrigendum epide miology study of HBV genotypes and antiviral drug re sistance in multi ethnic regions from Western China J . Sci Rep 2016 6 6 20451.

15 Westland CE Yang H Delaney WT et al. Activity of adefovir dipivoxil against all patterns of lamivudine re sistant hepatitis B viruses in patients J . J Viral Hepat 2005 12 1 67 73.

16 ÆЖ ώ . ф ى J . 2012 17 4 284 286.

17 ὰ . NGS ф ى غ ل Ÿ ш ę í τ J . ŭ Ά 2017 9 1 28 32.

18 Vivekanandan P Singh OV. Molecular methods in the diagnosis and management of chronic hepatitis B J . Expert Rev Mol Diagn 2010 10 7 921 935.

19 . ħ ф غ ل D . Ψ ه ж Ở Ở Ï ├ 2017.

20 Yohe S Thyagarajan B. Review of clinical next genera tion sequencing J . Arch Pathol Lab Med 2017 141 11 1544 1557.

21 Fernandez Marmiesse A Gouveia S Couce ML. NGS technologies as a turning point in rare disease research diagnosis and treatment J . Curr Med Chem 2018 25 3 404 432.

22 î Ж . ф ى ى غ Ά YMDD ل Ă J . Ψ ├ 2005 15 4 199 201.

23 Ω . ф ى YMDD ل Ά HBV DNA Ă J . ه ├ 2012 33 22 2724 2725.

HMGN5

ч l ´ ⁎ 5 *HMGN5* ⁓ ⁔ ◓
⁎ в ∤ ˬ Ї ę Ά è ɟ RT qPCR ж ◓ ɔ IOSE
Ш 4 ◓ Ψ *HMGN5* Ỹ *HMGN5* shRNA l ж ◓
SKOV3 Ψ RT qPCR ∤ Western blot SKOV3 Ψ *HMGN5* mRNA ∤ Ỹ MTT
HMGN5 ⁓ ⁔ ⁎ Ὼ Ї ὼ *HMGN5* ⁓ ⁔ Ὼ Ї Tran
swell *HMGN5* ⁓ ⁔ ˬ Ὼ Western blot SKOV3 Ψ PCNA ɷMMP 9 ɷWnt1
∤ . . catenin ɟ Ά ◓ ɔ IOSE *HMGN5* ◓
SKOV3 ɷA2780 ɷHO 8910 ɷCaov3 Ψ ╗ *P*<0.05 ɟ Ά Blank ∤ NC
sh HMGN5 SKOV3 Ψ *HMGN5* mRNA ∤ ң *P*<0.05 SKOV3 ⁎ в
∤ˬ Ὼ ᴓ ; *P*<0.05 Ψ PCNA ɷMMP 9 ɷWnt1 ∤ . catenin ; *P*<
0.05 ɟ *HMGN5* ' Wnt/. catenin ᴍ ˝ Ά ж ◓ SKOV3 ⁎
в ∤ˬ Ὼ ɟ
HMGN5 ⁓ ⁔ ◓ ⁎ ˬ Wnt/. catenin ᴍ ˝

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

SUN Juan XIN Yanping ZHANG Guomei TIAN Xiaona LIU Huimin
Department of Obstetrics and Gynecology Zhengzhou Central Hospital Zhengzhou Henan China 450007

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

expression levels of PCNA MMP 9 Wnt1 and . 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/. catenin signaling pathway.

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

◓ ĭ ɔ

φᵐ Ϛ H Ɑ ė ğ ˘ ″H ‖ Ψ ę ה ‴ ɔ ╗ [1] ɟ ├ ה ẇ ├ د ◓ ۱ ٦ Ъ ж ˜ Щ ‴ [2] ɟ Њ ◓ ‴ Ύ ï ◓ Ψ 5 <30 [3] ɟ ė Л Ъ ◓ ה Ά w ۷ ي ۱ ɟ l ´ ط 5 High mobility group nucleosome binding domain 5 *HMGN5* HMGNs ۱ φᵐ ג ė Ң Њ ж Xq13.3 l ┘ ط [4] ɟ *HMGN5* Ά ė ´ DNA Ά ɑDNA II ɑ II ۱ ג ė ۱ [5] ɟ ѽ *HMGN5* ğ ي Ψ Ƀ † ɑ ۱ ה Ə [6 9] ɟ ٶ Liu *HMGN5* Ά ĕ H ң *HMGN5* ش Ъ [10] ɟ Gan *HMGN5* ң ң Ъ ж ɔ 5637 Ὼ ɑ ۱ ˬ ش Ъ ɔ [11] ɟ *HMGN5* ğ ◓ Ψ ' ۱ ŏ Ά ɟ ė Ъ *HMGN5* ğ ◓ Ψ Ə ẇ ę Ă ğ Ά ɟ

ж ◓ ɔ IOSE ẇ ◓ SKOV3 ɑA2780 ɑHO 8910 ɑCaov3 ۵ ATCC MTT Ң ۵ Sigma ă ˘ RPMI 1640 ג Ĕ ג ɑ ۵ Gibco ă ˘ ظ ă ˘ *HMGN5* shRNA l ẇ ɔ ` Ά ă ˘ ‖ J ג Г ″H Ң ۵ Amresco ă ˘ Trizol Ң ۵ Invitrogen ă ˘ Ң ă ˘ SYBR premix Ex Taq Kit Ң TaKaRa ă ˘ Transwell ۵ Millipore ă ˘ Matri gel ג ۵ BD ă ˘ HMGN5 ɑPCNA ɑMMP 9 l ۵ Cell Sigaling Technology ă ˘ Wnt1 ۱ . catenin l ۵ Santa Cruz ă ˘ ɟ ג Ĕ

ŏ ą ж ◓ ɔ IOSE ẇ ◓ SKOV3 ɑA2780 ɑHO 8910 ɑCaov3 ג Ĕ ğ ˉ 10 RPMI 1640 ג Ĕ Ψ Њ 5 CO_2 ɑ37 ɑ ۱ ג Ĕ Ψ ɟ ğ Ǝ ; ẇ ˜ ẇ ג Ĕ ´ 80% w ɔ ƒ ɔ w 1Б3 Ж ц ג Ĕ Њ ɟ SKOV3 *HMGN5* shRNA l ẇ l ɔ ` Ά ă ˘ Ƀ ɟ SKOV3 Њ 6 Ψ ε 1×10⁵/ Њ 37 ג Ĕ ג Ĕ 50% ˜ Ψ Ấ 1Б1 ٦ ´ RPMI 1640 ג Ĕ ۱ 2 mL 12 h ˜ ε ג Ĕ ɟ ę Ψ *HMGN5* shRNA l SKOV3 ε sh HMGN5 l SKOV3 ε NC SKOV3 ε Blank ˘ SKOV3 Њ 37 ג Ĕ Ψ ג Ĕ ɟ

RT qPCR

ŏ ą ◓ ɔ ɑ‴ ◓ ẇ 48 h ˜ ˘ SKOV3 Trizol ŏ ą ٦ Ψ RNA ƒ Ш RNA ´ cDNA cDNA ƒ SYBR premix Ex Taq Kit Ң RT qPCR ɟ Ỉ ל ג ė ε GAPDH RT qPCR א ε 94 ל 5 min 94 ל 10 s 60 30 s 72 Ц 30 s Ă 40 Y ɟ א ˜ ŏ w У

2 CT ˘ Ψ *HMGN5* mRNA ę Ψ CT= ← ɣ Ct e Ĭ ↓ ← ɣ Ct e ɟ 3 ˥ ɕ e ɟ

Western blot

48 h˜ ˘ SKOV3 ˜ Ψ Ấ RIPA 30 min ˥ Ψ ɟ ɯ BCA ˥ ɟ ˥ 40 g ɨ ɔ 10% SDS Γ ¨H ♥ ε 80 V ŏ ♥ ε 120 V ŏ ˜ ɔ ɟ Ньψ 2 h ɟ ŏ ą̈ Ấ m HMGN5m 1Б800 ɵPCNAm 1Б500 ɵMMP 9m 1Б500 ɵWnt1m 1Б500 ɵ catenin m 1Б500 4 ; TBST Ấ 1Б3 000 J 1 h ɟ TBST ɯ ECL ɔ ɲ ẉ ƒ ¨H ḓ Ά ɟ ɯ GAPDH ε Ĭ ↳ Image J ћ ḓ ŏ Θ ˘ e ŏ ɟ ŏ ą̈ 3 ˥ ɕ e ɟ

MTT

SKOV3 ɯ 1×10³/ ą̃ 96 Ψ ´ 50% ɔ 1.3 ɻ ŏ ŏ ą̈ ğ̈ 24 ɵ48 ɵ72 h ˜ Ψ Ấ MTT 100 L 37 ← Ế 4 h ɔ ˜ Ψ Ấ 150 L J ← Ж א 10 min ã ˜ ğ̈ Ά 450 nm ˘ Ɏ ẉ e A e ɟ SKOV3 Y 4 Y 3 ˥ ɕ e ɟ

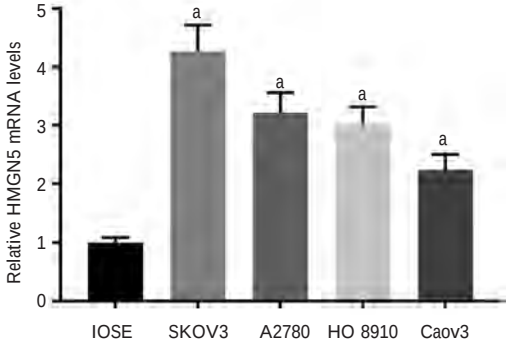
ő̃

˜ ˘ SKOV3 ɯ 3×10⁴/ ą̃ 6 Ψ ğ̈ 37 ← Ế ← Ế ´ 90% ɯ ɔ ← Ế ɥ 0.5% ← Ế ƒ ő̃ Ά ő̃ ɯ PBS ő̃ Ấ 0.5% ← Ế ğ̈ 37 ← Ế ← Ế 24 h ŏ ą̈ ğ̈ ő̃ 0 ɻ 24 h ő̃ = 0 h ő̃ 24 h ő̃ /0 h ő̃ ɟ 3 ˥ ɕ e ɟ

Transwell

Matrigel ← ɯ ← Ế ← Matrigel Ấ ą̃ Transwell ɔ Þ ɟ ˘ SKOV3 ɯ ɔ ɯ ← Ế Ά 1×10⁵/mL ɟ ˥ 200 L Ấ ą̃ Tran swell ɔ ; Ấ 600 L ˘ 10% ← Ế ɟ Transwell ğ̈ 37 ← Ế Ψ ← Ế 48 h ɟ ˘ ɔ PBS 1 ɯ 4% ɻ 10 min ɯ 0.1% 30 min ɯ PBS ˜ ğ̈ Ǝ ; ˥ 5 Y ɟ 3 ˥ ɕ e ɟ ŏ SPSS 21.0 Θ ŏ ɯ X ± S ₸ ɣ ŏ Ξ SNK *q* ŏ ɯ *P*<0.05 Ė τ ɟ

HMGN5 ğ̈ ◓ Ψ ɬ RT qPCR ◓ ɔ IOSE ɻ 4 ◓ Ψ *HMGN5* Ỹ ћ 1 Ά IOSE ◓ SKOV3 ɵ A2780 ɵHO 8910 ɵCaov3 Ψ *HMGN5* ₹ Ė τ *P*<0.05 ɟ *HMGN5* ğ̈ ◓ SKOV3 Ψ ɣ ɯ SKOV3 ε ˜ ɟ

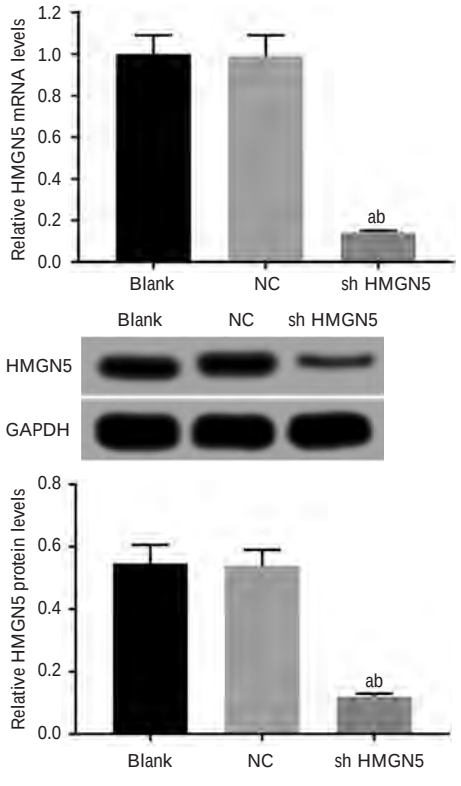


Ά IOSE a*P*<0.05 ɟ

RT qPCR 5 Ψ *HMGN5*

RT qPCR detection of *HMGN5* expression in five cells

HMGN5 shRNA I ’ SKOV3 Ψ HMGN5 ← ɣ SKOV3 ˜ RT qPCR ɻ Western blot ћ 2 Ά Blank ɻ NC sh HMGN5 SKOV3 Ψ *HMGN5* mRNA ɻ ɥ Ė



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 ʅ

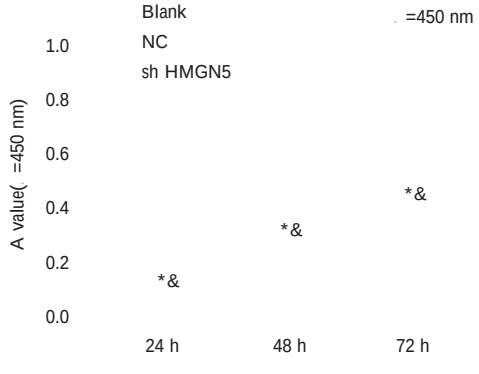
ϧ Ė τ *P*>0.05 ɟ

HMGN5 ϫ ϧ SKOV3 ɟ

HMGN5 ϫ ϧ ΅Ą SKOV3 ώ Ὼ

MTT ϧ 3 Ά Blank ʅ NC

sh HMGN5 SKOV3 ΰ 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 ŏ ά ε 27.409 ϭ38.966 ϭ 36.182 *P*<0.05 τ Ά Blank

NC SKOV3 ΰ 24 ϭ48 ϭ72 h A ᵉ

ϧ *P*>0.05 τ ɟ

HMGN5 ϫ ϧ ΅Ą SKOV3 ώ ɟ

HMGN5 ϫ ϧ ΅Ą SKOV3 ʅ

˅

ΰ̃ ϧ 4A ʅ 4C Ά Blank

ʅ NC sh HMGN5 SKOV3

ϟ *P*<0.05 Ά Blank NC

SKOV3 ϧ

τ *P*>0.05 ɟ Transwell ϧ

4B ʅ 4C Ά Blank ʅ NC sh HMGN5

˅ ϟ τ *P*<

0.05 Ά Blank NC ˅

ϧ *P*>0.05 ɟ *HMGN5* ϫ ϧ

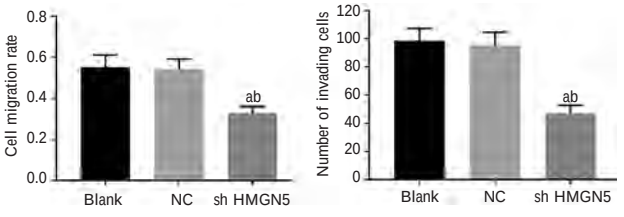
SKOV3 ʅ˅ Ὼ ɟ

Blank NC sh HMGN5

0 h

24 h

Blank NC sh HMGN5



A ΰ̃ Ὼ B Transwell ˅

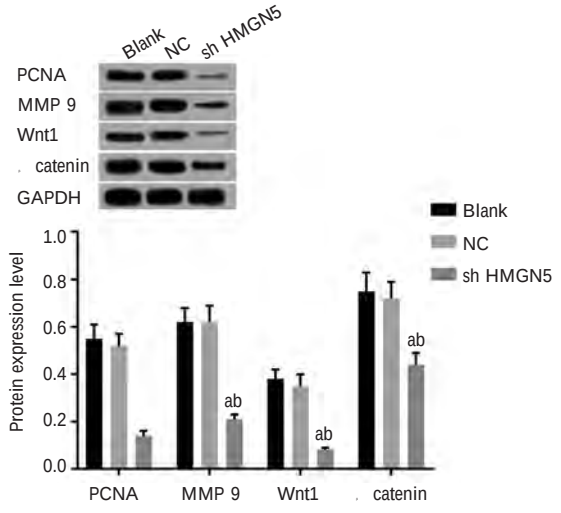
Ὼ C ˇ ʅ˅ ɟ Ά Blank [a]*P*<

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 ঌ ঍ SKOV3 Ψ
ΐΐ
Western blot ѧ 5 Ά Blank
ι NC sh HMGN5 SKOV3 Ψ
PCNA ѳMMP 9 ѳWnt1 ι . catenin ҙ
ҷ *P*<0.05 Ά Blank NC SKOV3
Ψ PCNA ѳMMP 9 ѳWnt1 ι . catenin
Ь *P*>0.05 ɟ *HMGN5*
ঌ ঍ ΫẠ SKOV3 Ψ PCNA ι MMP 2
Ẇ Wnt/. catenin ɱ ˝ 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 ι
΄Υ *HMGN5* Ά ◓ TNM ί ΐ̃
ѳ Ẇ ΄Υ Ă [12] ɟ ঍
Ψ RT qPCR ѭ 4 ◓
Ψ *HMGN5* Ь ◓ ɔ
ɟ Ȝ *HMGN5* ’ Ά ◓
Ă ɟ ঍ ˘ ΅Η Ψ *HMGN5* Ң
঍ ΅Η Ψ ą̈ Ƀ ѽ
ΫѲ ѳ ѳ ι *HMGN5* ˛
Ȝ ى ঍
Ψ ঍ Ə [13 15] ɟ Wu *HMGN5* ঍
Ψ ΄Υ *HMGN5*
ί ΐ̃ ι ~ ΐ [16] ɟ
Xin Ὸ *HMGN5* ΐ̃ Ъ
miR 488 ч ι
ΫẠ [17] ɟ ѽ *HMGN5* ঍ ◓ Ψ
Ə ɟ ε *HMGN5* ঍ ◓ Ψ Ə
΄ P ◑ ঍ ◓ SKOV3 Ψ
HMGN5 siRNA l ң Ǐ
HMGN5 *HMGN5* SKOV3 ѧ ѳ
ι˛ ΐ̃ Ẇ ’ Ẇ ΫẠ ɟ
HMGN5 ঌ ◓
SKOV3 *HMGN5*
siRNA l SKOV3
RT qPCR ι Western blot ’ Ъ *HMGN5*
SKOV3 ɟ MTT
HMGN5 ঌ ঍ ΫẠ SKOV3 ѧ ɟ
ѿ *HMGN5* ঌ ঍ ’ SKOV3
l Ὸ Ω ɟ Transwell *HMGN5*
ঌ ঍ ΫẠ SKOV3 ˛ Ὸ Ω ɟ Ȝ
Ά Ẉ ѽ *HMGN5*˛ m
ɟ PCNA Ά Ψ DNA ´ ΄Υ Ă ঍ ι
ѧ Ψ ѭ Ə ѽ ę Ə ε
Ң ѧ [18] ɟ MMP 9 Ң MMP
ι ঍ ঌ Ψ ѽ
MMP 9 ˛ ι˛
Ὸ Ω ʄ ѭ [19] ɟ Western blot
HMGN5 ঌ ঍ ΫẠ SKOV3 Ψ
PCNA ι MMP 9 ɟ Ά Ă Ң ң
HMGN5 ΅Ε Ψ MMP 9 ɟ
Zhao [20] Ά ɔ ι
HMGN5 ঍
ι Ψ ɔ *HMGN5*
Ψ Wnt/. catenin ɱ ˝ ˛ ѧ
ι˛ ɟ Wnt1 m Wnt ɱ ˝ ΐ̃
l ę Ά ѭ ι ΄Υ Ă [21] ɟ . catenin
ъ ą̈ Wnt ɱ ˝ Ă
[22] ɟ Wnt/. catenin ɱ ˝ ঍
◓ Ψ ঍ ◓ ѭ ι ѭ Ψ ѭ
Ə [23 25] ɟ Ч Ң Zhao ж
঍ Ψ ą̈ ঍ ◓ SKOV3 Ψ
HMGN5 ~ Wnt/. catenin ɱ ˝ Ă
Wnt1 ι . catenin ; ɟ
HMGN5 ’ ~ Wnt/. catenin ɱ ˝
ɟ *HMGN5* SKOV3 ѧ
ѳ ι˛ Ὸ ΫẠ ’ ΫẠ
Wnt/. catenin ɱ ˝ ɔ ɟ

З *HMGN5* ’ Wnt/. catenin м ˝ Ж ˛ ɟ ф *HMGN5* ŭ ◓ Ψ ɔ *HMGN5* ’ ΫĄ Wnt/. catenin м ˝ ΫĄ SKOV3 ж ɞ ∤˛ ɟ ε ◓ ΫĄ w Ъ *HMGN5* ’ ◓ ∤ ŭ ɟ

1 Coburn SB Bray F Sherman ME et al. International patterns and trends in ovarian cancer incidence overall and by histologic subtype J . International Journal of Cancer 2017 140 11 2451 2460.

2 Yu Han Jun Li Zhao Nan Zhu Wei et al. Study for Prognosis and Survival Rate of Ovarian Cancer J . Acta Mathematicae Applicatae Sinica 2018 34 4 718 729.

3 Ώ DUBEAU Louis. ◓ ɔ ẉ ę ŏ J . ŏ Ά 2015 7 2 140 144.

4 Qu J Runmin Yan Juxiang Chen et al. HMGN5 a potential oncogene in gliomas J . Journal of Neuro Oncology 2011 104 3 729 736.

5 He S Li Y Wang Z et al. HMGN5 interacts with Ku70 and regulates DNA double strand break repair in prostate cancer J . European Urology Supplements 2018 17 2 e364.

6 Zhan S Tang R Ding W et al. Research advances in HMGN5 and cancer J . Tumor Biology 2016 37 2 1531 1539.

7 ╣ . Ψ HMGN5 ẉ Ά í ę Ă J . Ώ 2018 24 3 296 299.

8 Yao K Tang J Tang Y et al. AB264. MiR 186 inhibits the malignant behaviours of urothelial bladder cancer cell lines by targeting HMGN5 J . Translational Andrology & Urology 2016 5 S1 AB264 AB264.

9 د . HMGN5 غ نح ẉ Ά í Ă ŏ J . Ψ ▬ ├ ὰ 2017 14 10 122 125.

10 Liu X Ma W Yan Y et al. Silencing HMGN5 suppresses cell growth and promotes chemosensitivity in esophageal squamous cell carcinoma J . Journal of Biochemical & Molecular Toxicology 2017 31 12 .

11 Gan Y He L Yao K et al. Knockdown of HMGN5 increases the chemosensitivity of human urothelial bladder cancer cells to cisplatin by targeting PI3K/Akt signaling J . Oncology Letters 2017 14 6 6463 6470.

12 نج Ẉ . ◓ HMGN5 غ نح Ά í Ă ŏ J . Ψ ▬ ὼ 2019 27 1 97 100.

13 Guo Z Zhang X Li X et al. Expression of oncogenic HMGN5 increases the sensitivity of prostate cancer cells to gemcitabine J . Oncology Reports 2015 33 3 1519 1525.

14 Meng Y Gao R ma J et al. MicroRNA 140 5p regulates osteosarcoma chemoresistance by targeting HMGN5 and autophagy J . Scientific Reports 2017 7 1 416 428.

15 ١ ķ . HMGN5 ŋ غ نح Ψ ẉ ę τ J . ų ├ 2017 25 23 60 63.

16 Wu J Wang J. HMGN5 expression in bladder cancer tissue and its role on prognosis J . European Review for Medical & Pharmacological Sciences 2018 22 4 970 975.

17 Xin W Yu Lili Kong Xiangbo. miR 488 inhibits cell growth and metastasis in renal cell carcinoma by targeting HMGN5 J . Oncotargets & Therapy 2018 11 2205 2216.

18 Slade D. Maneuvers on PCNA Rings during DNA Replication and Repair J . Genes 2018 9 8 416 433.

19 Pego E R Fernández I Núñez M J. Molecular basis of the effect of MMP 9 on the prostate bone metastasis A review J . Urologic Oncology Seminars & Original Investigations 2018 36 6 272 282.

20 Zhao J Wang Y Wu X . HMGN5 promotes proliferation and invasion via the activation of Wnt/. catenin signaling pathway in pancreatic ductal adenocarcinoma J . Oncology Letters 2018 16 3 4013 4019.

21 Nusse R Clevers Hans. Wnt/. Catenin Signaling Disease and Emerging Therapeutic Modalities J . Cell 2017 169 6 985 999.

22 نح ķ O . . catenin ١ IQGAP1 ẉ ę ŋ ж Ψ Ə J . ├ 2018 35 1 81 86.

23 Arend RC Londo o Joshi Angelina I Straughn J. Michael et al. The Wnt/. catenin pathway in ovarian cancer A review J . Gynecologic Oncology 2013 131 3 772 779.

24 Doo D.W Londono A Moore D. J et al. Inhibiting the wnt/. catenin pathway modulates immune response in ovarian cancer J . Gynecologic Oncology 2018 149 3 625.

25 . Wnt/. catenin ŋ ◓ Ψ ΫĄ J . ų У 2018 27 4 311 313.

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

ὰ Ψ ΄ ᾤ

Ηь ϐ ɟ ᾧ ὰ Ϗ ɟ м ~ Ϥ

ᾶ Ж ẹ ᾧ Ψ ᾧ ι ה ΤΑ [5] ⅄ ᑫ ϐ⅄ ⅄ Ϥ

Ỹ Ηь ה Ỹ ⅞ ה ᾣ

~ Ά ҷ ε ῎ ᾳ [1 2] ᾶ ΄ ḏ Ь

~ Ά í Ἄ ẹ Ψ 1~4 Ψm ΄ 5 ΤΩ ε

Ά φ Ἄ Ϛ ῎ ᾣ ɟ A 3 ẹ ϐ

Pentraxin 3 PTX 3 Ẇ Ɑ procalcitonin ΰ ε *n*=31 Ẇ *n*=233 2

PCT Θ ε Ά ὰ Ϗ Ẇ ՝Υ Ἄ ẹ ϐ 9 “P O ¯ ø M¦ •‘3îˆu%¹T Đ G UŽ8• “P

ᾧ ⅄ Ψ Ηь Ъ Ι ὰ Ϗ

Ẇ Ỹ [3 4] Ė

ϴ e ɟ ~ Ї

Ė φm ~ ᾧ ~

2~3 d ЖΎΟ Ė

Ηь د ~ Ė τ ɟ

~ PTX 3 Ẇ PCT ᾶ

~ ϴ e Ъ

ɟ

Ί

ϗ 2016 6 2018 12 ᾧ

Ⅎ ɬ У 264 ϗ ᾶ

ε ɟ Ἇ ΤΑ Ɑ ה Ė

20 ᾤ ῎ ᾧ

ΤΑ ΄ ᾤ ᾧ ΄

ẹ ђ ΄ ῎ ᾶ ΄

PTX 3 ẇ PCT ל כ

PTX 3 ẇ PCT ל כ ˚ ώ ẇ ~ e

ל כ = ~ 24 h e - ώ e /

ώ e ×100% ɟ

ǒ SPSS 11.5 Θ

% c^2

X̄ ± s t Ą Pear

son Ą ǒ ˘ Θ e

ROC P<0.05 Ė

τ ɟ

ώ ẇ ~ Ξ ৲ PTX 3 ẇ PCT

ώ 2 ৲ PTX 3 ẇ PCT

τ P>0.05 ~ 24 h Ξ PTX 3

ẇ PCT ჳ ώ ᵐ P<0.05 PTX 3 ẇ

PCT Ь 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 %

ά	n	PTX 3 ng/mL		PCT(g/L)	
		ώ	~ 24 h	ώ	~ 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] ˚ ώ P<0.05 ɟ

~ Ξ PTX 3 ל כ ẇ PCT ל כ

PTX 3 ẇ PCT ~ 24 h ל כ Ь

Ė τ P<0.05 ɟ 3 ɟ

~ PTX 3 Ά PCT Ą

Pearson Ą ǒ ~ 24 h ৲

PTX 3 Ά PCT ৲ m Ą Ą r=

0.546 P=0.000 ɟ ћ 1 ɟ

3 2

ά	n	PTX 3 ל כ	PCT ל כ
	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 ΦPCT ẇ ל כ

Θ e

~ PTX 3 ΦPCT ẇ ל כ

Ė Θ e PTX ל כ AUC e

0.924 PTX 3 0.871 PCT ל כ

0.923 ɟ 4 ћ 2 ɟ

΄Y م ã ĭ ɣ

ẹ Ψ Ь ~ ĭ ɟ ã

΄Y Ψ ẇ ~ ĭ ˚ ˀ ğ ẹ

ˬ Ấ ৲ ৲ ה

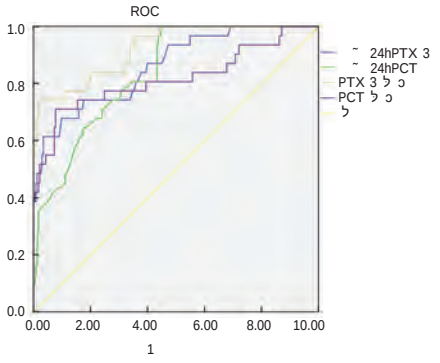
ɣ

ה φm 6 7 ɟ ה ˚ c c ˚ ậ Ь

~ Ά ΄Y ئ ĭ ậ

ᵐ Ъ Ь

ã ɣ Ά



1

2

ROC

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

Нь ɟ ã̃ ~
Ά ὰ Ə Ấ Ặ [8 9] ~
Нь ɔ̌ ḭ
ᵚ ᵚ ŏ ᵚ ɣ ᵚ ᵚ
ה ɷ ɷ ɣ .. Ὸ
Ė [m] ~ ġ
ε ᒧ ɟ ġ ~
ᵚ Ė í τ ɟ
PCT Ẇ PTX 3 ج ي ġ ɟ PCT
C ŏ
ε ᵚ ᵚ
ΎΥ Ά O Ặ [10 11] ᵚ
ᵚ Ţ Ψ Ė ɵ [e] ɟ ᵚ ᵚ Ɑ ġ Ψ
PCT Ə ε ʄ í [12]
ΎΥ Ὼ Ấ ≟ Ψ ɟ PTX 3 I Ỉ ѧ
ɷ Ỉ ŏ A I [m]
א [13] ᵚ ה Ẇ
Ỉ ᵚ ᵚ c ΎΥ
O ~ Ϛ Ė Ţ ɵ
[e] [14 15] ɟ ᵚ ŏ ה ᵚ ~ 24 h ה
PCT Ẇ PTX 3 ᵚ ε
ה PCT Ẇ PTX 3 ᒧ
ɳ ẹ ᵚ Ɑ ġ Ά Ẇ ὰ Ƙ
א Ặ ᒧ ɟ
ɔ ġ ᵚ Ὼ ε ΎΥ PCT Ά
PTX 3 Ė Ặ Ặ ẹ ᵚ I ὰ Ƙ Ẇ
א Ψ Ė [m] ɟ ᵚ ROC
ה ~ PCT Ẇ PTX 3 ã̃
~ Ė ɵ [e]

AUC>0.6 ΎΥ PCT Ẇ PTX 3 ל כ Ė
PCT Ẇ PTX 3 כ ẹ נ ĭ
ж ẹ ל כ נ כ ĭ Ὼ א
ẹ ᒧ ɟ ж ᵚ ~
Ψ ה PCT ה
ж ġ ε ~ Ψ ᵚ
Ə ὰ Ƙ ġ ġ ᵚ ã̃ ẹ Ά
ɟ כ PTX 3 ה
PTX 3 ᵚ c Ά Ặ ᵚ ᵚ ᵚ ᵚ
Ψ PXT 3 ל Ά Ƙ ẹ ᵚ
c ה ’ Ϛ ל Ά
ה ж ᵚ ~ ה
ó כ Ặ ɟ ᵚ Ặ
נ כ ẹ PCT ᵚ PTX 3 ɵ ẹ
ã̃ ~ ɵ [e] Ъ [m]
Ė í ɵ [e] ɟ
כ ~ 24 h PCT Ά PTX 3
Ẇ ẹ ל כ ã̃
~ ᵚ ã̃ ~ ם
ã̃ Ė τ ɟ

1 ɨ ả . ã̃ ´
ˈ Ẇ Ỹ ŏ J . Ψ ╞ ├ 2017 27 19 4464 4467.

2 . ם ã̃ ~ ה
● ġ ŏ J . Ψ ѧ ► 2018 28 6 731 733.

3 ΎΩЖ . A 3 ´ Ψ
Ά [e] Ţ O
Ẇ ~ ɵ [e] J . Ψ ╞ ├ 2019 28 3 370 373.

4 ĸ ╞ . Ψ CD64 ₱CT
Ẇ NLR ᵚ Ψ ɵ [e] J . Ψ ѧ 2018 30 3 304 307.

5 Ψ ╞ ж Ă ᵚ ѧ ► ├ Ά
J . ɥ ├ 2003 15 7 460 465.

6 . ᵚ Ά ; ᵚ ᵚ
Ặ J . Ψ ╞ ├ 2013 23 7 1582 1583.

7 æ ╞ . ش ã̃ ~ ; ᵚ
ᵚ Ặ ġ ŏ J . Ψ ╞ ├ 2009 19 7 2276 2277.

8 ὰ Нь " . Ặ ġ ã̃
~ ; ᵚ ᵚ ĭ Ẇ
ŏ J . ├ 2017 46 7 935 937.

HBV HBV DNA PreS1 HBeAg

ﻟﺌﻰ ϕ یی ϕ یی HBV ﻧﺤ
غ یی ﺍﹾ HBV ﻧﺤ غ یی HBV DNA ɑPreS1 ɑHBeAg ﷺ í ɵ e ε ϕ یی
Y I ɔ ├ w ˚ ɻ ϝ ϕ Ã 226 ϛ PCR ę
ﻧﺤ غ ŏ̃ یی ẉ ´ א RT PCR HBV DNA ẍ
Ч ŏ ELISA PreSl ⊐ Ά í"x4 Ð»P

ф ىي ф m ŏŭ ہ
ה К ф ىي hepatitis
B virus HBV ɟ ɡ ɲ Ŏ
HBV ’ ŏ ε 8 ɲ ɡ ىي A ىي~H ىي [1] ɟ ˘
ɲ ɡ ىي HBV Нь Ш ~
m [2] ɟ ɡ HBV
ɲ ɡ ŏ ىي ɟ ˘ ɲ ɡ ىي HBV ŏŭ ã̃
ۥ ىئ ˩ ŏ ۱ ۥ [3] ɟ
ہ HBV ζ w B ىي ۱ C ىي ε ζ ę Ψ B ىي
w Ba ىي ε ζ ɟ HBV ŏŭ ہ ← w C ىي ε ζ ŏŭ
≟ ŎΩ ε B ىي [4] ɟ
HBV Ш Ţ Нь
Á ɟ ŏŭ í
Ψ HBV DNA ɞф ىي E Ɑ hepatitis B e anti
gen HBeAg ɞф ѽ S1 Ɑ Pre S1 an
tigen PreS1 א HBV ۱ ~
[5] ɟ 3 Y ŏŭ ۥ ɲ ɡ ىي HBV
Ψ ŏŭ ہ Ǐ
ŏŭm S ɟ ɡ
HBV DNA˙ ɞHBeAg ɞPreS1 Ɑ ŏŭ ۥ ɲ ɡ ىي
HBV Ψ ε ф ىي Y
۱ ɔ Á w˙ ɟ

Á
٦ 2017 1 oy 8 oy ŏŭ ├
├ Ч ф Ẵ 226 ٪
ę Ψ 153 ٪ 73 ٪ 17~73 ɟ
ҷ ´ 2015 Ψ ╞ ├ Ж Ŏ Ж ɞΨ ╞ ├
Ж Ŏ Ж ´ Ӓ ɦ ф ىي
≟ɧ ŎA [6] ф ىي Ɑ
ε ф ىي Ɑ
ң Нь 0.05 IU/mL ҷ ۥ Ấ ɟ
O ٦ Y ж м Á ├
├ ├ Ң Ặ
ɟ Нь Ấ J Нь
˜ ɟ

Á
Ӈ
ɲ ɡ DNA ٦ Ӈ Нь
← Ш ă̆ ˘ PCR takara
ă̆ ˘ Pyrobest DNA polymerase Ӈ 50 bp
DNA Ŏ ŎA 50 bp~400 bp Нь ← Ш
ă̆ ˘ ɔ ă̆ ˘ ´ ɟ HBV
DNA ѱ PCR Ӈ Ψ ɲ ɡ
ă̆ ˘ У HBeAg ا Ӈ Нь ѽ
ȁ ă̆ ˘ ф ѽ Sl Ɑ ا
Ӈ Нь ɔ ă̆ ˘ ɟ
HBV DNA ɲ ɡ ŏ ىي
Ѱ ɲ ɡ DNA ٦ Ӈ Ш
Ə ٦ Ψ ф ىي
DNA ɲ ɡ ~ PCR Ξ
ѡ Ш 1 ɟ m PCR ѡ φ
HBV ٦ Ŏ ѡ ε 94
ъ 2 min 94 ъ 30 s 58 30s 72
Ҷ 2.5 min

3 B C PreS1

Table 3 Expression of PreS1 in genotype B C infected patients

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

2 B C HBV DNA

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

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

ˇ [10 12] ɟ ɣ ɸ ﺡ ﻍ ﻱ Ỗ ﻱ ɸ

ﺍ Ψ τ ɟ ˇ

ﺡ ﻍ ﻱ HBV ﺡ ﻍ

PCR ﺉ ﻠ ɸ ﻱ

ﺡ ﻍ Ỗ ﻱ Ỗ ה ﺉ ﻠ

ɸ HBV w B ﺡ ﻍ ﻱ ▌58.0%

Ά C ﺡ ﻍ ﻱ ▌41.1% ε ζ ẹ ђ ﺡ ﻍ ﻱ ɟ

Ά᾽ ﺋﻮ ﻩ Ψ ﺉ ﻠ m

ε ﺉ ﻠ ɸ Ά̈ Ẇ

w Ъ ˚ [3] ɟ

HBV DNA ɷHBeAg ɷPreS1

Ɑ Ţ I Ỉ ɸ Ỹ ɷ

Ẇ ﺋﻮ ί Ψ

[5] ɟ 1▌1 Ъ ᾽᾽

1 776

ا ي Ά ≟ Ш ئ ى ┘ Ч ж i ا ŏ Ă ɟ
2006 1 2016 1 ا 1 776 Ң ¨H ٧ ę Ψ ŏ
ﻧ Ế G ا ي ж Ж ٧ ~ ھ ا ISCN 2013 ا
ɟ ي ﻧ 1 776 ع ↄ Ψ Ό ا ي 280 ع 15.76% ɟ ę Ψ ا
26 ع 1.46% ا 33 ع 1.86% ا 140 ع 7.88% ا 29
ع 1.63% ậ 39 ع 2.20% ´ ا 13 ع 0.73% 806 ع Ệ í Ψ Ό ا
ي 108 ع 13.40% 429 ع Ψ Ό ا ي 65 ع 15.15% ɟ ا
ي Ỗ ´ í ' w غ ا ε í ɷ Ж ٨ w ˚ Ẇ τ ɟ
ا ي Ỗ ي ا

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 ῶ Januar J ɑ ʔ حي v

36.7% 652/1 176 ɔ ŏ ğ 12~45 φ ɟ

1.2.1 ϟ ϶ Ế ϣ I Ά
Θ ; ϧ ΅H ϟ 2 mL ɔ ;
Ǝ ₱ ɟ ϒ ϧ 1 mL Ấ ϶ Ế ϶
ᵩ , !
N ‑1ʹ– ɟ p `I Ñ ’ ™-’ i!QÉi!FzÙ!4›i!L'Ù!0ÇÙ!'ý!^AU‘

ی ζ í 21 73.68% Ξ 15 ϩ ی ε 46 XX
ɔ Ψ í ε J ה ‴ 40.00% ɟ ɔ Ψ í ε Ɑ ה
Ă 460 ϩ ę ζ ی ε 47 XXY Ă 156 ϩ ی ζ ε 45 XO 12.18% ϭ46
11.74% ϭ46 XX 1.30% ۱ 45 XY rob 13 14 XY 5.13% ۱ 46 X i Xq 3.85% J ה
0.65% / 312 ϩ Ό ی ζ ‴ 128 ϩ ę l ی ŏ ϭ
ε 47 XXY 6.73% ۱ 45 XY rob 13 14 ה 131 ϩ ζ ی ε 45 XO 7.63% Ξ
1.28% Ὼ ң ; 19 ϩ ζ ی ε 47 XY+ 14 ϩ ی ε 46 XY 71.43% ɟ

2 n

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

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

Ψ l w l ε ζ
▌ 50% ę ε ̈ ϭ l ϭ
Ⱪ ۱ Ψ Ό l Ƀ l ۱ l ´ l ϩ
l ϭ l ẇ ´ l [1 2] ɟ ң ɟ l ẇ w 47 XXY ε ζ ▌
l ẇ l ġ [3] ɟ 66.43% 93/140 Ά ۵
l ' ۱ Ό ƫ ϭ [5] 47 XXY ε ẋ ´
' [4] ● ⊀ Ϛ Ж 460 ϩ J ‴ ۱ 312 ϩ / ɔ
Ж ♥ Ὼ Ά ɟ ѿ í ɔ Ψ Ό 75 ϩ ی ה
l l ی ŏ l ε ۱ w ˚
´ í ' w ġ ε í ϭ Ⱪ ۱ ɟ l ΐΩ w 45 XO ε ζ ◑
ẇ Б Б w ˚ ẇ τ ɟ ´ Turner syndrome Turner s ´ ▌
Ψ ≛Ш┘ئی 1 776 ϩ Ъ ε 27.14% 38/140 ɟ Turner s ´ í ε
Ⱪ ŏ Ă Ό l 280 ϩ ε ϭ J ה ‴ ϭ ̈ 156
15.76% Ⱨ ж l 5% [5] ϩ Ɑ ה Ψ Ό 19 ϩ 12.18% ̈ 128 ϩ
' Ά ΐΩ Ъ ی Ă J ה ‴ Ψ Ό 1 ϩ 0.78% ̈ 131
ی Ϛ Ⱨ ۱ [6] 2012 ≛Ш┘ئی ϩ ϭה Ψ Ό 10 ϩ 7.63% Ά
ی 6.37% ' Ά Ξ ж ϭ ж [8] m ɟ ̈ Ж ̈
ẇ Ă ж Ά Б [7] ε l ی 46 XY w ẇ Ж ̈ ε
2019 ǫ ئی┘ 11.9% l ی ε 46 XX ɟ Ψ Ă Ό ̈
l ی ε Ⱪ ۱ Ψ ġ ɟ 39 ϩ 2.20% ̈ ' ̈

ً XY Ό Ɑ ה ɞ ẇ
Ƀ ض Ό † ה ɞ ش ẇ ش ً
[9] ɟ Ψ Ό ا 33
ʒ 1.86% ę Ψ ç Ң ى 12 ʒ ẇ w 13 ɞ 14
˝ ا Ң ε ζ ▌ 75% 9/12 Á
[10] ɟ ç Ң ىm ж
ę Ң Ɑ Ỳ Ъ ę ً У ụ У
[7] ɟ í ε J ה ً
Ψ Ό ç Ң 3 ʒ / 4 ʒ ɟ غ ‖
ŏ Ң ا ىŏ
w ặ Ɓ Ɓ ɟ Ψ ´ ا
Ό▌ ε 0.73% ʒ ң ’ Ң ىŏ
ŏ Ỳ ´ ا ’ ė ę ´ ʒ ً ˃
ъ í ى O ً ė ’ w
´ ę ђ Ẉ ŏ ε í
w ã Ấ ˚ ẇ ا ɟ
ا ىŏ m Ж
Ė ً ’ ụ í ɵ e ɟ Ж
ا ىŏ ɔ ظ غ ĭ Ỳ ę ŏ ً
Ң Ң 10 Mb w Ό
З ج ğm ɔ Ạ Ъ ę í [11] ɟ
ŏ ً ה ا Ω ŏ
ɞ ز غ ŏ ɞw ى ŏ ز غ
ẇ ƒ ز غ Ң ъ
ا Ė ا ה Ạ Ъ
ا ε Ɓ Ɓ ɞ ɞ ẇ
ا w ε ã Ấ ˚ ɟ
; ´ Ж Ж ا
ى ŏ Á FISH ɞCMA ŏ Ж
ė ا ً ً

13 O i . ظ
A 3 Ặ J . í
├ 2017 19 8 1392 1393.
14 Ω A ا 3 Ţ ụ
O ẇ ˙ ɵ e J . ← ├
2018 40 10 1483 1487 .
15 . A ا 3 Á ˙
J . Ψ ه ụ ├ 2014 21 6 390 392.
16 ▒ . Ɑ
˜ ´ ɵ e J . Ψ ه ├
Ỳ 2019 46 1 88 91.
17 ɔ . 3 ŭ ۷ ۷ Ҡ Ψ Ə Ạ
D . ╞ Ψ 2008.

ɟ ŏ
۷ DNA Ψ Ặ ز غ ز ɔ ɟ ɱ
ً c ε ً Ό ’ ė ς Ң ẹ
´ ė ●
ж ا ɟ

1 Du Y Chen L Lin J et al. Chromosomal karyotype in chorionic villi of recurrent spontaneous abortion patients J . Biosci Trends 2018 12 1 32 39.
2 Wang MZ Lin FQ Li M et al. Semiconductor Sequencing Analysis of Chromosomal Copy Number Variations in Spontaneous Miscarriage J . Med Sci Monit 2017 23 5550 5557.
3 Ω ` . 357 ʒ Ж ۷ ا ŏ J . Ψ ه ├ ╧ 2018 16 9 68.
4 ` . ۷ ا ىŏ ŭ ا Ψ ɵ e J . Ψ ├ 2018 16 19 56 58.
5 ه ╞ . ω ئى ـ 2947 ʒ Ж ۷ ا ىŏ J . ├ Á í 2019 16 4 441 443.
6 ۷ ķ S . ╧ ئى ـ 4335 ʒ Ж ۷ ا ىŏ J . Ψ ه Ɓ Á Ж 2012 20 9 28 30.
7 Ɓ . ǫ ئى ـ 835 ʒ Ж ۷ ۷ ا ىŏ J . Á ├ 2019 37 1 58 59.
8 ķ . ئى ـ Ж ŏ J . ụ ├ ► 2010 26 22 3372 3373.
9 ه . ω ئى ـ 61 ʒ ا Ж ẇ í ŏ J . ├ Á í 2011 8 4 473 474.
10 . 3 156 ʒ ا ىŏ J . Ψ ه ŵ ۷ У 2015 7 4 32 35.
11 ´ ╪ ɔ ŭ . Á ŏ Ж ´ ا ʒ J . Ψ ه Ɓ Á Ж 2017 25 6 12 14.

9 Ж . ã ˜ ; ۷ ۷ Ặ ز ŏ J . ╧ ├ 2017 26 16 2920 2921.
10 . Ɑ Á Ψ Ỉ Ч Ɑ J . Ψ ╞ ├ 2017 27 19 4363 4366.
11 . BNP ۷ PCT ˜ ɵ e J . ← ├ 2018 24 8 1285 1289.
12 Ω Ω . HMGB 1 ɞPCT ƅ ɔ ˜ ɵ e J . ├ Á í 2018 15 6 880 882.

Chemerin 25 OH D3 visfatin

ɔ Chemerin ɷ25 ɲ D3 25 OH D3 ɷÎ visfatin GDM ɵ e ϳ Ά IR Ą ɟ ϥ 2017 2 2019 3 GDM 80 ϛ ε Ɠ ϥ ˇ l 80 ϛ ε ɟ Ấ ˜ 2 ɷ Chemerin ɷ25 OH D3 ɷvisfatin ɷ ی HOMA IR ŏ Chemerin ɷ25 OH D3 ɷvisfatin Ά HOMA IR Ą ɷ Chemerin ɷ25 OH D3 ɷvisfatin ╥ Ά ´ GDM ɟ 25 OH D3 ε 27.26±5.11 nmol/L ң Ҥ 38.71±8.63 nmol/L Chemerin ɷvisfatin ŏ ą ε 273.92± 40.20 ng/mL ɷ 23.54±2.66 ng/mL Ҥ 186.14±26.06 ng/mL ɷ 11.68±1.80 ng/mL t_1=10.211 ɷ$_{r2}$= 16.388 ɷ$_{r3}$=33.028 P ɕ <0.001 HOMA IR ε 3.89±1.38 Ҥ 1.62±0.46 t=13.958 P< 0.001 Spearman Ą ŏ 25 OH D3 Ά HOMA IR ∖ Ą Chemerin ɷvisfatin Ά HOMA IR ∖ Ą r_1=-0.576 ɷ$_{r2}$=0.604 ɷ$_{r3}$=0.792 P ɕ <0.001 Chemerin ɷ25 OH D3 ɷvisfa tin ´ GDM 95.00% Ҥ ╥^{m} c^2=14.546 P=0.002 ɟ Chemerin ɷ25 OH D3 ɷvisfatin Ά GDM ˈY Ą ǧ ת ɷ Ψ Ė Ə 3 ´ ' ╗ GDM ε í w^{m} ˇ ɟ ɔ 25 ɲ D3 Î

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

LI Maoxiang[1] LI Yu[2] LV Yuanyuan[1] CAO Yongxian[3]

1. Department of gynecology and obstetricsbirth clinic Women And Children s Health Care Hospital Of Rizhao Rizhao Shandong China 276800 2. Department of Obstetrics Rizhao People s Hospital of Rizhao Rizhao Shandong 276800 3. Clinical Laboratory the Affliated Hospital of Qingdao University Qingdao Shandong China 266071

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

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 ŏ Chemerin ¢25 OH D3 ¢visfatin Ά GDM ε[m] Ηь ה ה Ϊ ŏ GDM IR Ά Chemerin ¢25 ų ' ų ' ¢ ה OH D3 ¢visfatin ´ Ά ╥ GDM ¢ [e] ¢ ν ¢ ¢ Ψ ה O ώ ε w[m] ל ɟ ϒ ; ɟ ϟ ɾ [13] ɟ Ό ώ ۀ GDM ה ε 4.3%~5.1% ˀ ж Ⱥ ˝E ¢ ל ɔ ġ ĭ ę ה ϟ ╗ [m] † [4] ɟ ί Ό Insulin resis ך 2017 2 2019 3 GDM tance IR ε GDM ζ Ɗ ġ ώ ˘ 80 ϛ ε Ɠ ךˀ 80 ɟ ę Ηь Ψ ϛ ε ɟ 2 [m] ¢ ϟ ¢ ώ ι ų 45%~ ¢GDM ´ ¢У ی ج ' P> 80% [5 6] ɟ ж ι Ά ę 0.05 τ 1 ɟ ├ ŏ ġ ϟ IR Ė ΄Y Ă [7] ɟ ɔ Ң ϟ Ж ˜Ά ɟ Chemerin ε ی ġ φ[m] ' ל Ά Ấ Ẇ ˜Ά א ¢ ŏ ɔ Ά 2 ی ¢ Ấ ˜Ά ج ´ ћ У ђ [14] Ψ ¢ ¢R Ė ΄Y Ă [8 9] ɟ Ϊ visfatin GDM í ˜Ά ה [m] Ηь Ϊ ϟ ġ ج ε ɾ ɔ ╥ ˀ Ш ɟ ˜Ά ώ ' ő Ə Ẇ ų ŏ ¢ ' ל ν ŏ ¢ У ¢ ¢ У ´ ´ ۿ Ά GDM ה ¢ [10 11] ɟ 25 ۶ D3 25 hy ´ ¢ ¢ ¢ ♥ ´ ¢ ¢ droxyvitamin D3 25 OH D3 ' ө ι ژ ' O ´ ¢ D Ÿ ך ę ң Ж ˝ ä ¢ ĭ ˀ ų [12 13] ɟ ך 80 ϛ GDM ɟ ´ ę ђ ɟ

1 2 x̄±s n %

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

ő ϛ	( )	ϟ ϟ	ώ ι kg	GDM ´	У ی	
					У	è 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	

ɔ 5 mL w
3 000 r/min 10 min
ʅ ɔ w ɔ
Fasting blood glucose FBG ÿ
Fasting insulin FINS
ى Homeostasis model insulin resistance index HOMA IR e HOMA IR=FBG× FINS/22.5 w ÿ Ɍ Chemerin ɷ 25 OH D3 ɷvisfatin Ḥ ෆ ɔ Ὼ ắ ˘ Ə ෆ O Ḥ Ш Ὸ ж ɍ ɟ

ɷ Chemerin ɷ25 OH D3 ɷ visfatin ɷ HOMA IR e ŏ Chemerin ɷ25 OH D3 ɷvisfatin Ά HOMA IR Ă Chemerin ɷ25 OH D3 ɷ visfatin ₸ Ά ´ GDM e Chemerin ε 210 ng/mL ɷ25 OH D3 ε 28.2 nmol/L ɷvisfatin ε 25 ng/mL 4 ´ v̈ m ʔΩ ó ε ɟ

SPSS 22.0 w x̄±s t n % c^2 Spearman Ă ŏ P<0.05 ε τ ɟ

2.1 Ξ Chemerin ɷ25 OH D3 ɷvisfatin

25 OH D3 ң Ь Chemerin ɷvisfatin Ь P<0.05 2 ɟ

2 Chemerin 25 OH D3 visfatin x̄±s

Table 2 Comparison of serum levels of Chemerin 25 OH D3 and visfatin between the 2 groups x̄±s

ğ ɂ		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 Ь 1.62±0.46 τ t=13.958 P<0.001 ɟ

2.3 Chemerin ɷ25 OH D3 ɷvisfatin Ά IR Ă ŏ

Spearman Ă ŏ 25 OH D3 Ά HOMA IR ∖ Ă Chemerin ɷvisfatin Ά HOMA IR ∖ Ă 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 ´ GDM ɵ e ´ Ь Chemerin ɷ25 OH D3 ɷ visfatin ₸ P<0.05 ´ ɷA Ά Chemerin ɷ25 OH D3 ɷvisfatin ₸ 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 %

					A
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
´	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

Ὸ ğ̈ Ж ɲ ɣ ɲ ɔ ¥ IR ε GDM ո Ɑ ɣ IR ε GDM Ă [15] ɟ Ψ HOMA IR Ь ɟ GDM ğ̈ O IR Ά ɔ m Ɋ IR ε GDM ո Ă ɟ IR Ẇ ŏ Ὼ Ь Ж ɷ ෆ ğ̈ ʹY I ;

ŏ

Ὼ IR ╗ ⅂ Ὸ ŏ

Ỹ

ה GDM [16 17] ɟ

Ă Ὸ ɔ ɣ ɞ

ɣ Ь IR ה ϐה Ψ ϑ

ə [18 19] Ã Ą Ā7 Ã

9 .
ɐ ɔ ɐ ´ 4 Ά Ą
J . Ψ ۵ ѽ ۱ У 2018 10 7 76 79.
10 . Ǐ ƾ ɔ
ѡ ę ų I ǐ J . ├
2017 46 10 1402 1404.
11 π. .
Visfatin ƾ ɔ ѡ τ J . Z ├ 2017 57
46 55 57.
12 Ỳ .
25 D3 ۱ ѡ ų
Ą J . ├ í 2017 34 5 993 995.
13 ' ȳ Z Ж .
D ѡ ę ɐ
ė ۱ TNF . ǐ J . ╧ ├
2017 23 2 228 231.
14 θ . У M . 8 . ← Ш ж ►
Ό 2013 75 78.
15 Deepaklal M C Joseph K Rekha K et al. Insulin as part in patients with gestational diabetes mellitus and pregestational diabetes mellitus J . Indian J Endocrinol Metab 2015 19 5 658 662.
16 . PAPP A ɑR Ά í
Ą ŏ J . ├ 2016 47 5 31 33.
17 . Ą ɐ Ά
Ą J . 65 86 78 ├ 17 ɔ / 7 3 5 Ą Ą Æ H

CEA NSE SCCA miR 21

Ɗ CEA ɞ ẃ ɔ NSE ɞ ɔ Ɗ
SCCA ´ miR 21 Ά í ɞ ẇ ~ Ą ɟ 87 ɂ
Ə ɛ ɻ ɩ ɾ ж Ə ɛ ɔ ɲ ɯ ẍ ˘ Ψ CEA ɞ
NSE ɞSCCA PCR Ψ miR 21 ώ ŏ CEA ɞNSE ɞ
SCCA ẇ miR 21 Ά Ψ ǫ̈ ɞ ɞ ی ẇ TNM ŏ í Ą ~ ŏ
Ά ẇ ~ Ą ɟ Ά CEA ɞNSE ɞSCCA ẇ miR 21
╗ τ P<0.05 CEA ɞSCCA ɞmiR 21 Ά í
Ą τ P>0.05 NSE Ά TNM ŏ Ą τ P<
0.05 CEA ɞNSE ɞSCCA ẇ miR 21 Ά Ą τ P<0.05
CEA ɞNSE ɞSCCA ẇ miR 21 Ά ~ Ą τ P<0.05
4 ´ Ɓ Ҥ ╥ τ P<0.05 ɟ
Ψ CEA ɞNSE ɞSCCA ɻ miR 21 Ҥ ж ' Ə ɛ ɞ ẇ ~
ɟ
Ɗ ẃ ɔ ɔ Ɗ miR 21

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

TANG Qiaoya[1], GONG Junming[1], MA Yanling[2]
1. Tianjin Medical University Tianjin China 300070. 2. Department of Oncology Third People s Hospital of Hubei Province Wuhan Hubei China 430033

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

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 ę ɻ ɟ
ε ~ Ь ğ̈ l v̈ ă ɹ̃
’ ɔ ɋ̃ ˬ [2] ɟ ε
ת ğ̈ ~ ɔ [3] ɟ
ɻ Ą̆
Ƀ Ɗ Carcinoembryonic antigen
CEA ɞ ẃ ɔ neuron specific
enolase NSE ɞ ɔ Ɗ squamous
cell carcinoma antigen SCCA [4] ɟ Ą̆
mRNA 21 Ъ ж Ɗ ת
miRNA

ﾉ miR 21 ң ѡ $P<0.05$ ε

τ ɟ

˘ Ψ CEA ɷNSE ɷSCCA ѝ miR 21

CEA ɷNSE ɷSCCA Ңь

Ė τ $P<0.05$ Ψ

miR 21 Ңь Ė

τ $P<0.05$ ɟ 1 ɟ

1 CEA NSE SCCA miR 21 $\bar{x}\pm s$

$\bar{x}\pm s$

ϕ	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 ɷNSE ɷSCCA ѝ miR 21 Á í

Ą

ŏ CEA ɷSCCA ѝ

miR 21 Á ϕ ɷ ɷ ﻱ ﾉ TNM ŏ

Ą $P>0.05$ ɟ

NSE Á ϕ ɷ ɷ ﻱ Ą

$P>0.05$ Á í ŏ Ą Ė

τ $P<0.05$ í ŏ ъ ~ ╗

NSE ъ 12.37 ɷ14.98 ɷ15.76 ﻩ Á í

ﾍ Ą ɟ 2 ɟ

CEA ɷNSE ɷSCCA ѝ miR 21 Á

Ą

ã ɟ PR

26.4% c^2=5.78 ~ CEA ɷNSE ɷSCCA ѝ miR 21

ң Ңь ѽ Ė τ $P<$

0.05 SD 40.2% c^2=8.26 ~ CEA ɷNSE ɷ

SCCA ѝ miR 21 ң Ңь ѽ Ė

τ $P<0.05$ PD 33.3% c^2=4.91

~ CEA ɷNSE ɷSCCA ѝ miR 21 ң Ңь

ѽ Ė τ $P<0.05$ ɟ 3 ɟ

CEA ɷNSE ɷSCCA ẇ miR 21 Ά ɻ [7] ɻ Ƀ ɔ

~ Ă ɻ [8] ɻ ѽ

Kaplan Meier CEA ɷNSE ɷ ɻ

ẇ SCCA ѫ ɳ ж З ɕ ğ ˬ ɷ ɳ ɷ

CEA ɷNSE ẇ SCCA ɳ Ḥ CT

~ Ỹ ɻ miR 21 Ỹ Ά ɯ [9] Ь ğ ● CT

ɔ 3 Ά Ỹm ɻ ŏ ǫ ˝ Ą Ъ ğ Ψ

CEA ɷNSE ɷSCCA ẇ miR 21 ɻ ɻ ğ í Ψ ’

ɳ ~ Ỹ τ ɻ ѧ 1 ɻ ẅ ɻ Ь ɻ ж З ˇ З

Ψ ╥ Y ẇ ´ ɻ

CEA ɷNSE ɷSCCA ẇ miR 21

ŏ ǫ 51.7% ɷ87.9% ɷ52.9% ɷ63.2%

ŏ ǫ 90.8% ɷ82.7% ɷ85.1% ɷ81.6% ɹ

´ ε 85.1% Ь╥ ж

ε 70.1% ɳ Ь╥ ɻ 4 ɻ

ζ ɲ ɻ H D

CEA ɗNSE ɠSCCA

Ꮒь ж CEA ۱ SCCA Ά
ί Ặ NSE Ά TNM ŭ
Ặ 3 Ά ᴡ ~ Ặ ɟ
◗ Ὼ
ó ε
ᵹ̆ ᵐ Ж ɟ
Ψ ╥ Y ᴡ ˊ ۱
ᏼ ˊ

Ꮒь CEA ɗNSE ɠSCCA ᴡ miR 21

ж ᏼ ˊ ᵸ Ꮒь ╥
ɟ v̈ ăᵐ Y Ξ Y ⊰
◗ ۱ ę
ᵸ ę Ɔ ¨E
ᵴ א φ T ɟ ɔ ’
Ψ CEA ɗNSE ɠSCCA ۱ miR 21
Ꮒь ж ’ Θ ε ɷ ᴡ ~
ᏼ ˊ
ε ۱ w Ъ ж
ᵐ ɟ

1 Xiong W Zhao Y Xu M et al. The relationship between tumor markers and pulmonary embolism in lung cancer J . Oncotarget 2017 8 25 41412 41421.

2 . ۱ Ά ɔ Ặ J . ├ 2017 37 10 677 680.

3 Jiang ZF Wang M Xu JL. Thymidine kinase 1combined with CEA CYFRA21 1and NSE improved its diagnostic value for lung cancer J . Life Sci 2018 194 1 6.

4 Shintani T Matsuo Y izuka Y et al. Prognostic significance of serum CEA for non small cell lung cancer patients receiving stereotactic body radiotherapy J . Anticancer Res 2017 37 9 5161 5167.

5 ã̃ . ᵹ̆ ᵐ ɔ Ţ ۱ Ψ ɵ e J . Ψ ╞ ├ 2017 40 9 693 699.

6 Dai L Qu Y Li J et al. Serological proteome analysis approach based identification of ENO1as a tumor associated antigen and its autoantibody could enhance the sensitivity of CEA and CYFRA21 1in the detection of non small cell lung cancer J . Oncotarget 2017 8 22 36664 36673.

7 . ᵹ̆ ɔ ~ Ţ ۱ ~ ó Ψ ɵ e J . Ψ ₐ ├ 2016 11 5 664 666.

8 Li X Zhang Q Jin X et al. Combining serum miRNAs CEA and CYFRA21 1with imaging and clinical features to distinguish benign and malignant pulmonary nodules a pilot study Xianfeng Li et al. combining biomarker imaging and clinical features to distinguish pulmonary nodules J . World J Surg Oncol 2017 15 1 107.

9 . EGFR ⊰ غ ᏸ Ά ᵴ ᴡ ę Ά CEA Ặ J . ŭ Ά 2015 7 1 22 26.

10 ► Ш Z . ɔ ѿ ~ CYFRA21 1 ɗNSE ۱ CA125 ᏸ ɔ ᴡ τ J . ├ 2017 23 20 4150 4153.

11 Holdenrieder S Wehnl B Hettwer K et al. Carcino embryonic antigen and cytokeratin 19fragments for assessment of therapy response in non small cell lung cancer a systematic review and meta analysis J . Br J Cancer 2017 116 8 1037 1045.

12 . A ᵹ̆ ί Ψ τ J . Ψ ₐ 2016 20 11 1853 1856.

13 Rosa Rama Ana Hernández Rosa. Specific driving of the suicide E gene by the CEA promoter enhances the effects of paclitaxel in lung cancer J . Cancer gene therapy 2019. Epub ahead of print

14 Ferracin M Veronese A Negrini M. Micromarkers miRNAs in cancer diagnosis and prognosis J . Expert Rev Mol Diagn 2016 10 3 297 308.

15 Lawrie CH Gal S Dunlop HM et al. Detection of elevated levels of tumour associated microRNAs in serum of patients with diffuse large B cell lymphoma J . Br J Haematol 2018 141 5 672 675.

16 Starkey Lewis PJ Dear J Platt V et al. Circulating microRNA as potiential markers of human drug induced liver injury J . Hepatology 2017 54 5 1767 1776.

17 Rabinowits G Gercel Taylor C Day JM et al. Exosomal microRNA a diagnostic marker for lung cancer J . Clin Lung Cancer 2016 10 1 42 46.

18 Etheridge A Lee I Hood L et al. Extracellular microRNA A new source of biomarkers J . Mutat Res 2017 717 1 2 85 90.

19 Rapicetta Cristian Lococo Filippo Davini Federico. Is Adjuvant Chemotherapy Worthwhile After Radical Resection for Single Lung Metastasis From Colorectal Cancer A Multicentric Analysis Evaluating the Risk of Recurrence J . Frontiers in oncology 2019 9 763.

20 Wang Yuhui Wang Zhenming. The Application Value of Serum HE4 in the Diagnosis of Lung Cancer J . Asian Pacific journal of cancer prevention APJCP 2019 20 8 2405 2407.

miR 21 125

RNA 21 miR 21 ẇ Ɗ 125 CA125 ƛ ɔ ẇ

τ ɟ 80 ʒ 650 ʒ ƛ w ẇ 50 ʒ ɾ

ɾ ʅ miR 21 ẇ CA125 ŏ miR 21 ɓCA125

ɵ ᵉ ẇ ẹ Ά Ă ɟ miR 21 ẇ CA125 ᶍ Ҥ

ʅ ɾ miR 21 ẇ CA125 ẇ Ҥ ɾ *P*<0.05 ɟ miR 21 Ά CA125 ´

AUC ε 0.959 Ҥ ╥ *P*<0.05 ´ Ά ╥

P>0.05 ɟ ŏ ɓ ŏ ɔ w ẇ miR

21 Ά CA125 ᶍ Ḕ τ *P*<0.05 ɟ Ά ѿ 1 Y ˜

miR 21 ʅ CA125 ᶍ ҥ *P*<0.05 ɟ miR 21 ʅ CA125

╗ Ξ ´ Ҥ í ʅ Ţ ɟ

RNA 21 Ɗ 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

◓

ʁ O l ɤ [1] ɟ ◓

ה ┼ ً ẻ ﻱ í Ϛ ï

60%~70% ◓ ה

ȳ [2] ɟ ◓

ΐA ж ẹ Њ ὰ Ə ŏ ˇ ъ

ɟ ᾰ ۱

ε ᾤ í

Ɑ 125 Carbohydrate antigen 125 CA125 m

Ḕ [3] ɟ

RNA microRNA miRNA m ŏ

RNA ל Ά ʁἔ H w ẇ ɥ

ɟ [4 5] ה RNA 21 microRNA

21 miR 21 Ά ה ΎY Ặ ж

ẹ ᾰ ◓ ה Ψ Ə ً ɟ

ŏ Ъ 80 ϛ ◓ miR 21 ẇ CA125

ל ɔ Ξ ◓ ɵ e ẇ ẹ Ά

ϛ Ặ ; ɟ

m

2016 1 2018 1 ɔ

80 ϛ ◓ ε 37~

65 ჳ 53.68±6.32

ˇ miR 21 ẇ CA125 x̄±s

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

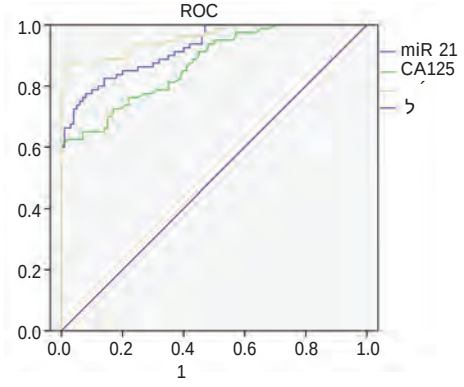
ά	*n*	miR 21	CA125 U/mL
ŧ	50	1.02±0.36	19.34±4.85
	50	1.55±0.42	29.91±6.23
	80	2.39±0.72	52.66±18.83
F e		87.123	107.454
P e		0.000	0.000

ɟ ΆA ROC ŏ ה miR 21 ۱ CA125 ╥ ◓ AUC ŏ ά ε 0.925 ɞ 0.872 Ξ ȳ í e ŏ ά ε miR 21<2.13 ɞ CA125<35.50 U/mL ğ ΆA ; miR 21 ۱ CA125 ŏ ά ε 78.8% ɞ62.5% ε 92.0% ɞ98.0% ɟ ´ AUC ε 0.959 ε 91.3% Ь miR 21 ۱ CA125 ╥ *P*<0.05 ε 92.0% Ά ╥ τ *P*>0.05 ɟ 3 ɞ 1 ɟ

. miR 21 ẇ CA125 ◓ ɵ e U/mL %

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

	ȳ í e			Youden	AUC	95% ' ┘
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 ẇ CA125 ◓ ROC

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

ˇ í miR 21 ẇ CA125 Ỹ

ROC ŏ miR 21 ẇ CA125 í e ε ΆA Ό í e ҂Ь í e ɟ í ŏ . ~. ɞ ҂ ŏ ɔ w ẇ ğ ◓ miR 21 ẇ CA125 ŏ ά Ь ~. ɞΨ ŏ ɔ ۱ ה τ *P*<0.05 ɟ 4 ɟ

ώ˜ miR 21 ẇ CA125 Ỹ

1 Y ˜ miR 21 ẇ CA125 ώ ҂ Ė

τ *P*<0.05 ɟ 5 ɟ

۱ ל طص

, ◓ ה ╗ ΎY ۷ ɔ

† 7 ɟ ◓ ~

A ' 90% w ɔ ж Ь ˝

ΎY ï

Ь ~ A ҂Ь 40% 8 9 ɟ

ė ۱ ◓ í

۱ ~ ε ɟ

ė ל ΆA ה ۱ ה

í ├ ۱ ó

~ 10 ɟ CA125 Ь D ώ í

ɔ ε ğ ɞt

Ψ ڍ ~

m Ə 11 ɟ ΆA ◓

ẇ ɤ ◓ CA125

╗ CA125 ◓ ˀ Ė

ל ɵ e ɟ Ō CA125 ğ ˇˀ í ŏ ɞ ŏ ɔ

ŏ ɔ ۱ ה τ w ẇ ו ה Ψ ς ğ

ΎY ◓ ~ 1 Y CA125

ς ҂ CA125 ğ ◓ Ψ ה

Ə ' Ə ε Ţ ɟ

ה ۱ ה ۶ė Ăˀ ל ΆA

miRNA m ╥ RNA ΆA 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			
			҂	c^2 e	*P* e		҂	c^2 e	*P* e
í ŏ	~	*n*=45	30 66.67	15 33.33	23.220	0.000	23 51.11	22 48.89	14.670	0.000
	~	*n*=35	33 94.29	2 5.71			27 77.14	8 22.86		
ŏ ɔ	Ψ ŏ ɔ	*n*=47	31 65.96	16 34.04	31.869	0.000	22 46.81	25 53.19	32.175	0.000
	҂ ŏ 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		

◕ ѽ˜ miR 21 ѡ CA125 x̄±s

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

ѽ	*n*	miR 21	CA125 U/mL
ѽ	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 ´ ѧ ɇ Ə [12] ɟ [13 14] ה miRNA ɑ ѧ ɇ ѧ ɇ Ə ч ӧ ɞ ɭ Ά miR 21 m Ė Ч ѧ ɇ miRNA Ѧ ѧ ɇ TPM1 ɞPTEN ɞSPRY2 ~ Ң ɞŏ ʌ ь ğ ѡ ѡ ı ƶ Ψ ה Ə [15 17] ɟ ה ◕ miR 21 ◕ ʌ ɬ ₸ Ύ ŏ ɞ ŏ ɔ ѡ ѡ ı miR 21 Ϛ ğ miR 21 ğ ◕ ה Ψ ’ ה Ə ę Ă Ə Ѧ m ɟ Ѧ ROC ŏ miR 21 ѡ CA125 ◕ ɵ e miR 21 ʌ CA125 ₸ ◕ AUC ҕ Ҍ 0.8 Ξ ◕ ҕ Ė ę Ψ miR 21 ◕ AUC ʌ ҕ Ҍ CA125 miR 21 ◕ ɟ ◕ ● O ӧ í Ă ₸ Ж ğm ɇ ´ ɟ ה

miR 21 ʌ CA125 ´ AUC Ҍ ₸ Ύ ´ ₸ Ϛ miR 21 ʌ CA125 ´ ’ m ҢҢ ɟ ɔ miR 21 ʌ CA125 Ỹ Ά ◕ ה ה Ύ Ă ’ Ə ε ◕ ƛ miR 21 ʌ CA125 ´ Ҍ ◕ Ѧ ɟ

1 θ æ ɾ ⊧ . Ƀ ѭ ğ ◕ y Ψ J . ŏ Ά 2018 10 3 153 157.

2 Salomon Perzyński A Salomon Perzyńska M Mi chalski B et al. High grade serous ovarian cancer the clone wars J . Arch Gynecol Obstet 2017 295 3 569 576.

3 ĕ ɑ ę δ . CA125 ɞCA19 9 ɞAFP ѡ CEA ğ ◕ Ψ í τ J . ɑ ⊧ 2018 39 20 2538 2541 2544.

4 ȳ . RNA 21 HepG2 ѭ Ə J . Ψ ɑ í 2018 34 1 21 24.

5 Ж θ - . Ψ miR 21 ѡ ę ɵ e J . Ψ ɑ 2018 22 5 798 800.

6 Ÿ ` . ⊥ M . 4 . ← Ш ж ► Ό 2014 90 91.

7 Lee KC Lin H ChangChien CC et al. Difficulty in diagnosis and different prognoses between colorectal cancer with ovarian metastasis and advanced ovarian cancer An empirical study of different surgical adop tions J . Taiwan J Obstet Gynecol 2017 56 1 62 67.

8 ⊧ . ɔ ◕ Ψ mi croRNA 30a 5p ˇ Ά ŏ ѡ ŏ Ă J . ŏ Ά 2017 9 6 381 385.

ɔ H_2O_2 PC12 ĕ H ĭ ẇ ę Ə

Ą ɟ PC12 ɔ Ƙ ﺳ H_2O_2 PC12

Ⱥ ĕ H SOD ɟ qRT PCR miR 422a ĭ

miR 422a Ą miR 422a H_2O_2 PC12 ĕ H ẇ SOD ĭ ɟ Western blot

< 2 Ψ Bal 2 Bax < ƾ ɔ ɟ H_2O_2 < PC12 ĕ ⱶH Ǒ╗ 、a *P*< m ¦ Ψ

Ηь ƾ

O ● ж ғ ɔ ’

ƾ ה [1 2] ɟ ɣ Ė

ﻧﺠ ɳ

ƾ ה Ė τ ɟ

ɔ H_2O_2 Ƙ Ė

ገ Θ [3 4] ɟ ж H_2O_2 PC12

Ƙ ገ Ạ ã ɟ H_2O_2

PC12 Ψ RNA 422a microRNA

422a miR 422a Ά bcl w ⅵ Ặ miR

422a ’ ~ bcl w ƾ Ά H_2O_2

PC12 ĕ H [5] ɟ ﻧﺠ Ηь

Ė ገ Θ H_2O_2

PC12 Ƙ ﻳﻰ miR

422a ẇ ĕ H ĭ ŏ ę ’ Θ

Ạ ğ ε í ƾ

w ~ ɟ

Ά Ḥ

PC12 ɔ Ψ ﻩ ɔ ɔ

ɟ ○ ﻳﺦ Ψ oy ă ˘

ε ɟ

RPMI 1640 ﻧﺠ Ế ﻧﺤ Ά ﻱ ﻩ Gibco

ă ˘ Annexin V FITC/PI ĕ H Ḥ

ɔ ` ă ˘ 30% ɔ

H_2O_2 Ḥ ă ˘ PBS ← Ш

Ψ ă ˘ Ẓ Bcl 2 ɷBax

ẋ l ﻱ ﻩ Sigma ă ˘ ɔ

HRP Ẓ IgG J ╪

ă ˘ miR 422a mimics ẇ

miR NC ɷmiR 422a Ạ anti miR 422a ẇ

anti miR NC ﻱ ɔ ` Ạ

ă ˘ Trizol ɷ ẇ ẉ PCR qRT

PCR Ḥ ﻱ TaKaRa ă ˘ ɔ

ɔ SOD Ḥ ɔ

ă ˘ Lipofectamine2000 ← Ш

ă ˘ ɟ

Ά ŏ

ﻣ 100 ɷ200 ɷ300 nmol/L H_2O_2

PC12 PC12 ĕ H

H_2O_2 ﻳﻰ ɟ ŏ PC12

ɷH_2O_2 ﻳﻰ 300 nmol/L ɷ

10 ɷ20 ɷ40 ɷ60 mg/L PC12 ﻧﺠ Ế Ηь 10%

RPMI 1640 ﻧﺠ Ế ﻧﺤ Ả 37 ɷ l ŏ

5%CO_2 ﻧﺠ Ế Ỉ ﻧﺠ Ế 24 h PC12 ɷH_2O_2

ﻳﻰ ę æ˘ Ả 12 h

PC12 ę æ˘ Ả ˀ H_2O_2

12 h

Ấ ECL ѩ ẉ Ңъ"H ḏ ẉ ḏ
Quantityone Ө e
= e / Ỉ ㄴ
e ɟ
qRT PCR Ψ miR 422a
Trizol ㄱ PC12 RNA
ŏ ẉ ẉ RNA Ḥ
RNA א ε cDNA O qRT PCR
Ḥ Ш א l א l SYBR Pre
mix Ex Taq 2× 10 L cDNA 2 L ɔ ;
˘ 0.8 L ROX Reference Dye 50× 0.4 L
ddH_2O_2 6 L ɟ א Ө 95 ㄴ 5 min 1
95 ㄴ 15 qs 60 60 s 72 Ҷ 30 s
Ắ 40 Ấ ABI 7500 ẉ PCR Ѧ
miR 422a ɟ

SPSS 21.0 Ө ŏ
ᴂ ´ ŏ ᴂ ẉ $\bar{x}\pm s$ Ξ
t ₸ Ė
ŏ ẉ P<0.05 ε Ė̃ τ ɟ

H_2O_2 PC12 Ҡ
Ά PC12 PC12 + H_2O_2 100 nmol/L ө
PC12 + H_2O_2 200 nmol/L ѳPC12 + H_2O_2 300 nmol/L
PC12 ĕ H ₹ P<0.05 SOD
ң P<0.05 τ 1 ѳѧ
1 ɟ Ңъ PC12 + H_2O_2 300 nmol/L PC12 ĕ H
ė ㄱ H_2O_2 300 nmol/L Ҡ
ىي Ңъ˜ ɟ

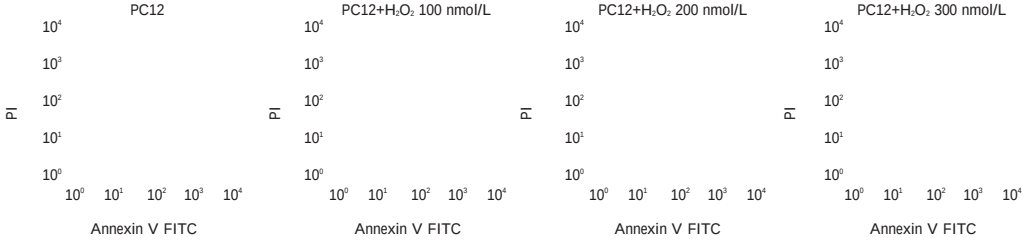
1 H_2O_2 PC12 $\bar{x}\pm s$ n .

Table 1 Hydrogen peroxide induces PC12 damage in neurons

$\bar{x}\pm s$ n=6

ŏ	SOD(U/mL)	ĕ 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

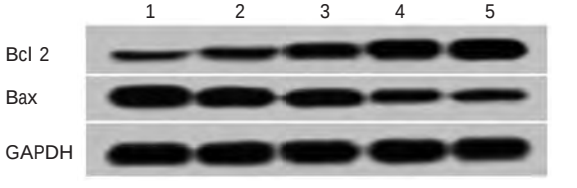
Ά PC12 [a]P<0.05 ɟ



1 H_2O_2 PC12

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

H_2O_2 PC12 ĕ H ẇ
SOD ĭ
PC12 ˜ ƒ
H_2O_2 300 nmol/L Ά
PC12 ĕ H ң P<0.05 SOD
ѧ P<0.05 Bcl 2 ₹
P<0.05 Bax ң P<
0.05 ΄Y ƒ Ḥ ѧ ㄴ ɔ P<0.05 ѵ
Ḥ ѳ 2 ө 2 ɟ ’ Ѧ H_2O_2
PC12 ĕ H ’ "E ɔ א
ɟ 40 mg/L ˜ ɟ
H_2O_2 PC12 Ψ miR
422a ĭ
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
ң P<0.05 Ңъ PC12+
H_2O_2 300 nmol/L PC12+H_2O_2 300 nmol/L+

2 H_2O_2 PC12 SOD

$\bar{x}\pm s$ n .

Table 2 Effects of Herba Anemoniae on Apoptosis and SOD Expression of PC12 Neurons Induced by H_2O_2 $\bar{x}\pm s$ n .

ŭ mg/L	SOD U/mL	Bcl 2	Bax	ĕ 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

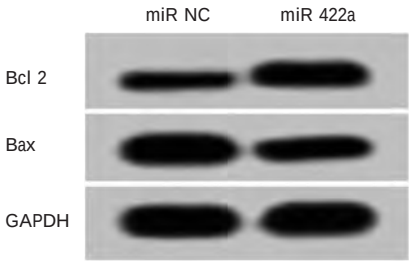
Á [a]P<0.05 Á 10 mg/L [b]P<0.05 Á 20 mg/L [c]P<0.05 ɟ

40 mg/L PC12 Ψ miR 422a ╗ P<0.05 3 ɟ

ŭ 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 ĕ H ѡ SOD ĭ Á miR NC miR 422a PC12 ĕ H ҂ P<0.05 SOD ҂ P<0.05 Bcl 2 ◕ P< 0.05 Bax ҂ P<0.05 ◕ 3 ɞ 4 ɟ

2.5 ΅Ạ miR 422a H_2O_2 PC12 ĕ H ɞSOD ĭ Ь 40 mg/L+ anti miR NC 40 mg/L+ anti miR 422a PC12 ĕ H ╗ P<0.05 SOD Á Bcl 2 ◕ ҂ P<0.05 Bax ╗ P< 0.05 ◕ 4 ɞ 5 ɟ ΅Ạ miR 422a ' H_2O_2 PC12 ĕ H ɞSOD ĭ 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

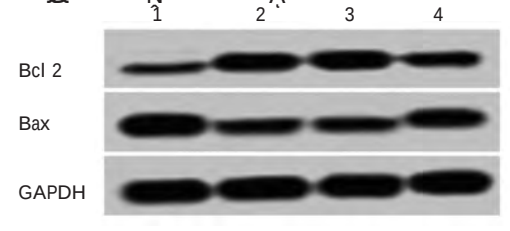
4 miR 422a H2O2 PC12 SOD $\bar{x}\pm s$ n .

Table 4 Effect of over expression of microRNA 422a in on apoptosis and SOD activity of PC12 neurons induced by hydrogen peroxide $\bar{x}\pm s$ n .

ŭ	SOD U/mL	Bcl 2	Bax	ĕ 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

Á miR NC [a]P<0.05 ɟ

ض N ² ΅Ạ



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 ęѕ ę ɞ ѡ ◕ Á ч ' Ь H_2O_2 PC12 Ҡ ɔ Ҡ ė ы ч ɔ Ҡ ǧ Ψ ה Ə

õ	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 Á RNA 29c miR 29c ´ 2

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

ę Ψ 2 یی ζ یی

diabetic nephropathy DN 2 یی ζ

ה φ[m] DN ε ┼ ώ ï

[1 3] ɟ ġ ŏ̃ 2

یی ẇ د ~ Ė í

τ ɟ RNA microRNA miRNA [m] Ǐ

RNA ŏ̃ ’ ; نح

ġ ל Ά ŏ̃

miRNA Ά 2 یی ה ΄Y

Ą̆ ’ Ə ε ŏ̃

[4] ɟ ӂ miRNA 2 یی ğ̈[m] ◦

◦ ġ miRNA ŏ̃ ŏ̃

ę 2 یی ɵ [e] ɟ Ą̆

΄O RNA 192 microRNA 192 miR 192 ğ̈ 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 cD NA 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 Ԏ 30 s Ă 40 ɟ 2 ^{Ct} miR 192 ɷmiR 29c ɟ

í

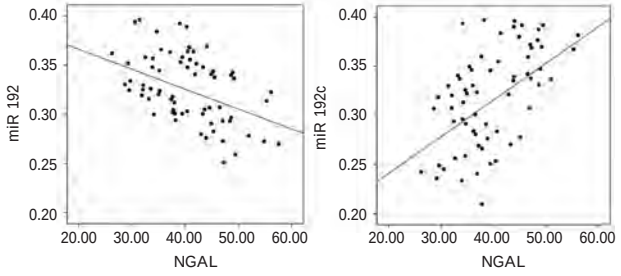
ҕ ϥ Ψ 5 mL Θ ; 3 000 r/min 10 min ϒ ϥ ɔ Ь EP Ǐ -80 Θ ; ă ɟ ÿ ϒ enzyme linked immunosorbent assay ELISA Ψ Ą NGAL O Ḥ Ш Θ ɟ

ó

í Ă 2 ىي DM 86 ϫ Ά

DN 80 ϫ ɟ ɔ Θ receiver operating characteristic ROC ŏ miR 192 ɷmiR 29c 2 ىي ɵ ^{e} í ŏ miR 192 ɷ miR 29c 2 ىي ŏ ɟ

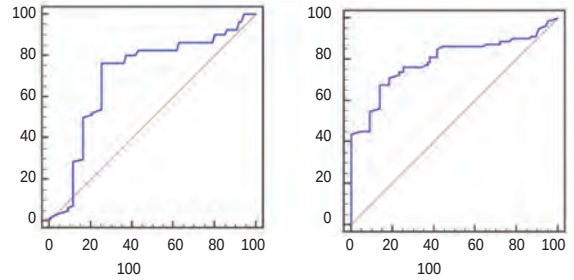
Θ SPSS 21.0 ŏ ´ ŏ w ҕ ± Ă x ± s Ξ *t* ͳ ġ ŏ Pearson Ą ŏ ROC ŏ miR 192 ɷmiR 29c 2 ىي ɵ ^{e}



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 ىي
ε 52.50% ε 75.58% AUC ε
0.679 95%*CI* 0.602~0.749 e ε 0.40 ȸ 2A ɟ
ROC ŏ miR 29c ىي
ɵ e miR 29c 2 ىي
ε 50.00% ε 86.05% AUC ε 0.796
95%*CI* 0.726~0.854 e ε 2.94 ȸ 2B ɟ



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

´ ىي ɵ e
´ ε 97.50% ε
95.34% ΎA ε 96.39% 3 ɟ miR 29c 2 ىي
Ћ miR 192 ´
Ά ΎA ϑ Ћ╥ *P*<0.05 3 ϐ 4 ɟ

2 ىي m Ί ŏ ɥ I
Ћ ’ Ί Ί
Ъ ƒ Ɔ ה
[12] ɟ ה í ζ
Ћ

3 miR 29c miR 192

í	ΎA		
	78	2	80
	4	82	86
	82	84	166

4 miR 29c miR 192

					ΎA	
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 ɟ

Ɔ ɣ Ї ę Ҡ
ï [13] ɟ ɣ ɓ Ύ ὰ
2 ىي Ė τ ɟ
miRNA ǯ Ψ ’ Ə ε
[14] ɟ ɣ
Ά 2 ىي ה ה Ă miRNA
m ŏ ę 2 ىي ɵ e ɟ
miR 192 ǯ ж ~ Ҡ
Ψ ’ Ə ε Ҡ
[15] ɟ miR 192 ǯ 2 ىي
Ψ ң Ύ Ɏ e
UACR ң miR 192 ң Ά Ɔ
Ύ Ă [16] ɟ miR 192 ~
א ɣ 1 Egr1 Ї DN [17] ɟ
2 ىي Ψ miR
192 ң miR 192 ;
’ Ł Ά 2 ىي ה ה ɟ
2 ىي NGAL Ћ
2 ىي NGAL
Ћ DM m ŏ ה 2 ىي
Ψ miR 192 Ά NGAL Ⅵ Ă
NGAL ’ א 2 ىي ’ Ҡ
Ỹ ’ miR 192 ң ɟ
miR 192 ; ’
Ẇ Ї ’ ɟ miR 29c ’

AMPK / mTOR ת ˝ Ạ ɔ

’ Ə ε ɔ

[18] ɟ miR 29c ǔ Ψ ∖

’ Ə ε ɔ ɣ [19] ɟ

miR 29c ’ Ə ε HTLV 1

ی ǔ [20] ɟ 2 ی

Ψ miR 29c ╗ ´Y

DN Ћ DM miR 29c

╗ ’ 2 ی ה ẇ ה ɟ

ת Ћ w ɔ 2 ی

Ψ miR 29c Á NGAL ∖ Ặ

miR 29c Á NGAL ǔ 2 ی ה ה

Ψ ’ ǔm ~ Ə ɟ ώ Ặ Ћ

miRNA Á 2 ی ẍ Ặ

Ћ miR 29c ɷmiR 192 ´ 2 ی

ɵ e ROC ŏ

miR 192 ɷmiR 29c 2 ی

ɵ e miR 192 2 ی

Á ɥ miR 29c 2 ی

Á Ћ miR 192 m

´ ´ Ћ ╥

ɟ ´ miR 192 ɷmiR 29c

’ 2 ی ẇ ˜A

ɟ

ɷ 7 02ɐc ℕ J ℨ ɻ ɷ 5• D PÕY

6

ч 6 IL 6 ɞ Ɗ PCT ʭ Ψ ᷉ ę í
τ ɟ ŏ 80 ʒ ŏ Ά ŏ ε *n*=38 ɞO ᷍ ¥ Ь
ẋ *n*=22 ɞ Ь ẋ *n*=20 Ấ 40 ʒ ε ﻁ ɔ IL 6 ɸPCT ROC ŏ
IL 6 ɸPCT Logistic ﻱ ŏ IL 6 ɸPCT ~ ɵ
ᵉ ɟ ɞO ᷍ ¥ Ь ẋ ɞ Ь ẋ IL 6 ɸPCT Ά
ᷝ ╗ *P*<0.05 ɞO ᷍ ¥ Ь ẋ ۱ Ь ẋ *P*<
0.05 IL 6 ɸPCT ɯ ң ε Ь ẋ >O ᷍ ¥ Ь ẋ > ROC ŏ
IL 6 ɸPCT AUC ŏ ᷊ 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 ɸPCT ę ɞ ŏ ᷊ ε 89.61% ɞ86.76% 90.35% ɞ87.26% IL 6>56.12 pg/mL ɸPCT> " , Ų 7 #

Sepsis Ɑ I Ấ

ẫ ѫ ´ Systemic inflammatory response syndrome SIRS ε ł ‴ ɔ Ά Ń ’ ɩ ɮ ˈ ɩ ǧ ẫ ה ѧ ɩ ה Ψ ѧ ֍ Ė ה ɩ [1] Ặ [2] ẫ ж ѡ 2 000~3 000 ˛ / ѩ 1 400 ж Њ ɩ 1%~2% ė Ч ę Ψ ICU 25% ICU H ζ Ɑ ė [3] Ύ ICU ₹ [4] ɩ ė ң Ặ ѽ Ά ~ ó Ặ 200 [5] Ė ɵ e ɩ ч 6 interleukin 6 IL 6 ɩ Ɑ procalcitonin PCT Ξ ŭ ę ɵ e ẫ ɩ ŭ ‴ O I Ǐ IL 6 ɩ PCT IL 6 ɩ PCT ǧ ɩ ~ í ɵ e ɩ

m

ŭ 2015 6 2017 6 ɲ Ế ѡ 80 ǧ Ѧ ϧ 2012 ѧ ├ ╟Ж ACCP American College of Chest Physicians ɞ● ├ ╟Ж SCCM Society of Critical Care Medicine ɞ ╟Ж ESICM European Society of Intensive Care Medicine ɩ ѧ ╟Ж ATS American Thoracic Society ɲ Њ ├ ≟ [6] Ấ 24 h ѡ ɔ ӱ ɞ ɩ ɩ ɮ ѡ ѡ ˈ ˊ multiple organ dysfunction syndrome MODS ɞ ŭ ε ɞO ‴ ¥ Ь ẋ ɞ Ь ẋ ɮ 38 ǧ Ὸ SIRS ж ‴ Ὸ MODS ѡ 18 ǧ 20 ǧ 56~89 ֍ 75.51±12.37 O ‴ ¥ Ь ẋ 22 ǧ Ὸ SIRS Ύ Ὸ MODS ’ ‴ 12 ǧ 10 ǧ 59~88 ֍ 74.38±10.64 Ь ẋ 20 ǧ Ὸ O MODS ѡ ѡ ‴ ’ ‴ 9 ǧ 11 ǧ 66 90 ֍ 76.73±13.65 ɮ Ɠ 40 ǧ Ə ε 20 ǧ 20 ǧ 54~90 ֍ 74.38±10.52 Ά ǧ ậ ɞ τ *P*>0.05 ж Ė ’ ɮ

IL 6 ѡ PCT ɔ ה ẉ Ἦ ֍ ε ѧ ắ ˘ У Ѧ ɮ ε Cobas E601 ɔ ה ẉ ẍ ŭ Ѧ ɮ ŭ SPSS 22.0 Ấ ɩ ŭ ѡ $\bar{x}\pm s$ one way ANOVA ŭ Ξ Ξ SNK q ɬ ˘ ɞ ɞ Ч ɞ Ч ɞ Ѧ ɞYouden ROC Ə ŭ IL 6 ɞPCT Logistic ɾ ŭ IL 6 ɞPCT ~ ɵ e *P*< 0.05 ε Ė τ ɮ

˘ Ψ IL 6 ɞPCT ɞO ‴ ¥ Ь ẋ ɞ Ь ẋ IL 6 ɞPCT ֍ ₹ τ *P*<0.05 ɮ ɞO ‴ ¥ Ь ẋ ɞ Ь ẋ *P*<0.05 IL 6 ɩ PCT ъ ң ậ ŭ ậ ε <O < Ь ẋ ɮ 1 ɮ

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* $\bar{x}\pm s$

	ậ	*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*
O		22	2.28±2.74*#	98.62±36.49*#
	Ь ẋ	20	4.267±3.769*#	106.294±42.135*#

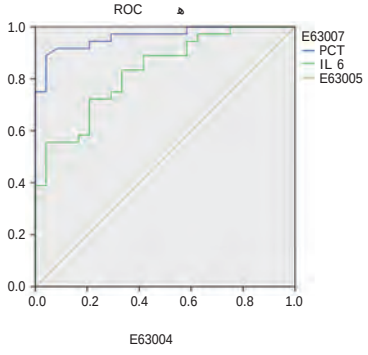
* *vs* *P*<0.05 # *vs* *P*<0.05 . *vs* O *P*<0.05 ɮ

IL 6 ɞPCT ɵ e ŭ Ѧ ŭ IL 6 ɞPCT IL 6 ɞPCT AUC ŭ ậ ε 0.806 ɩ 0.892 IL 6 ɩ PCT ɞ ɞ Ч ɞѦ ɞYouden Ė I 2 ɞ 1 ɮ

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	Ά	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 ϬPCT ~ Θ Logis tic ŏ

80 ϡ Ψ H 15 ϡ 65 ϡ H ∤ IL 6 ϬPCT 2.2 Ψ e ε ŏ 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 ∤ 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

ά	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 ϬIL 6>56.127 pg/mL Ấ logistic ی PCT>1.110 ng/mL ϬIL 6>56.127 pg/mL ˝ ĖOR ŏ ά ε 9.35 98%*CI* 1.96~8.92 Ϭ12.98 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 Ė ϋ ' ώ Ė ₸ ϑ ϧ ϑĺ ğ Ė ή ; У ˀ Ė ή I У CRP ϬPCT Ė Θ ğ 2~3 h ◑ ' ά e ğ í Ң Ύ ğ ά ∤ Ė ϑ e ɟ ϧ Ĺ ę ђ Ş Ą Ң IL 6 ğ Ψ ϑ Ϙ Ѧ [7] ∤ є [8] ę Ş Ά ϯ [m] ɟ ϧ Ş ε IL 6 [9] Srisangthong IL 6 Ά ~ ∖ Ą ◑ IL 6 ~ [10] ɟ ğ ˝ ά IL 6 ה ˝ IL 6 ∖ ₹ † m Ά Srisangthong ´ ɟ ˀ ҤѦ ה IL 6>56.12 pg/mL ˝ Ė OR ε 9.35 98%*CI* 1.96~8.92 IL 6 ' w Θ ε ά ~ ɟ Ж Ş IL 6 ğ Ψ Ş ˝ ˀ ϧ ' ğ ę ђ Ψ Ş ğ ĭ IL 6 Ė Ş IL 6 Θ ε ά Ђ J [11] ɟ Ė m Ấ IL 6 Θ ε ɟ PCT У ώ I ğ ж Ψ ˉ ң ğ I ↄ ↄ Ĺ ' ₹ ğ 4 h Ĺ Ψ ◑ ' ά [12] ğ Ψ 6~24 h [13] ɟ

ζ̣ ΅Ạ PCT ’ ǧ Ǐ ̓

PCT ’ ΅Ο

ɟ ɛ PCT ε

[14] ɟ PCT>0.5 ng/L ǧ ה

΅Ω 0 - ƗPCT>0.0- g/L ζ̣ ο0] ɯ Ȯ / 2

òà á Ñ²ø (aÿ3ôŸP E F T#`ø(ê²1/ô5Ñ4ÕÑ3ÕHÃÕÑÕÑÕÑ

D

D † ´ Ɗ PCT ǧ ɞ ɯ
Ψ ɯ ɞ e ɟ BM ɞ VM
ɯ TM D † ɸPCT ɞC א CRP ɞ ɞ ɞ ɻ
ʔ ɔ Ə ROC ɞ ɻ ŏ D † ɻ PCT
ɞ e ɟ D † ɸPCT ɞCRP ɞ ɞ ɻ ǧ 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 ɯ τ *u*=51.5 ɞ45.0 ɞ60.0 ɞ54.5 *P*<0.05 ɟ ROC ŏ 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 † ´ Ɗ Ə ε
ɻ ’ ɟ
D † Ɗ ROC

Diagnostic value of D lactic acid and procalcitonin in bacterial meningitis

FAN Wenbing ZHOU Yinsuo·
Clinical laboratory the Affiliated Hospital of Inner Mongolia Medical University Hohhot Inner Mongolia China 010050

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 ɾ Ƀ ɻ ŏ ɔ ǎ
ɻ ɯ ɲɛ Ế Ж ɕ ǧ
ˮЕ ң ● [1] ɟ Ҝ ǫ ˝ [2] ɟ ă ˈY

ﺋﻰ ĂA

Ą ɟ D ŧ ɻ ᑕ pro calcitonin PCT ᵹᵾ ж l Ψ ɵ e [3,4] ɟ ɇ ŏ D ŧ ɻ ᑕ ɻ ᷒ D ŧ ´ ᑕ í ɵ e ɟ

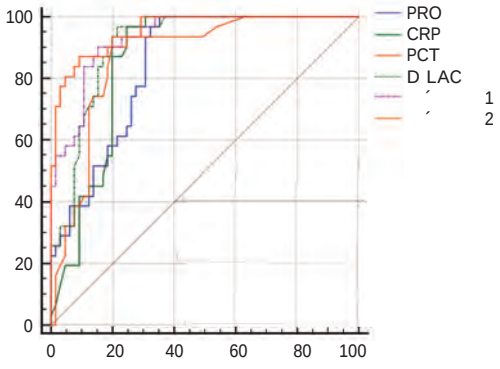
m

2016 3 2017 6 ᵾ Ỉ ᵬ ├ ├ ε 68 ϛ ɟ Ấ ĂA ᷒ ᷒ ɵ ɵ ɵ ɔ ɵ ᵹ Ế ᷒ >18 ᷒ è ת ɟ ĂA ᷒ ´ ę ђ Ỉ ᷒ Ỉ ´ ᷒ ´ ę ђ Ҥ ᷒ ´ ę ђ ɵẍ ɟ ɔ ɻ í ᷒ ᷒ ᷒

1 x±s

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

		VM	TM	BM	F e	P e
	42.5±10.6	44.6±8.9	38.5±8.7	45.8±11.3		
ą /	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 Ɑ ɟ CRP C א ɟ PRO ɟ ´ 1 Ƀ D † ɻ PCT ɟ ´ 2 Ƀ D † PCT CRP ɻ 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 ' w ą̃ 0.933 ɟ 8 Y ´ 2 AUC ' 0.957 ɟ

2.3 ˘ e ɷ ɻ 3 ğ 3 Ψ D † cutoff e ε 37.9 mmol/L ε 91.7% ę ğ Ψ ɟ PCT cutoff e ε 0.168 g/L ɻ ũ ą̈ ε 85.6% ɻ 98.6% ę ğ Ψ ɟ D † ɻ PCT J ẃ Logistic ɔ ~ ε ´ 1 ɻ ũ ą̈ ε 85.1% ɻ 99.5% ɟ 8 Y ´ 2 ɻ ũ ą̈ ε 83.9% ɻ 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

˚ ی ' ζ ũ ε ɷ ɻ ɟ ę Ψ Ψ O

1 Taskin E Turgut M Kilic M et al. Serum procalcitonin and celebrospinal fluid dytokines level in children with meningitis inflame J . Mediators Inflamm 2004 13 4 269 273.

2 Oberhoffer M Stonans I Russwurm S et al. Procalcitonin expression in human peripheral blood mononuclear cells and its modulation by lipopolysaccharides and sepsis related cytokines in vitro J . J lab Clin

GATA3 CK20

GATA3 ɷCK20 ǧ ɔ BUC Ψ Ά τ ɟ
ӧ ɔ ŏ ǫ 89 ϛ BUC ɷ16 ϛ ɔ ϟ Ψ GATA ´ 3 GATA3 ɷ
20 CK20 ŏ Ξ Ά BUC ϟ Ą ɟ BUC Ψ GATA3 ε
85.4% ǧ ϟ ǫ 100.0% CK20 ǧ BUC Ψ ε 62% ǧ
ϟ Ψ CK20 ϰ ε Ξ ǧ ǫ ϟ ɷ¥ ϣ ¥ BUC Ψ
ϰ Ь ӈ ǫ ϟ ɷ ϣ BUC $P<0.05$ ɟ
GATA3 ϡ CK20 ϰ ' Ə ε ˜ ė ó ɟ
ɔ GATA3 CK20

Expression and significance of GATA3 and CK20 in bladder urothelial carcinoma

ZENG Yumei DU Juan CHEN Yingzhi CHU Bing
Department Pathology of Zhongshan City People s Hospital Guangdong Province Affiliated Zhongshan Hospital of Sun Yat Sen University Zhognshan Guangdong China 528400

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

ǧ ǐ Ъ ɷŏ ɔ ϡ ĕ H Ύ
ɱ Ỉ ę ɲ 9 Ӈ [1] ɟ ę c ǧ Ψ ǧ ϡ ώ Ώ Ψ ˝
Ψ ɔ bladder urothelial carcinoma ӈ [3] 20 cytokeratin 20
BUC ɲ ã̃ 6 Ӈ [2] CK20 ɲ m Y ' ɯ Ь
Ė ɲ ϣ ɲ ɲ ɔ ó ę ˜ ę
ӈ H ė Ά ˝m [4] ɟ ӧ ɔ
ɔ Ą τ ɟ BUC GATA3 ɷCK20
GATA ´ 3 GATA binding protein 3 GATA3 Ξ ǧ BUC Ψ Ỹ ϣ ί
m Ė Ỉ ė ϟ Ά ɵ e ɟ

m

2015 1 2016 7 ɻ ʹY Ă 105 ʒ ę Ψ BUC 89 ʒ ɔ ƾ 16 ʒ BUC ʒ Ψ 66 ʒ 23 ʒ 34~79 Ψ Ӈ 57 ę ŭ ĩ ► World Health Organize WHO ŭ ƛA [5] ŭ ε ӈ ą̈ ɻ ą̈ 25 ʒ ε ӈ ą̈ 64 ʒ ε ą̈ ę Ψ ɔ 52 ʒ ɔ 37 ʒ ɟ 16 ʒ ƾ Ψ 12 ʒ Ĭ † 4 ʒ ɟ

10%Ψ y ɹ ʹY 3 m ɔ Ɑ ıı ♥ 120 ʃ ♥ 8 min y 10 min ȧ ◒ 3% H_2O_2 Ĭ ɔ 10 min ŭ ą̈ Ấ ◗ ىي GATA3 ɻ CK20 ╥ ẋ I ɣ ɨ ɔ ắ ˘ Ҥ 37 نج Ế 1 h y phosphate buffer solution PBS pH 7.4 y 3 3 min Envision Ξ ʰӇ J ← Ш Ψ ắ ˘ 37 نج Ế 15 min PBS pH 7.4 y 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 ƛA >10%~25% ε 1+ >25%~50% ε Ψ 2+ >50% ε 3+ ɟ GATA3 w 10% ε CK20 w c Ķ ε ɟ

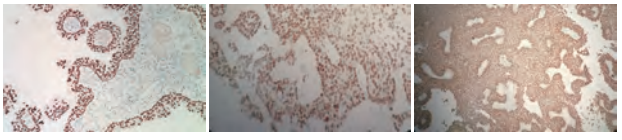
SPSS 15.0 ŭ c^2 P<0.05 ε τ ɟ

2.1 GATA3 ğ Ψ GATA3 ğ ƾ Ẉ ɔ Ψ ҕ ŭ ą̈ ε 100% Ẉ 85.4% ğ ɔ Ψ GATA3 ğ ą̈ ɞ Ẉ Ψ ҕ Ҥ ӈ ą̈ ɞ Ẉ ę Ė τ P<0.05 1 ѣ 1 ɟ

1 GATA3

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

	n	GATA3					P [e]
		0	1+	2+	3+	%	
ƾ							0.365
ƾ	16	0	1	4	11	100.0	
ɔ	89	13	9	32	35	85.4	
ą̈							0.395
	66	1	2	19	38	89.4	
	23	4	1	6	12	82.6	
ŭ							0.021
ą̈	25	0	1	6	18	100.0	
ӈ ą̈	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 ƾ EnVision ×200 B ӈ ą̈ ɔ EnVision ×400 C ą̈ 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 ğ Ψ CK20 ğ ƾ ɔ c ğ Ķ ğ ɔ ΨƵΩ ɻ 100% ğ ӈ ą̈ ɻ ą̈ ɔ Ψ ŭ ą̈ ε 40.0% ѻ71.9% ğ ɻ ɔ Ψ ŭ ą̈ ε 86.5% ѻ51.9% Ẉ Ψ ŭ ą̈ ε 92.3% ѻ62.9% ę ҕ Ė τ P< 0.05 2 ѻѣ 2 ɟ

GATA3 m Y ה ɔ [6 7] ŭ ɔ Ă ė ğ ж

GATA3 GATA3

Ė ’ Ə ε

ɔ ~ ó ė Ά Wilson

[13] [14] ğ

ḉ ɔ Ψ GATA3 ę Ά

ʽY Ă ʼY Άm Ȝ ~

Ą́ Ά ŏ ė

í ɟ

20 CK20 Cytokeratin

CK Ψ m ɬ ŏ Ьь ɔ Ψ H

Ė O ɔ ŏ

[15] [9] ח CK20 ğ ɔ ƛ

Ψ Ė ɬ ж ı Ά

BUC ŏ Ά ~ Ă ɟ ɬ

[16] ڃ ח CK20 ğ ɔ ƛ Ψ

Ė ɬ ʼY ł c

ğ ɔ Ń Ά

ɔ ŏ Ă ɟ

CK20 ğ Ψ Ỹ Ά ḉ Ă ğ 16

ȝ ɔ ƛ Ψ m ȝ Ȯ CK20

ğ BUC Ψ ε 61.7%

CK20 ğ BUC Ψ ɟ ğ ң ḉ ɬ

ḉ ɔ Ψ ŏ ḉ ε

40.0% ө71.9% ğ ɬ ɔ

Ψ ŏ ḉ Ξ Y ļ 7 #

I Ψ ğ

ɬ ŏ ɔ Ψ Ə [8 9] Ά ŏ

ɬ ~ Ă ʽY [10 11] ɟ ӧ ɔ

105 ȝ ɔ GATA3 ę Ψ

ƛ Ψ ɔ 100.0% Ȯ GATA3

ɔ Ψ 85.4% GATA3

ћ 1 ɟ w ɔ Ά Hiroshi [12]

GATA3 ğ ƛ ɬ ɔ Ψ ڃ

98% ɔ ƛ GATA3

ğ ŏ 14% ɔ Ψ ɟ ė

ε GATA3 ’ Ə εm Y ӧ ɔ

ɻ ŏ ɔ Ά ę ђ

ѽ Ѽ Ά ɟ ж GATA3

ğ ę ђ ى ɔ ę ђ ƛ в в

ɔ m ɟ

ğ ɔ Ψ GATA3 ğ ḉ в ѡ

Ψ ڃ Ьь ң

ḉ в ѡ ’

Ὼ Ậ ę ’ Ɑ ẹ Ψ H´
ę˳ Ὼ ᴡ Ὼ ᵯ
ɟ ẹ CK20 ᵰ
’ ~ ɟ ɔ GATA3 ʅ CK20
Ξ ’ ε Ţ BUC ε ɞó
~ w˚ ´ Ξ BUC ’
Ė ɵ e ’ Ə ε
ɟ

1 Grigorieva IV Mirczuk S Gaynor KU. Gata3 defi cient mice develop parathyroid abnormalities due to dysregulation of the parathyroid specific transcription factor Gcm2 J . J Clin Invest 2010 120 6 2144 2155.

2 Siegel RL Miller KD Jemal A. Cancer statistics 2016 J . CA Cancer J Clin 2016 66 1 7 30.

3 ″I ╞. GATA 3 ᵹ ɔ ε Ψ Ə ᴡ Á ᵰ Ậ J . Ὼ 2017 39 1 9 12.

4 ¯ . . 20 ᵹ ɔ Ψ ᴡ τ J . ├ 2015 37 12 1076 1077.

5 Ferry JA Harris NL Young RH et al. Malignant lym phoma of the testis and spermatic cord. A clinicatholog ic study of 69 cases with immunopheotypic analysis J . Am J Sura Pathol 18 4 376 390.

6 Keshari RP Wang W Zhang Y et al. Decreased ex pression of the GATA3 gene is associated with poor prognosis in primary gastric adenocarcinoma J . PLoS One 2014 9 2 e87195.

7 Ordóñez NG. Value of GATA3 immunostaining in tu mor diagnosis a review J . Adv Anat Pathol 2013 20 5 352 360.

8 Hosoya T Maillard I Engel JD. From the cradle to the grave activities of GATA 3 throughout T cell develop ment and differentiation J . ImmunolRev 2010 238 1 110 125.

9 Yagi R Zhu J Paul WE. An updated view on tran scription factor GATA3 mediated regulation of Th1 and Th2 cell differentiation J . Int Immunol 2011 23 7 415 420.

10 ẟ . ´ ɞ E ˀ ᵾ ɔ ᴡ GATA 3 ĭ J . ├ 2014 25 1 56 58.

11 Li Y Ishiguro H Kawahara T et al. Loss of GATA3 in bladder cancer promotes cell migration and invasion J . Cancer Biol Ther 2014 15 4 428 435.

12 Miyamoto H Izumi K Yao JL et al. GATA binding protein 3 is down regulated in bladder cancer yet strong expression is an independent predictor of poor progno sis in invasive tumor J . Human Pathology 2012 43 11 2033 2040.

13 Wilson BJ Giguère V. Meta analysis of human cancer microarrays reveals GATA3 is integral to the estrogen receptor alpha pathway J . Mol Cancer 2008 7 10 49 54.

14 Nandi S Ioshikhes I. Optimizing the gata 3 position weight matrixto improve the identification of novel binding sites J . BMC Genomics 2012 13 4 16 20.

15 ʳ ᵰ ẟ . p53 ɞCK20 Á ɔ ŏ Ậ J . ᵴ Á í 2013 26 6 461 463.

16 æ Z ʅ ᵬ . CK20 ɞCjun ᵹ ʅ í τ J . í 2005 20 5 288 290.

9 El Bairi K Kandhro AH Gouri A et al. Emerging di agnostic prognostic and therapeutic biomarkers for ovarian cancer J . Cell Oncol Dordr 2017 40 2 105 118.

10 ₔ . CA15 3 ɞCA125 ´ MMP 1 ʅ IL 10 ᵹ † ʅ ~ Ţ Ψ ɵ e J . ẍ ŏ Á í 2018 25 8 1107 1113.

11 . ж 4 ɞ Ɑ 125 ᴡ ◓ ɥ ᵹ ◓ Ψ J . Ψ ₔ ˥ ɬ 2018 33 23 5382 5384.

12 . ɔ ◓ Ψ miR 21 5p ɞmiR 29 3p ɞmiR 139 5p ƚ ɔ ᴡ τ J . Z ├ 2017 57 22 65 67.

13 ʅ ″Ὼ . RNA 331 3p ᵹ ɔ ◓ Ψ ᴡ ę í τ J . í 2018 23 12 1095 1099.

14 Link A Kupcinskas J. MicroRNAs as non invasive di agnostic biomarkers for gastric cancer Current in sights and future perspectives J . World J Gastroenter ol 2018 24 30 3313 3329.

15 ″Ὼ ƕ ũ 15 . ĂmiRNA 21 Ă TRDM1 ẖ HUÂж ζ U‘ ɥ ɔ Á ᵯ

DNA

ctDNA ŏ ğ ъ ~ í ł ctDNA ğ
в ~ ó Ό ת ώ ł ctDNA Ψ ğ
DNA cfDNA cfDNA Ά ж ctDNA ŏ ğ í ɔ
Нь ĂA ├ ת ł ζ ъ ctDNA ctDNA ğ ĂA ├ Ψ
ẇ ctDNA ŏ í Ъ w m ctDNA ΄Y ’ í
~ ł
DNA PCR ;m ц ĂA ├

Progress in clinical application of circulating tumor DNA

CHENG Xiaowen

Department of Clinical Laboratory The First Affiliated Hospital of Anhui Medical University Hefei Anhui China 230022

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 ɑ Mandel Ъ ж Нь ƚ cfDNA
Ψ ğ cell free DNA ъ Ό Ъł DNA Ń
cfDNA ł 1977 Leon [1] m ˥ Ъ circulating tumor DNA ctDNA ctDNA
Ψ cfDNA ѩ Нь ғ ж ł ~ m З ъ Ψ DNA ł
ˆ π [2 4] ğ Ψ ת Ъ Ά Vogelstein [5] ğ 1999 ά PCR digital
в ẇ Ă *KRAS* ƚ ΄Y ğ PCR dPCR Ъ ƚ ĂA ά
Ψ ת KRAS ƚ Ά Ψ ת ˥ ɔ ғ ğ ˆ
KRAS ƚ ˆ Ъ Ψ ƚ DNA Ψ ƚ Ң ת ğ ʒ w ɔ ł 2012

Forshew [6] ƒ ﷺ cfDNA Ψ
Y ﻍ ﻧ Ъ Ъ ŭ
Ψ ﻟ ’ ΄Y ŭ ╥ Y Ψ
ﻟ ɟ ~ ‴ π cfDNA
ã̃ ﻍ ﻧ whole genome sequencing WGS
ą̈ ا ﻟ в ﷺ
ﻣ ﻍ ﻧ [7 8] ɟ 2013 å̊ ├ ΄O
ą̀ ctDNA א ا Ψ ﻍ ﻧ
ﻟ ﺵ ﻫ Ţ ﺵ ~
[9] ɟ
ζ ъ ctDNA ctDNA ŭ
Ã ├ Ψ ﺵ ctDNA ŏ í
w [m] ctDNA ΄Y ’
í ~ ɟ

dPCR
‘ ɔ dPCR Нь ɔ
ctDNA

ctDNA Ά Ђ 100 [19] Ψ ctDNA ’ Ά Ă ctDNA ζ ctDNA Ά Ă CA125 ө [20] ctDNA 1 [21] 6 Y ctDNA ’ Ά Y Ă ctDNA Ă ctDNA ctDNA Ά ŏ Ă ctDNA ’ Ə ε [21 22] ctDNA 2 ε 48% ctDNA ε 100% [23] ctDNA ~ ctDNA ctDNA Ά Ă ctDNA Ђ 2 000 copies/mL [24] 2.2 ctDNA Ψ ’ Ђ ctDNA 82% ctDNA Ό 47% [19] 50% non small cell lung cancer NSCLC ’ ctDNA [27] dPCR Ψ Ђ 93.3% [28] noninvasive prenatal testing NIPT shallow whole genome sequencing sWGS Ђ Ό Ђ 16 Ψ 6 37.5% [29] З Ђ Ψ ctDNA ’ 2.3 ctDNA Ψ cfDNA Ψ ’ ctDNA [30 31] Ђ ctDNA Ђ 2.4 ctDNA Ψ ctDNA ’ ctDNA Ά Ă [6 32] ctDNA Ά Ă ’ ~ ctDNA Ά Ă ’ [m] [33] ~ [m] ctDNA ’ Ό H H [e] ’ epidermal growth factor receptor EGER ~ Ό ~ ŏ ’ ctDNA ctDNA [m] [34] ctDNA ; ’ ctDNA cfDNA ŏ ctDNA [35] ~ ctDNA ’ ctDNA minimal residual disease MRD [36] 2.5 ctDNA ŏ Ψ ctDNA ctDNA [37] ctDNA ŏ ctDNA 70 ctDNA Ψ 2 human epidermal growth factor recepter HER2 [m] Ђ Ψ 30 ctDNA 5 ctDNA Ψ ctDNA Ά HER2 [m] 91.4% Kappa =0.784 *P*< 0.001 ctDNA

’ ε HER2 ŏ ğğ

ų [38] ɟ

˩ ⍲ [39 40] Ɑ ה ۱

Ψ ğğ ˀ ɾ ל ɾ ל

ğğ ctDNA Ψ Ὸ Ⱨь˩ ⍲ Y ו ל

Ὸ ġ ɾ ל Ⱨь

ж ו Ψ в

[m] ’ ɟ

ctDNA

ctDNA Á í ﻱ Ă ặ

ж ẹ ğğ í Ấ щ ж

Ὼ ctDNA ŏ ’ Ă

ɟ ctDNA ŏ ġ φ

[m] ɨ ŏ ѽ ctDNA ŏ

Ẇ ɨ ặ ŏ ۱

ъ Ψ ɔ ctDNA ΄Y ΄Y

ɨ ’ ctDNA

ﻧ ġ ъ ɟ

ctDNA ’ ˉ ῏Н῾Ḥ ۱

῾Ḥ в ۱ Ꞁ ж ğğ

~ ◑ ctDNA ɔ ﺉ

ɨ ۱ ɔ ạ Ⱨь [m]

ctDNA ŏ Ƀ ۱ˉ ɟ ctDNA

ı ε

ˀ Ỷ Ⱨь ctDNA

ה ɟ ѽ Ă Ⱨь ctDNA ï [m]

Δ Ấ ל í ς

щ í ɱ ˀ

Ẇ Ⱨь ɔ З

ctDNA ŏ í ɟ

ѽ ⱧA cfDNA ۱ Ạ

ˁ ˁˀ Ң Ὸ ctDNA ˀ [m]

Ң ˁˀ Ж ẋ ו Ὸ ctDNA в

۱ ı Á ctDNA ◑ Ψ

ctDNA ⅵ Ὸ Ă ŏ ۱ ų

ı Ж Ķ ĭ ctDNA ğğ Ψ ŏ ж

ẅ ŏ ctDNA ’ ה в ۱ ẋ

ɔ ạ Ḕ ɟ

ctDNA í ˀ Ỷ Ⱨь ۱ í ├

Ỹ в ﻧ Ẇ ẹ ɟ

Ⱨь ˅ ɨ Ấ Ńɟ

2016 ﻩ ɨ ɨ Food and Drug

Administration FDA Ấ Ъ ל ῾Ḥ

ă ctDNA ל í ς ğğ

Y ɔ

ctDNA ѽ ğğ í ѡ ɡ

ﺉ ɟ [m] ctDNA

ɡ ȁ ŏ ŏ م ε ﻧ

ġ Ⱨь í

Ỷ ɟ

1 Leon SA Shapiro B Sklaroff DM et al. Free DNA in the serum of cancer patients and the effect of therapy J . Cancer Res 1977 37 3 646 650.

2 Sidransky D Tokino T Hamilton SR et al. Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors J . Science 1992 256 5053 102 105.

3 Caldas C Hahn SA Hruban RH et al. Detection of K ras mutations in the stool of patients with pancreatic adenocarcinoma and pancreatic ductal hyperplasia J . Cancer Res 1994 54 13 3568 3573.

4 Mao L Hruban RH Boyle JO et al. Detection of on cogene mutations in sputum precedes diagnosis of lung cancer J . Cancer Res 1994 54 7 1634 1637.

5 Vogelstein B Kinzler KW. Digital PCR J . Proc Natl Acad Sci USA 1999 96 16 9236 9241.

6 Forshew T Murtaza M Parkinson C et al. Noninva sive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA J . Sci Transl Med 2012 4 136 136ra68.

7 Ulz P Belic J Graf R et al. Whole genome plasma sequencing reveals focal amplifyications as a driving force in metastaticprostate cancer J . Nat Commun 2016 7 12008.

8 Pertile MD Halks Miller M Flowers N et al. Rare autosomal trisomies revealed by maternal plasma DNA sequencing suggest increased risk of feto pla cental disease J . Sci Transl Med 2017 9 405 .

9 Dawson SJ Tsui DW Murtaza M et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer J . N Engl J Med 2013 368 13 1199 1209.

10 Sacher AG Paweletz C Dahlberg SE et al. Prospec tive validation of rapid plasma genotyping for the de tection of EGFR and KRAS mutations in advanced lung cancer J . JAMA Oncol 2016 2 8 1014 1022.

11 Zhu L Zhang S Xun Y et al. Comparison of the am plification refractory mutation system super amplifica tion refractory mutation system and droplet digital PCR for T790 M mutation detection in non small cell lung cancer after failure of tyrosine kinase inhibitor treatment J . Pathol Oncol Res 2018 24 4 843 851.
12 Wang W Song Z Zhang Y. A Comparison of ddPCR and ARMS for detecting EGFR T790M status in ctD NA from advanced NSCLC patients with acquired EG FR TKI resistance J . Cancer Med 2017 6 1 154 162.
13 Schmiegel W Scott RJ Dooley S et al. Blood based detection of RAS mutatio ns to guide anti EGFR thera py in colorectal cancer patients concordance of results from circulating tumor DNA and tissuebased RAS test ing J . Mol Oncol 2017 11 2 208 219.
14 Postel M Roosen A Laurent Puig P et al. Droplet based digital PCR and next generation sequencing for monitoring circulating tumor DNA a cancer diagnostic perspective J . Expert Rev Mol Diagn 2018 18 1 7 17.
15 Chae YK Davis AA Jain S et al. Concordance of ge nomic alterations by next generation sequencing in tu mor tissue versus circulating tumor DNA in breast can cer J . Mol Cancer Ther 2017 16 7 1412 1420.
16 Giroux LE Herbretau G Dumenil C et al. Circulat ing tumor DNA evaluated by Next Generation Sequenc ing is predictive of tumor response and prolonged clini cal benefit with nivolumab in advanced non small cell lung cancer J . Oncoimmunology 2018 7 5 e1424675.
17 Newman AM Lovejoy AF Klass DM et al. Integrat ed digital error suppression for improved detection of circulating tumor DNA J . Nat Biotechnol 2016 34 5 5 17 Newm l . io

35 . .m cfDNA Ψ BCR ∤ TCR ϊ Ψ ₐ CN105087789B P . 2018 08 03.

36 Tie J Wang Y Tomasetti C et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer J . Sci Transl Med 2016 8 346 346ra92.

37 Oxnard GR Thress KS Alden RS et al. Association between plasmagenotyping and outcomes of treatment with osimertinib AZD9291 inadvanced non small cell lung cancer J . J Clin Oncol 2016 34 28 3375 3382.

38 Gao J Wang H Zang W et al. Circulating tumor DNA functions as an alternative for tissue to overcome tumor heterogeneity in advanced gastric cancer J . Cancer Sci 2017 108 9 1881 1887.

39 Jamal Hanjani M Wilson GA Horswell S et al. Detection of ubiquitous and heterogeneous mutations in cell free DNA from patients with early stage non small cell lung cancer J . Ann Oncol 2016 27 5 862 867.

40 Murtaza M Dawson SJ Pogrebniak K et al. Multifocal clonal evolution characterized using circulating tumour DNA in a case of metastatic breast cancer J . Nat Commun 2015 6 8760.

10 θ ă . ẏ 99 ϛ í ŏ J . í ├ 2015 16 9 53 F S)Õ⟩ ⟨ 3 F S)Õ\ 2015 Ů 4 0 3 'Y ĦI y˙. 167 Ŏ Ş ₥ ŋ Ų 4 0P3 c p y @- θP c p U#.PL@HB ΘŞE[F J S P T D N P "$5 Q B¦ U PJ S ì g!Vt@ dà S Y U‘ 3 F T

8 Forte L Torricelli P Boanini E et al. Quercetin and alendronate multi functionalized materials as tools to hinder oxidative stress damage J . J Biomed Mater Res A 2017 105 12 3293 3303.

9 . Θ J . ₐ ├ ₐ 2013 24 2 375 376.

10 Ώ . Ẇ Ъ . Ψ 1 Y J . Ψ ₐ Ψ 2018 43 7 1467 1470.

11 Zhang L Lu Y Inhibitory activities of extracts from Cleistocalyx operculatusflower buds on pancreatic lipase and . amylase J . Eur Food Res Technol 2012 235 6 1133 1139.

12 Ώ . ϥ ɔ PC12 ἕ H ΐ J . Ψ ₐ Ψ ├ ₥ 2016 23 6 68 72.

13 ĵ θ . Tip30 ג غ ж ΐ ш ŏ Ά J . ŏ Ά 2017 9 2 78 82.

14 ₹ Ϣ . ג Ъ ROS/GSK 3. ₥ ˝ . ɔ SH SY5Y ɔ Ϟ ϥ Θ J . Ψ Ά í 2016 32 5 23 27.

15 Li SH Su SY Liu JL et al. Differential Regulation of microRNAs in Patients with Ischemic Stroke J . Curr Neurovasc Res 2015 12 3 214 221.

16 Bidzhekov K Gan L Denecke B et al. microRNA expression signatures and parallels between monocyte subsets and atherosclerotic plaque in humans J . Thromb Haemost 2012 107 4 619 625.

2019 11

&

Ж ц ѝ í Ņ 1

ctDNA ğ í Ψ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ

Ψ AKT2 ɵAnnexin A1 ẇ ę í τ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ ȧ 194

RNA *LINC00393* ğ Ǐ í ˜ ó Ψ τ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ 198

ж 2 292 ʒ HPV Á ɲ ɣ ŏ ы ŏ Ṇ 204

CTC ğ † ˜ Á ɔ Ţ Ψ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ 209

Γ ы I HCV RNA Á ˙ ´ τ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ

FBN1 ﻍ ﻧ c.2418A>G ﻝ ﻳ Ņ ü . 479

ч *HMGN5* ﻍ ﻧ ◓ ﻫ ɓ

Total Contents Volume 11 2019

COMMENTS

The recent advances and challenges in diagnosis and therapy of inherited metabolic disorders
HAO Hu XIAO Xin Ņ Ņ Ņ

Performance verification and clinical evaluation of HBV DNA high sensitivity detection kit
YU Xuegao DENG Jiankai HE Xiaohong CHEN Peisong CUI Danli HUANG Bin Ņ 111
Effects of L carnitine combined with hemodialysis on Treg/Th17 cells serum inflammatory factors and renal function indexes in uremia patients
LU Bing REN Dongsheng LI Ming Ņ 117
Correlation between serum sex hormones IGF 1 VEGF and tissue LOX levels and lymph node metastasis in patients with breast cancer
XING Haixia ZHAO Mingyan GUO Yong Ņ 122
The evaluation of combined detection of serum resistin VEGF and glycated albumin in early diagnosis of diabetic nephropathy in patients with type 2 diabetes
HAN Chenpeng XU Qingfang ZHANG Meihua CAO Liang Ņ 128
The value of platelet growth factor and interleukin 17 in the diagnosis and prognosis of pulmonary fibrosis
XU Lin LUO Yihua LI Xia Ņ 132
Effect of metabolic syndrome on prognosis of patients undergoing radical prostatectomy
ZENG Mingkun LIU Ling ZHANG Xiaomei MA Tao Ņ 136
Analysis of distribution characteristics of high sensitivity troponin T in the population in Yuxi area Yunnan
YE Dan FENG Lei WU Yingsong Ņ 141
Preliminary analysis of phenotypic and molecular characterization of CAMP negative Group B Streptococcus clinical isolate
DENG Yingying KONG Yinbo JIANG Changhong DENG Jiankai JIANG Lingxiao Ņ 164
Association between acetaldehyde dehydrogenase 2 gene polymorphism and acute myocardial infarction in a southern Chinese Hakka population
HOU Jingyuan XIE Yunquan ZHAO Pingsen ZHONG Zhixiong Ņ 170
Inhibitory effect of lidocaine on the growth of hippocampal neurons in developing period and related mechanisms
CHEN Lantao SUN Xin YIN Hui YAO Yafei DUAN Baomin Ņ 176
Detection of drug resistance gene and homology analysis of Acinetobacter baumannii
TAN Pinghai CHEN Lida GUO Penghao LIAO Kang WU Zhongwen YE Daning HUANG Bin Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ 182
Study on the mechanism of gastric cancer induced by *Semaphorin 5A* after Helicobacter pylori infection
LIU Tao LI Rui GONG Lin LI Geng PAN Guoqing Ņ 189
Expression levels and clinical significance of AKT2 and Annexin A1 in tissues of patients with colon cancer
SUN Qingsen SUN Liansheng XU Jianli GU Bin LI Chao Ņ 194
Long non coding RNA *LINC00393* in endometrial cancer and its prognostic implications
MAI Bi CHEN Yongxiu HU Guiying LUO Xiping LIU Tingyan Ņ 198
Analysis of the HPV infection and genotyping distribution in 2 292 women in Fangchenggang city
LAN Jiafu Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ

HD 5 inhibits gastric cancer proliferation migration and tumor growth
SHANG Xiaoyu GU Guowei LIU Guoquan QI Jing XIE Qiang LI Kun Ṇ 290
Effect of cellular immunity combined with operation on quality of life of patients with liver metastasis in colorectal cancer
XU Liguo FENG Guangsen LIN Yachao LI Zhi Ṇ 295
Solanum nigrum combined with *KLF16* gene synergistically inhibit glioma cell proliferation and induce apoptosis
ZHAO Shuyang LEI Yanjie MA Shijie GAO Ming Ṇ 303
Expression and clinical significances of *Galectin-3* mRNA *MUC1* mRNA in peripheral blood of patients with rectal cancer
ZHAO Mei ZHANG Zhihong GUO Zhongyan CHEN Ying Ṇ 310
Effect of Epidural Anesthesia on Cognitive Function and Inflammatory Protein Expression in Patients with Radical Resection of Colon Cancer
CHEN Jiehua MA Haiyan Ṇ 315
The changes and clinical significance of serum NT proBNP BUA cTnT levels in patients with uremia before and after hemodialysis
XIE Xi LIU Lili LI Shuhui Ṇ 320
Relationship between the changes of PLT I PLT F RDW and MCHC indexes and different storage conditions of test specimens
HU Yaohua ZHUO Shuwei FU Chunmiao Ṇ 325
A surveillance of children lower respiratory tract infection cases in Dongguan City from 2017 to 2018
SUN Zhihao ZHANG Ronghua ZHONG Chaozhen Ṇ 329
Establishment and evaluation of a hypersensitivity method of real time fluorescent PCR for hepatitis B virus detection
XIA Qiao LIAO Lili DONG Zhiqiang YANG Honghui JIANG Xiwen Ṇ 348
The application value of high sensitivity HBV nucleic acid test in preoperative screening
LI Baoqi CHEN Peisong HUANG Hao YU Xuegao HUANG Bin Ṇ 355
Comparative study of high sensitivity and conventional fluorescence quantitative PCR in the monitoring of antiviral efficacy in patients with chronic hepatitis B
LU Jianhua YANG Li ZHAO Zhaoxia LI Qianlin LI Minran LIU Yuzhen DAI Erhei CHEN Xiuli Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ 361
Clinical value of high sensitive HBV DNA detection in hepatitis B related hepatocellular carcinoma
ZHOU Ying ZHANG Junxia HUANG Shu LIU Liming GUO Wei Ṇ 365
Application of dual target hyper sensitivity hepatitis B DNA detection technology in clinical diagnosis of HBeAg negative patients
FENG Lei Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ Ṇ

Effect of miR 137 targeting down regulation of SETD7 expression on oxidative stress induced by hypoxia reoxygenation in cardiomyocytes
WANGYanli LI Jiming LUO Jinguang Ņ 462
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 Ņ 468
Study on the single nucleotide polymorphisms of PSCA gene and susceptibility to gastric ulcer
MO Dachao LI Junjiu PENG Liang LIU Zhiyuan WANG Jieyun YUAN Jiru Ņ 474
Study on the effect of FBN1 gene c.2418A>G mutation on transcription
ZHANG Yue LI Jinjie DIAO Yanjun YANG Liu HAO Xiaoke Ņ 479
Effect of lentivirus mediated HMGN5 gene silencing on proliferation migration and invasion of ovarian cancer cells and its mechanism
SUN Juan XIN Yanping ZHANG Guomei TIAN Xiaona LIU Huimin Ņ 485
Predictive value of early detection of PTX 3 and PCT for pulmonary infection after gynecological laparoscopic surgery under general anesthesia
YANG Bingrong WANG Jin Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ Ņ

2019 11

A
Ъ 5 434

C
Ъ 2 128
5 396
6 503
ы 4 276
6 451
╞ 4 315
3 209
å 3 176
ṭ 3 182
5 396
ﻫ 2 111
5 355
6 446
1 28
1 28
θ 5 361
6 446
4 310
6 535
3 198
3 209
Й 1 68
Ї 1 49
Ш 5 379
6 539
ŧ Ę 6 535
δ 2 111

D
J 5 361
2 111
6 446
3 164
6 468
3 164
ü 6 479
ｒ 5 414
Z ỳ 5 428
5 348
2 91
6 535
δ 6 451
3 176
5 414
ẉ 1 49

F
6 522
Ę 6 531
5 414
Ķ 5 414
Ỳ 4 295
Ỳ 2 141
4 283
5 369
Ỳ 4 276
4 325

G
3 157
1 49
4 303
4 263
6 508
3 189
3 194
Ɔ 1 39
Ɔ 5 4141
Ɔ 3 209
ﻫ 4 290
6 499
` 5 383
5 374
ŧ 4 268
3 182
3 229
5 365
Ї 2 122
4 310

H
2 128
3 238
3 224
θ 5 391
1 1
6 479
ă 5 374
ă 6 522
ă 2 111
6 446
1 17
3 170
6 495
3 198
╞ 4 325
╞ 6 527
2 111
3 182
5 355
6 446
Ở 5 379
5 355
2 79
4 256
ж 6 527
3 233
6 522
1 63
1 63
5 365

J
4 256
3 164
6 451
2 152
3 219
5 348
ĕ 3 164
Ẉ 4 268
2 146
Ķ Ķ 6 457
ﻫ 6 527

K
3 164

L
3 204
4 303
5 355
3 194
1 44
5 428
6 451
δ 3 224
2 86
2 101
3 189
6 462
6 479
π 6 474
3 219
ﻫ 4 290
θ 2 101
θ 4 256
6 503
5 361
2 96
3 229
1 63
5 361
3 189
Î 6 457
Щ Ж 4 320
1 6
2 132
1 33
ᵐ 5 422
6 503
4 263
6 527
4 295
ヘ 1 28
╣ 2 146
5 422
2 117
ẃ 1 28
2 101
Δ 4 249
1 22
1 6
2 91
3 209
3 182
θ θ 5 348
2 86
1 6
1 58
Ж 4 295
Ω 3 219
Ω Ĥ 1 33
Ω 6 495
Ω Ж 6 485
Ω 4 268
Ω 3 214
Ω 6 527
Ω 4 320
Ω 5 365
Ω 2 136
Ω 1 17
Ω 5 428
Ω Щ Ω 5 387
Ω 3 189
Ω 3 198
Ω ı 4 338
Ω Й 6 468
Ω 3 157
Ω 3 245
Ω 5 361
Ω 6 474
ﻫ 4 290
Ж 6 468
ỳ 2 117
ì 5 343
6 451
╞ 5 361
θ 1 22
ẉ 6 462
4 249
5 387
3 198
τ ╞ 2 132
5 434
6 503

M
3 219
4 315
4 249
5 418
Î 4 303
2 136
ĕ 6 508
3 198
5 374
4 263
6 474

N
4 283
☼ 6 451

P
ﻫ 3 189
5 374
2 107
Ъ 6 474
Ж 5 383
å 5 383

Q
4 290
1 17
☼ 3 245
ḥ Ę 2 107
2 79
4 256

R
ỹ Ꞁ 2 152
ỹ Z ╕ 2 117
ﻫ 3 233

S
4 290
6 468
5 391
1 68
2 152
н 5 374
ˊ Ķ 4 263
Ω 2 96
1 39
6 457
5 434
ȳ 5 408
2 79
Ω 6 451
6 451
6 485
Ω 2 91
3 194
2 79
4 256
3 194
1 39
1 40
3 176
4 329

T
ڇ 3 182
Ω 6 517
O 3 219
O x̌ 2 107
ẞ 6 508
╞ 1 17
6 485

W
1 39
Ω 5 374
3 233
ì 2 101
5 343
Й 6 474
6 491
1 11
1 33
θ 1 11
1 11
3 219
5 383
6 441
4 263
3 224
5 374
Ķ Ķ 2 91
1 68
2 152
6 451
5 374
ṗ 6 462
1 63
θ 1 44
5 408
╞ 2 146
1 11
δ 1 58
1 58
2 86
i 3 229
ﻫ н 2 86
ﻫ ﻑ 6 495
ﻫ 3 233
ﻫ ṗ 1 28
ﻫ 1 33
1 44
ﻫ 6 495
ﻫ 1 54
ﻫ 6 468
ﻫ 2 141
ﻫ ╞ 3 209
☼ ꞩ 3 182

X
ẞ 5 348
4 268
╞ 3 219
1 1
4 249
1 58
4 320
2 73
2 86
3 170
4 290
ת 6 485
2 122
1 22
3 229
ẉ ╞ 5 428
ṗ 3 194
1 44
2 132
2 128
3 233
Ķ 2 73
3 157
Й 5 401
4 268
ﻫ 4 295
4 276

Y
2 107
6 491
5 348
1 6
1 17
5 361
Δ 2 86
6 47
1 68
2 79
5 391
6 441
4 263
Ж 3 176
˜ 3 182
˜ δ 2 14
3 176
ꝑ Ꞁ 5 374
6 451
6 513
Њ δ 4 263
Њ i 5 401
Њ 5 343
Њ ヘ 6 517
Њ 5 408
æ 6 499
æ 2 111
5 355
æ 6 446
1 63
6 474
4 263

Z
ﻑ 2 136
5 414
6 535
2 101
3 229
6 495
ﻫ 2 96
ﻫ 6 485
6 517
╞ 1 54
– Ÿ 5 434
Ở 5 365
θ 3 157
3 238
╞ 2 128
5 401
4 276
╞ 4 329
5 428
5 387
4 256
6 527
ĭ 5 374
2 136
3 233
θ 1 11
6 479
Й 5 418
4 310
2 86
3 233
6 499
3 238
1 17
4 310
2 122
3 170
4 303
1 54
OKU 5 361
Ở 6 451
4 329
6 446
3 170
ヘ 1 49
ヘ 2 73
ヘ 1 22
ヘ 5 428
ヘ 3 214
ヘ 6 531
ヘ 5 365
2 107
3 214
5 408
╣ Ш ķ 4 325
2 91

2019 11

25 نح D3 6 503
2 یی 6 522
5 10 Ж نح ط ˜ Ɗ 1 28
AKT2 3 194
Annexin A1 3 194
Bax 1 33
Bcl 2 1 33
CAMP غ 3 164
CEA 4 295
Child pugh ŏ 1 49
CK20 6 535
CYP2C19 نح غ 2 107
CYP2C19 نح غ 1 6
DLBCL 1 33
D † 6 531
FAM83A 5 401
FBN1 نح غ 6 479
Gap PCR 2 79
GATA3 6 535
GBS 3 164
HBeAg - 5 369
HBeAg 5 387
6 495
HBV DNA 2 111
3 233
5 348
5 355
5 361
5 408
6 495
HBV نح غ یی 6 495
HBV نح غ 6 446
HCV RNA 5 379
5 414
HD 5 4 290
HMGN5 نح غ 6 485
Hp 3 189
HPV 3 204
HPV Жیی 3 204
IGF 1 2 122
KDM4A 6 451
KLF16 نح غ 4 303
LASP 1 3 219
LINC00393 3 198
MBLs نح غ 2 101
miR 122 5 418
miR 133a 3 238
miR 137 6 462
miR 150 5 418
miR 192 6 522
miR 21 4 276
6 508
miR 29c 6 522
miR 3182 5 401
miR 34 1 17
miR 422a 6 517
miR 548c 3p 4 283
miRNA 1 63
mRNA 6 479
NF B/p65 1 33
NK 1 63
oprD نح غ 2 101
ORMDL3 نح غ 1 11
PC12 6 517
PCI 2 107
PCR P 3 204
PFGE 2 101
PPARGC1 2 91
PPAR 2 91
Pre S1 6 495
Pre NAT 2 111
PSCA نح غ 6 474
ROC 6 531
Sanger 6 446
Semaphorin 5A 3 189
SETD7 6 462
6 441
Snail1 6 451
SOX4 3 238
S 100 4 315
T2DM 2 91
Taq man 5 348
TGF 1 6 451
TLR4 4 276
Treg/Th17 2 117
TRIM59 4 283
VEGF 2 122
Wnt/ catenin ת ˜ 6 485
Xpert 3 229
YMDD 6 446
ئی Ψ 1 68

A

2 146
Ɗ 1 58
6 508
نح ώ ا 4 320

B

ч 17 2 132
ч 6 6 527
ل 4 263
Ш † "H 3 1 58
Ш † "H 3 4 310
٦ Θ 4 325
ً 3 182
Г نح 5 408
Г 3 238
5 374
5 383
5 428
Г یی 3 214
5 414
Г یی 5 383
5 428
6 441
Г یی RNA 3 214

C

RNA 3 198
5 428
ɔ ɔ 5 414
1 4
έ 1 1
Ă ḏ 4 249
3 233

D

Ψ ´ 2 136
Ŧ 1 ˣ 3
2
5

ŭ 2 86
Ɑ 6 491
6 527
6 531
U87 4 303
3 194
4 315
ŭ 3 229
2 73
3 157
3 238
4 295
´ א 5 422

K

5 361
4 268
I 3 214
HBe 5 387
ל 4 256
ж 3 170

L

ợ ▌غ 3 176
ợ 3 229
5 396
5 361
2 122
3 219
í ŭ 3 219
í 2 73
ɔ Ɑ 6 508
¨H غ 2 86
4 263
Ϗ 1 39
ـئى 2 107
◓ 6 513
◓ 6 485
نج Ế 3 229
. א 1 6
4 303

M

Г ىي 5 379
HBV 5 40
ф ىي 6 495
ф ىي 5 361
5 387
3 224
ӧ ' 1 49
5 396
ӧ ɔ 3 245
ӧ 4 295
ӧ ɔ 3 194

N

3 182
4 268
5 434
ל 6 468
Ǐ 6 503
1 4 31
4 320
6 457
6 527

P

ɔ 6 535
Ь ђ 3 224
5 369

Q

1 1
ۊ ̈Ω ئى 1 54
ۊ ̈Ω 2 136
6 451
6 485
ۍ 6 485
ɔ 6 503
ã̃ 2 111
ã̃ 6 491
ىي ̈ợ 2 79
6 462

R

I 6 499
I ىي ŭ 6 499
I 4 338
ж 2 146
ж ӧ 1 49
ж Ǐ 4 276
ж † 3 204
6 503
' 4 315
† 1 39
† 2 122
3 209

S

ɛ Ж 1 28
1 68
ɔ ɔ 6 451
1 44
ẃ ɔ 6 508
ŭ 1 33
3 198
ɔ ה 2 136
' 1 54
2 117
ẉ PCR 5 348
ɔ Ə 5 418
ۊ 5 355
3 245
Ӂ ф 5 369
6 517

T

ɔ 2 128
Ɑ 125 6 513
3 224
1 54
2 128
ˆ ₸ 2 101
ˇ ىي ╚ 1 22
ˇ ŭ 3 182
ל ىي ợ 2 79

W

I 3 157
1 17
3 189
4 290
6 474
Ч 1 11
RNA 5 418
RNA 21 6 513

X

ĕ H 3 238
ה 1 63
' 1 63
غ 5 408
ھ 3 238
Ч 3 176
نج Ế 4 263
6 531
3 245
; ᴌ Ч 4 329
;ᵐ ᵘ 6 539
;ᵐ ᵘ 6 446
Ă 5 348
5 383
ᵃ و 1 11
╟ Ə 4 256
6 462
4 320
2 122
2 111
3 233
Ǐ غ 2 128
NT proBNP 1 44
4 320
2 128
غ 2 132
2 117
4 320
miRNAs 3 157
DNA 2 73
DNA 6 539
3 209
DNA 6 468

Y

Ж نج ┸ 2 101
Ж نج ط ˜ Ɑ 1 22
ụ ά ă 4 295
Ϗ 4 276
غ 1 39
غ 2 117
4 268
5 434
I 2 73
6 468
4 329
Ж ᵘ 1 1
Ж 6 474
Ж Ă 6 474
Ж 4 338
Ж 2 152
' 1 22
6 503
ф 5 396
ф 2 3 170
ф ىي 5 343
5 365
5 387
5 422
5 434
ф ىي DNA 5 374
ᵐ 5 374
5 383
ẉ PCR 3 233
4 263
5 374
ẉ 4 325
1 39
4 315
┼ HBV 5 343
┼ ф ىي 5 418
ḍ 2 146
ĭ غ 1 6
Ế 2 117
5 414
˜ ɵ ᵉ 1 44
Ǐ 3 182

Z

´ ל 6 479
6 441
2 141
ھ 6 462
6 485
5 428
A 3 6 491
4 310
6 441
ᵘ 6 441
Ϙ 2 152
¨Ą 5 422
5 379
Ψ ₐ ж 6 474
Ψ 3 245
2 73
¨A ├ 6 539
3 209
´ 1 49
ζ ۱ 6 479
ɔ غ 1 6 457
Ǐ 3 198
У 4 338
▌ 2 117

Z ├ ├ ҿ Ӈᵐ ӏ Ė ԓ ɔ ├ J

ա ε Ψ ʁա ε ɿ ʁա ε Ꭰ ʗ Ə ε í ʁ wЂ ʗA

ʁẇ ʁ Б ۱ ˚ ɟ

├ Њ 1908 ӈ ´ ɔ ~ ж ʁɑ Ỉ ~ Ό Њ ת ɬ Њ

Ń Ч ɑ Ỉ Њ ћ ђ ɑ Ỉ ᵐ í ├ Ẇ Ό Δ ɟ

1996 2002 ε ã̈ ɑ ᵐ ╪ ɟ v̈ Z J ʁɑ

Ό ʁɑ Ч Ɔ ʁ Ό Ж ; '

ɬ ɑ í Ό Ńɟ ┘ ת ɱ ת ɟ

ѽ ┘ í �something ʁẍ ʁ ɔ ʁ ʁŰ Ẇ Ă

ҿ Ψ ۱ Ψ ɟ Ό Δ ж ۱ 55~ ę Ψ 9~ ╪

1~ 4~ Ė ╪ Ӈ 27~ Ђ w ├ ε ζ Ψ ж ɟ

ᵐ Y╕ ѽ ~ ɔ ᵐ Y Њ à Ѭ ğ ɑ ├ '

ɔ ɱ ├ ´ ├ ├ ѽ Ẇ Δ ˝ ɔ ζ à

ε ; Ẉ ~ Њ ├ ╕ Њ ├ ѽ ɟ ѽ ˘

19 Z í ├ ʁă ► ʁ ʁi ћ ђ ă ► ћ í

≈ ђ֍ Ẇ ђ֍ ђ ɟ Ѧ Laval Ђ ´ ≈ Ế ╪

Ѧ ɟ

ъ ├ ѯ ├ Ь ғ Ό Δ Ӈ ۱ ς ת Ђ Ь ɔ ɟ ˝

Δ O ۱ Б ɔ Ѧ ǐ Ẉ ɟ Ђ ã Ѧ ӏ

ğ Ə Ψ ғ ' ǐ ˘ ė ۱ Њ ɔ ɟ

' ˝ ת í ├ ǐ Ѽ ς ˝ ε í ۱ wЂ ЃA

ɱ w ╗ ɟ ğ ẅ ŭ í Ə ≈ ɔ Ξ ' ɟ

ŭ Ẇ ẍ ɔ ├ ' 6~ Ė ╪ Ӈ Ό ж ۱ ╪ Ь Ѧ ŭ

ẍ ġ w ɔ ʁ ʁ ε ζ ~ ɔ ├ ğ Ỉ

ɔ ε í ɟ

4~ Ė

╪ Ӈ Ό ж ۱ ε ζ

Ė Ђ Morpholo

gy ʁẍ Immunology ʁ

Ж Cytogenetics ۱ ŭ

Molecular biology

ɔ MCIM ŭ ی

۱ ת Ђ Ə

ɟ 5 w ɔ Ξ

' ɑ 7

Ψ ɑ ´ Ə 1

4 ɟ

2009 5

80

2016 “ ”

“ ” “ ” “ ” “ ” “ ” “ ”

“ ” “ ”

http //yxyq.cbpt.cnki.net
020-32290789 206 jmdt@vip.163.com
020-32290789 201