

# 间质表皮转化因子通过 PI3K/AKT/MMPs 信号通路促进肺腺癌细胞的侵袭转移

林祥博<sup>1)</sup>, 石文静<sup>2)</sup>, 杨志一<sup>3)</sup>, 张丽萍<sup>3)</sup>, 周丹丹<sup>3)</sup>, 郑 荃<sup>3)</sup>, 尹崇高<sup>4)</sup>, 李洪利<sup>5)</sup>\*

(潍坊医学院<sup>1)</sup>生物科学与技术学院生物制药专业;<sup>2)</sup>附属医院中医科;<sup>3)</sup>病理学教研室;

<sup>4)</sup>护理学院外科护理学教研室;<sup>5)</sup>医学研究实验中心, 山东 潍坊 261053)

**摘要** 间质表皮转化因子(mesenchymal to epithelial transition factor, MET)在多种癌症中异常表达,影响肿瘤的发生发展,但 MET 影响肺腺癌的分子机制并不明确。本研究收集 3 例淋巴结转移的肺腺癌组织(lung adenocarcinoma tissues, LAD)和 3 例无淋巴结转移的肺腺癌组织,用于微阵列基因芯片分析。结果显示,与无淋巴结转移的肺腺癌组织相比,有淋巴结转移的肺腺癌组织中有 1 314 条 mRNAs 表达上调,400 条 mRNAs 表达下调。其中, MET 在有淋巴结转移的肺腺癌组织中表达显著升高。随机选取 8 个差异表达基因,对收集的潍坊医学院附属医院 2014 年 2 月至 2017 年 2 月间有淋巴结转移的肺腺癌组织和无淋巴结转移的肺腺癌组织各 30 例通过 qRT-PCR 实验进行微阵列基因芯片验证。结果显示,所选 mRNAs 的表达与微阵列结果一致,验证了微阵列基因芯片结果的准确性。通过 Western 印迹进一步检测 MET 的表达。结果显示,相较于正常肺上皮细胞,肺腺癌细胞中 MET 的表达显著升高。利用质粒转染,敲减肺腺癌细胞 A549 中的 MET, Transwell 侵袭实验结果显示,敲减 MET 后肺腺癌细胞的侵袭能力明显降低;对各细胞组进行 EGF(epidermal growth factor)处理并检测 PI3K/AKT/MMPs 信号通路, Western 印迹检测结果显示,敲减 MET 后,肺腺癌细胞中基质金属蛋白酶-2(matrix metalloproteinase 2, MMP-2)和 MMP-9 的表达显著下降, AKT 的磷酸化水平也显著下降。上述结果表明, MET 可通过激活 PI3K/AKT 信号通路进而增加 MMPs 的表达促进肺腺癌的侵袭转移。

**关键词** 肺腺癌; 基因芯片; 间质表皮转化因子; PI3K/AKT/MMPs 信号通路

**中图分类号** R734.1

## MET Promotes Invasion and Metastasis of Lung Adenocarcinoma Cells through PI3K/AKT/MMPs Signaling Pathway

LIN Xiang-Bo<sup>1)</sup>, SHI Wen-Jing<sup>2)</sup>, YANG Zhi-Yi<sup>3)</sup>, ZHANG Li-Ping<sup>3)</sup>, ZHOU Dan-Dan<sup>3)</sup>,  
ZHENG Quan<sup>3)</sup>, YIN Chong-Gao<sup>4)</sup>, LI Hong-Li<sup>5)</sup>\*

<sup>1)</sup> Biological Pharmacy, College of Biological Science and Technology, Weifang Medical University;

<sup>2)</sup> Department of Traditional Chinese Medicine, Affiliated Hospital of Weifang Medical University;

<sup>3)</sup> Department of Pathology, Weifang Medical University; <sup>4)</sup> Surgical Nursing College of Nursing, Weifang

Medical University; <sup>5)</sup> Medical Research Center, Weifang Medical University, Weifang 261053, Shandong, China)

收稿日期: 2019-02-10; 修回日期: 2019-04-05; 接受日期: 2019-04-22

国家自然科学基金项目(No.81702932, No. 81402389, No. 81641111); 山东省自然科学基金(No. ZR2015HL065); 潍坊市科学技术发展项目(No. 2018GX077); 国家级大学生科技创新基金项目(No.201810438032)和潍坊医学院大学生科技创新基金项目(No. KX 2018044, No. KX2018049)资助

\* 通讯作者 Tel: 13506477591; E-mail: ycglihongli@163.com

Received: February 10, 2019; Revised: April 5, 2019; Accepted: April 22, 2019

Supported by National Natural Science Foundation of China (No. 81702932, No. 81402389, No. 81641111); Natural Science Foundation of Shandong Province (No. ZR2015HL065); Science and Technology Development Project of Weifang (No.2018GX077); National Student's Innovative Training Program (No. 201810438032) and College Students' Science and Technology Innovative Foundation of Weifang Medical University (No.KX2018044, No.KX2018049)

\* Corresponding author Tel: 13506477591; E-mail: ycglihongli@163.com

**Abstract** Mesenchymal to epithelial transition factor (MET) is abnormally expressed in many cancers, but the molecular mechanism of MET affecting lung adenocarcinoma is not clear. In this study, 3 cases of lung adenocarcinoma tissues (LAD) with lymph node metastasis and 3 cases of LAD without lymph node metastasis were collected for microarray gene chip analysis. The results showed that 1 314 mRNAs were up-regulated and 400 mRNAs were down-regulated in LADs with lymph node metastasis, and the expression of MET was significantly increased in lung adenocarcinoma tissues with lymph node metastasis, compared with those without lymph node metastasis. Eight differentially expressed genes were randomly selected, and qRT-PCR was used to verify the results of microarray gene chip in 30 cases of LADs with lymph node metastasis collected from February 2014 to February 2017 in Affiliated Hospital of Weifang Medical University. The results showed that the expression of the selected mRNAs was consistent with the microarray data, which verified the accuracy of the microarray gene chip results. The expression of MET was further detected by Western blot, and the results showed that the expression of MET in lung adenocarcinoma cells was significantly higher than that in normal lung epithelial cells. Transwell invasion experiment results showed that the invasion ability of A549 lung epithelial cells was significantly reduced after knockdown of MET. After treated with EGF (epidermal growth factor), results showed that the expression levels of matrix metalloproteinase 2 (MMP-2) and MMP-9 in lung adenocarcinoma cells were significantly decreased after the knockout of MET, and the phosphorylation level of AKT was also significantly decreased. These results indicated that MET can promote the invasion and metastasis of lung adenocarcinoma cells by activating the PI3K/AKT signaling pathway and thereby increasing the expression of MMPs.

**Key words** lung adenocarcinoma; microarray gene chip; mesenchymal to epithelial transition factor (MET); PI3K/AKT/MMPs pathway

肺癌是男性最常见的癌症死亡原因(16%),是女性因癌症致死的第二大原因(13%),对人群健康和生命构成巨大威胁<sup>[1]</sup>。肺癌从医学角度分为非小细胞肺癌和小细胞肺癌,其中最常见的是非小细胞肺癌类型是肺腺癌(lung adenocarcinoma)。它发病于细支气管或肺泡上皮细胞,血运丰富,具有典型的外周转移性<sup>[2]</sup>。有限的早期诊断数量及癌细胞侵袭是肺腺癌高死亡率和预后不良的主要原因<sup>[3,4]</sup>。因此,研究肺腺癌的潜在标志物及其侵袭转移机制具有重要意义。

间质表皮转化因子(mesenchymal to epithelial transition factor, MET),又称细胞间质表皮转化因子(cellular-mesenchymal to epithelial transition factor, c-Met)或者肝细胞生长因子受体(hepatocyte growth factor receptor, HGFR)等,是 PTKs 家族的一员,总长度超过 120 kb<sup>[5]</sup>。已有多项研究证明, MET 在多种不同来源的肿瘤中呈过表达,并参与肿瘤细胞的侵袭转移,如结肠癌<sup>[6]</sup>、胆道癌<sup>[7]</sup>和乳腺癌<sup>[8]</sup>等。但 MET 影响肺腺癌侵袭能力的分子机制仍不明确。本研究应用微阵列基因芯片技术,筛选出在有淋巴结转移的肺腺癌中高表达的 MET,通过探讨 MET 对肺腺癌细胞侵袭的影响,明确 MET 影响肺腺癌细胞侵袭能力的机制,为肺腺癌分子靶向治疗提供理论基础。

1 材料与方法

1.1 患者病历资料

收集潍坊医学院附属医院 2014 年 2 月至 2017 年 2 月间有淋巴结转移的肺腺癌组织及无淋巴结转移的肺腺癌组织(>5 cm)各 30 例作为研究对象。患者术前未经过任何化疗和放疗,年龄 40~70 岁,临床资料完整。所有的肺腺癌患者均已经签署知情同意书。

1.2 试剂与细胞株

Lipofectamine 2000 Reagent 购自美国 Invitrogen 公司;β-肌动蛋白(β-actin)、兔抗人基质金属蛋白酶(matrix metalloproteinase, MMP)-2、MMP-9、兔抗人 MET、AKT、p-AKT 抗体均购自 Abcam;Transwell 小室购自 Corning 公司;Matrigel 购自 BD 公司;正常肺上皮细胞 BEAS-2B 及肺腺癌细胞 A549 均由本实验室保存,并通过细胞鉴定。

1.3 微阵列基因芯片分析

应用人类 LncRNA 阵列 V3.0 (Human LncRNA Array V3.0)进行微阵列分析,检测 3 例有淋巴结转移的肺腺癌组织,以及 3 例无淋巴结转移的肺腺癌组织中具有明显差异表达的 mRNAs<sup>[9]</sup>,将原始数据进行归一化处理,通过差异表达倍数(fold-change)筛选差异表达基因,挑选要求为 Fold-change (linear) ≥ 2.0。

将差异基因数据做聚类分析,对样本和基因分别进行分级聚类之后,通过聚类热图(heat map)直观地展示基因在不同样本中的表达情况。

1.4 qRT-PCR 检测

提取有淋巴结转移的肺腺癌组织,以及无淋巴结转移的肺腺癌组织各 30 例的总 RNA 进行

Table 1 qRT-PCR primer sequences

Gene	Forward sequence (5'-3')	Reverse sequence (5'-3')
LIMK2	CCCTGAGATGCTGAACGGAAA	GATGGTGACCTTCCCTTGGC
RASSF5	CTCGCGCCTCTGTGTCC	GTGAATTTACAGTTAGTGCAGCG
IPCEF	GATGGCAGTGCTCTGTTC	ACAGTGACGACCCCTTCAGT
HNRNPAB	GGCGAGTTTGGGGAGATTGA	TGTACTACCCTGACCTCCACC
MET	TGGGCACCGAAAGATAAACCT	ATCTGGGTGTTCCAGCACAG
CD1E	ACGGAAGTGAAGCCAGAGG	TCCAGGGTTGCTCGGAGATA
MBD1	TTGAACGTCAGCACGCAGAT	CAGCAGTAGCCAGTTTGCA
KIAA0895	GTCACGGTGGCAGGTTCTAA	GTGAAGCTTTTCCGTCACGC
GAPDH	GTTGGAGGTCGGAGTCAACGG	GAGGGATCTCGCTCCTGGAGGA

1.5 细胞培养及转染

细胞培养参照先前文献<sup>[11]</sup>。将 A549 细胞分组为(1)A549 组,不转入质粒;(2)Scr/A549 组,转入空载质粒;(3)SiMET/A549 组,转入 MET 小 RNA 干扰质粒。

1.6 Transwell 侵袭检测

实验过程参照先前文献<sup>[12]</sup>。使用 0.25%胰酶对细胞进行消化。用无血清培养基重悬细胞,将转染后的 150 μL(4×10<sup>5</sup> cells/mL) SiMET/A549 和 Scr/A549 细胞分别加入到 2 个已加入基质胶的 Transwell 小室中。下室补充含 20% 胎牛血清的 RPMI1640 培养基 500 μL,放于 5% CO<sub>2</sub>、37 ℃ 的培养箱中培养 24 h,4%多聚甲醛固定 20 min,Giemsa 染色 35 min,PBS 清洗 3 次,随机选取 5 个视野于显微镜下拍照计数,取平均值为实验结果。实验重复 3 次。

1.7 Western 印迹

将各组细胞抽提总蛋白质,各组蛋白质样品经电泳、转膜、封闭 1 h 后敷一抗 4 ℃过夜,再经洗膜 3 次,常温敷二抗 1 h 后洗膜 3 次并曝光。所用抗体浓度如下:β-肌动蛋白(1:1 000)、间质表皮转化因子(1:500)、基质金属蛋白酶-2 (1:500)、基质金属蛋白酶-9 (1:500)、p-AKT(1:500)、AKT(1:500)。

1.8 统计学方法

所有实验数据用均数加减标准差( $\bar{x} \pm s$ )表示,并使用 SPSS 17.0 进行统计学分析。采用独立

逆转录,RNA 提取及逆转录过程参照先前文献<sup>[10]</sup>。PCR 条件为 95 ℃ 5 s、63 ℃ 30 s、72 ℃ 30 s 进行 35 个循环。初步筛选 LIMK2、RASSF5、IPCEF、HNRNPAB、MET、CD1E、MBD1、KIAA0895 进行检测(GAPDH 作为内参),基因引物序列见表 1。

样品 *t* 检验分析两组定量资料。多组比较采用单因素方差进行分析, $P<0.05$  时认定差异具有统计学意义。

2 结果

2.1 mRNAs 微阵列基因芯片分析

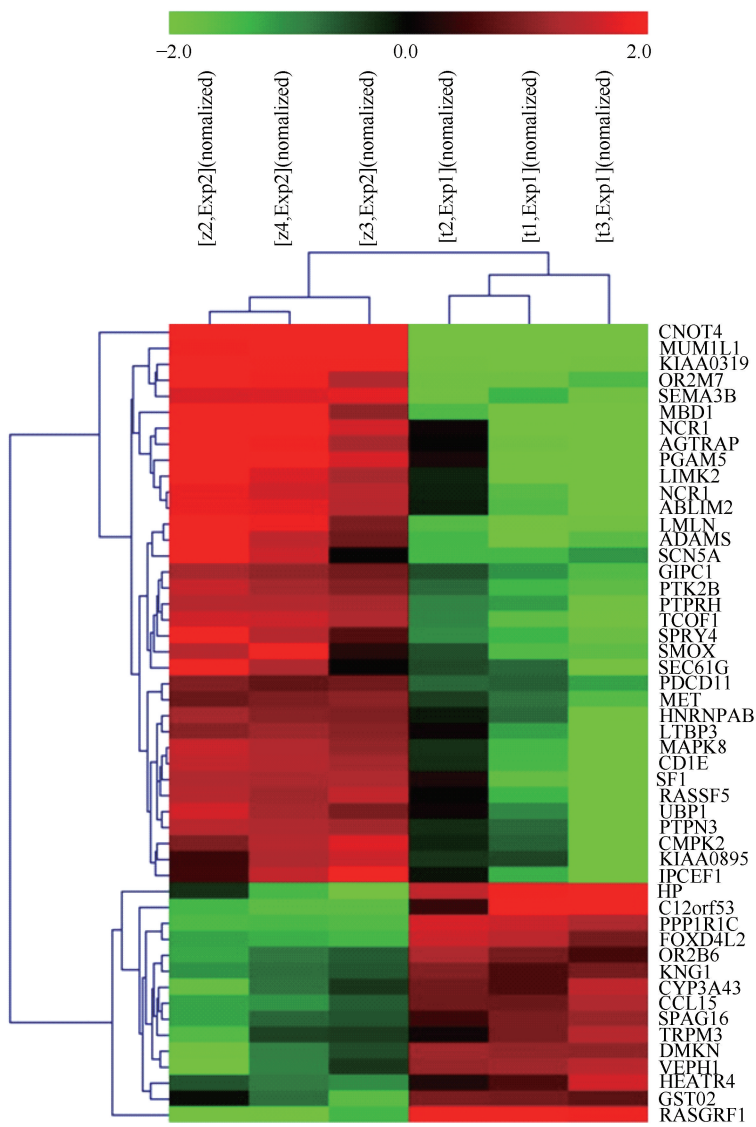
通过 mRNAs 微阵列基因芯片技术,对 3 个有淋巴结转移的肺腺癌组织(z2,z3,z4)和 3 个无淋巴结转移肺腺癌组织(t1,t2,t3)的总 RNA 进行检测,存在差异性表达的条件为差异倍数 $\geq 2.0$ , $P<0.05$ 。结果显示,相较于无转移的肺腺癌组织,有转移的肺腺癌组织中有 1 314 条 mRNAs 表达上调,400 条 mRNAs 表达下调。其中,MET 表达上调;热图显示部分差异表达的 mRNAs(Fig.1)。

2.2 qRT-PCR 验证基因芯片的准确性

为验证微阵列基因芯片结果,本文初步筛选了 LIMK2、RASSF5、IPCEF、HNRNPAB、MET、CD1E、MBD1、KIAA0895 进行 qRT-PCR。结果显示,所选 mRNAs 在标本中的表达特征与微阵列数据一致(Fig.2),验证了微阵列基因芯片结果的准确性。

2.3 间质表皮转化因子在肺腺癌细胞 A549 中高表达

用 Western 印迹检测人正常肺上皮细胞 BEAS-2B 和肺腺癌细胞 A549 中 MET 的表达量。结果显示,MET 在肺腺癌细胞 A549 中的表达量显著高于人正常肺上皮细胞 BEAS-2B(Fig.3)。



**Fig.1 Clustering heat map of partial differentially expressed mRNAs in LAD with or without lymph node metastasis** Human LncRNA Array V3.0 was applied to analyze the LADs with and without metastasis. The condition for the existence of differential expression is Fold change $\geq 2$ , $P<0.05$ . Each grid in the heat map represents a data. The figure shows some differentially expressed genes, among which MET is highly expressed

2.4 肺腺癌细胞 A549 的敲减成功

转染 24 h 时,低温提取对照组及敲减组细胞总蛋白质。Western 印迹检测敲减组细胞 SiMET/A549 的敲减效率,与对照组细胞 Scr/A549 相比,敲减组中 MET 的表达量显著降低 (Fig.4),表明敲减质粒成功转染。

2.5 间质表皮转化因子促进肺腺癌细胞的侵袭

利用 Transwell 实验检测 MET 对肺腺癌细胞侵袭能力的影响。结果显示,穿过基底膜的 SiMET/A549 细胞数量明显少于 Scr/A549 细胞 ( $P<0.05$ , Fig.5)。结果表明,MET 可以促进肺腺癌的侵袭转移。

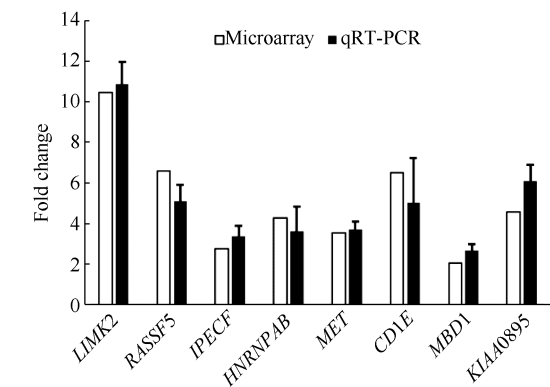
2.6 间质表皮转化因子可通过激活 PI3K/AKT-信号通路促进基质金属蛋白酶表达

通过使用 EGF 起始 PI3K 的激活,并通过 Western 印迹检测敲减 MET 后 AKT 的磷酸化水平的变化,以及 MMP-2、MMP-9 表达。结果显示,敲减组 AKT 磷酸化水平降低,MMP-2、MMP-9 蛋白表达量降低 (Fig.6)。

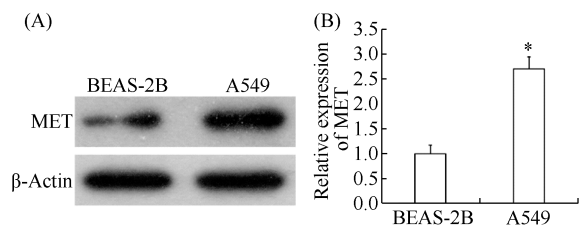
3 讨论

肺腺癌具有进展快、恶性程度高且极其容易转移的特点<sup>[2]</sup>。其肺腺癌发生和发展涉及多个环节和因素,是一个复杂的过程,其中癌基因激活和抑癌





**Fig.2 The accuracy of the gene chip was verified by qRT-PCR** Total RNAs of 30 LADs with lymph node metastasis and 30 LADs without lymph node metastasis were extracted, and the expression of 8 pre-screened genes was detected by qRT-PCR. Primer sequences are shown in Table 1. The expression characteristics of eight selected genes were consistent with microarray analysis. The accuracy of microarray gene chip was verified



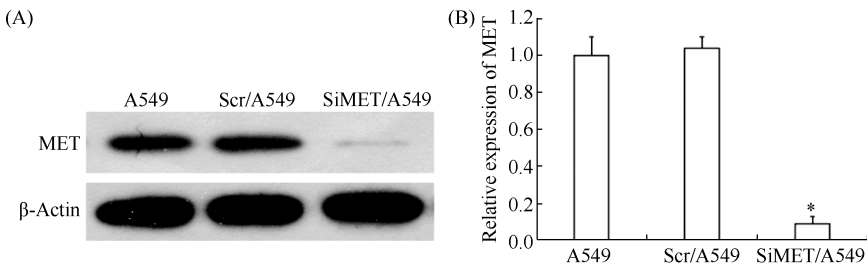
**Fig. 3 The expression of MET in A549 lung adenocarcinoma cells was significantly higher than that of BEAS-2B human normal lung epithelial cells** (A) Total proteins of BEAS-2B normal lung epithelial cells and A549 lung adenocarcinoma cells were extracted, and the expression of MET was detected by Western blot using  $\beta$ -actin as an internal reference. The experiment was repeated three times. (B) The relative protein levels of (A) were analyzed by Image J. \*  $P<0.05$ , vs BEAS-2B

基因缺失发挥很重要的作用<sup>[13]</sup>。

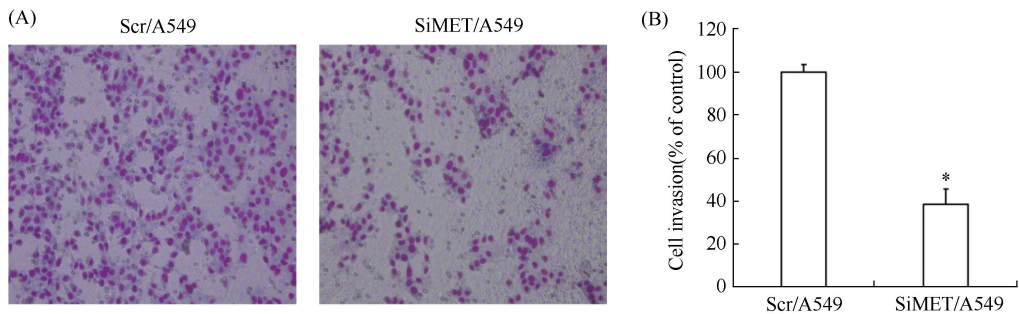
MET 及其配体肝细胞生长因子/分散因子 (HGF/SF) 于 1980 年代中期被发现<sup>[14]</sup>。研究发现,

MET 由通过二硫键连接的胞外亚基和穿膜亚基组成<sup>[15]</sup>。胞外亚基包括 SEMA (semaphorin) 等 3 个功能域,而细胞内片段则由酪氨酸激酶域和多功能对接位点组成<sup>[16]</sup>。其中,SEMA 结构域在受体结合方面发挥重要作用,影响下游通路发挥调控作用<sup>[17]</sup>。越来越多的研究发现,MET 参与多种癌症的发生发展进程,是一种潜在的癌基因。Miekus 等发现,降低 MET 在宫颈癌细胞系中的表达后,宫颈癌细胞的生长明显受到抑制<sup>[18]</sup>。Li 等发现,高表达 MET 可增加小鼠的成瘤潜力,且促进胰腺癌细胞的自我更新<sup>[19]</sup>。本研究通过基因芯片检测 3 例有淋巴结转移的肺腺癌组织,以及无淋巴结转移的肺腺癌组织中的基因表达差异,发现 MET 在有淋巴结转移肺腺癌组织中表达明显上调。Western 印迹结果显示,肺腺癌细胞 A549 中 MET 表达也明显上调。Transwell 侵袭实验表明,敲减 MET 后肺腺癌细胞的侵袭能力显著降低,证明 MET 参与肺腺癌细胞的侵袭进程。

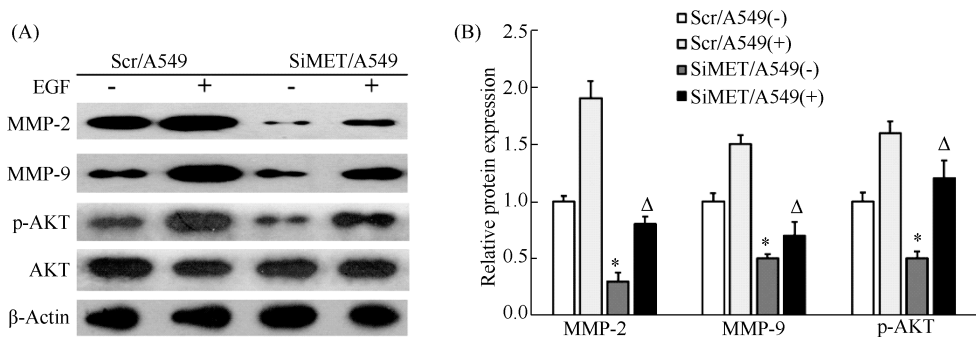
PI3K/AKT 信号转导通路在调节细胞生存、侵袭等方面发挥着重要作用,并在多种癌症中异常活跃,如胃癌<sup>[20]</sup>等。本课题组前期研究已发现,PI3K/AKT 信号通路在乳腺癌中异常表达,且可促进乳腺癌的侵袭<sup>[21-23]</sup>。近期另有研究发现,PI3K/AKT 信号通路的下游因子基质金属蛋白酶 (matrix metalloproteinases, MMPs) 例如 MMP-2、MMP-9 等在肿瘤细胞的发育过程中发挥重要的作用,例如乳腺癌<sup>[23]</sup>等,它可以降解几乎所有种类的细胞外基质蛋白质组分,打破组织学上肿瘤细胞侵袭屏障,从而促进肿瘤的进展,在肿瘤的侵袭转移中起到非常重要的作用<sup>[24]</sup>。本研究中,EGF 刺激各组细胞后发现,敲减 MET 组细胞中 MMP-2、MMP-9 的表达量明显降低,而且 AKT 的磷酸化水平也明显降低。



**Fig.4 The expression of MET in the knockdown group was significantly reduced** (A) Total proteins of A549 lung adenocarcinoma cells, Scr/A549 (Into the empty plasmid) and SiMET/A549 (Transfected MET small RNA interfering plasmid) cells were extracted, and the expression of MET was detected by Western blot using  $\beta$ -actin as the internal reference. The experiment was repeated three times. (B) The relative protein levels of (A) were analyzed by Image J. \*  $P<0.05$ , vs Scr/A549



**Fig.5 The invasiveness of A549 cells was markedly reduced after knockdown of MET** The cells were digested with trypsin and resuspended with serum-free medium. SiMET/A549 and Scr/A549 cells ( $4\times 10^5$  cells/mL) were added respectively to two upper chambers with Matrigel, and the F12K with 20% fetal bovine serum was added to lower chambers. After 24 hours culture, fixation, staining, photographing and counting were performed. The experiment was repeated three times. (A) Transwell assay was used to detect the effect of MET on the invasion ability of lung adenocarcinoma cells. (B) The histograms showed the numbers of penetrated cells in (A). \*  $P<0.05$ , *vs* Scr/A549



**Fig.6 The levels of MMP-2, MMP-9 and phosphorylated AKT were decreased after knockdown of MET in lung adenocarcinoma cells** (A) After transfection, the cells in each group were treated with or without EGF for 30 minutes. The total protein was extracted. Western blot was used to detect the changes of the expression levels of MMP-2, MMP-9 and p-AKT after the knockdown of MET. The experiment was repeated three times. (B) The relative proteins levels of (A) were analyzed by Image J. \*  $P<0.05$ , *vs* Scr/A549 (-); <sup>Δ</sup> $P<0.05$ , *vs* Scr/A549 (+)

由上述可知,MET 可通过 PI3K/AKT/MMPs 信号通路促进肺腺癌细胞的侵袭,提示 MET 可能成为肺腺癌临床治疗的新靶点,有望通过此机制控制肺腺癌的侵袭作用,进而为治疗肺腺癌提供新的思路。

参考文献 (References)

[ 1 ] Miller KD, Goding Sauer A, Ortiz AP, *et al.* Cancer statistics for Hispanics/Latinos, 2018 [J]. CA Cancer J Clin, 2018, **68**(6): 425-445

[ 2 ] Torre LA, Siegel RL, Jemal A. Lung Cancer Statistics [J]. Adv Exp Med Biol, 2016, **893**: 1-19

[ 3 ] Li H, Yin C, Zhang B, *et al.* PTTG1 promotes migration and invasion of human non-small cell lung cancer cells and is modulated by miR-186 [J]. Carcinogenesis, 2013, **34** ( 9 ): 2145- 2155

[ 4 ] Meng C, Tang C, Liang J. Progress of biomarkers in diagnosis of bone metastases of lung cancer [J]. Chin J Lung Cancer, 2018, **21**(8): 615-619

[ 5 ] Skead G, Govender D. Gene of the month: MET [J]. J Clin Pathol, 2015, **68**(6): 405-409

[ 6 ] Zeng ZS, Weiser MR, Kuntz E, *et al.* c-Met gene amplification is associated with advanced stage colorectal cancer and liver metastases [J]. Cancer Lett, 2008, **265**(2): 258-269

[ 7 ] Zhou W, Jiang C, Zhan N, *et al.* Human epidermal growth factor receptor 2, epidermal growth factor receptor, and c-MET overexpression and survival in biliary tract cancer: A meta-analysis [J]. J Cancer Res Ther, 2018, **14**(Suppl): S28-S35

[ 8 ] Holland JD, Gyorffy B, Vogel R, *et al.* Combined Wnt/beta-catenin, Met, and CXCL12/ CXCR4 signals characterize basal breast cancer and predict disease outcome [J]. Cell Rep, 2013, **5**(5): 1214-1227

[ 9 ] Yang Z, Li H, Wang Z, *et al.* Microarray expression profile of long non-coding RNAs in human lung adenocarcinoma [J]. Thorac Cancer, 2018, **9**(10): 1312-1322

[ 10 ] Zhang B, Li H, Yin C, *et al.* Dock1 promotes the mesenchymal transition of glioma and is modulated by MiR-31 [J]. Neuropathol Appl Neurobiol, 2017, **43**(5): 419-432

[ 11 ] 张国新,徐新伟,孟斌,等. miR-186-5p 通过靶向调控 PTTG1 抑制肺腺癌细胞的 上皮-间质转化[J].中国生物化学与分子生物学报 (Zhang GX, Xu XW, Meng B, *et al.* miR-186-5p inhibits epithelial mesenchymal transition in lung adenocarcinoma cells through modulating PTTG1[J] Chin J Biochem Mol Biol), 2017,**33**(4):380-385

[ 12 ] Zhang B, Yin C, Li H, *et al.* Nrl1 promotes invasion of breast cancer cells by binding to chemokine (C-C motif) ligand 18 through the PI3K/Akt/GSK3beta/Snail signalling pathway [J]. Eur J Cancer, 2013, **49**(18): 3900-3913

- [13] Wu YL, Zhang L, Kim DW, *et al.* Phase Ib/II study of capmatinib (INC280) plus gefitinib after failure of epidermal growth factor receptor (EGFR) inhibitor therapy in patients with EGFR-mutated, MET factor-dysregulated non-small-cell lung cancer[J]. Clin Oncol, 2018, **36**(31): 3101-3109
- [14] Gherardi E, Birchmeier W, Birchmeier C, *et al.* Targeting MET in cancer: rationale and progress [J]. Nat Rev Cancer, 2012, **12**(2): 89-103
- [15] Granito A, Guidetti E, Gramantieri L. c-MET receptor tyrosine kinase as a molecular target in advanced hepatocellular carcinoma [J]. J Hepatocell Carcinoma, 2015, **2**: 29-38
- [16] Szewczyk B, Skrzypek K, Majka M. Targeting MET receptor in rhabdomyosarcoma; rationale and progress [J]. Curr Drug Targets, 2017, **18**(1): 98-107
- [17] Niemann HH, Jäger V, Butler PJ, *et al.* Structure of the human receptor tyrosine kinase met in complex with the Listeria invasion protein InlB [J]. Cell, 2007, **130**(2): 235-246
- [18] Miekus K, Pawlowska M, Sekuła M, *et al.* MET receptor is a potential therapeutic target in high grade cervical cancer [J]. Oncotarget, 2015, **6**(12): 10086-10101
- [19] Li C, Wu JJ, Hynes M, *et al.* c-Met is a marker of pancreatic cancer stem cells and therapeutic target [J]. Gastroenterology, 2011, **141**(6): 2218-2227
- [20] Zeng L, Liao Q, Zou Z, *et al.* Long non-coding RNA XLOC\_006753 promotes the development of multidrug resistance in gastric cancer cells through the PI3K/AKT/mTOR signaling pathway [J]. Cell Physiol Biochem, 2018, **51**(3): 1221-1236
- [21] Zhang G, Li H, Sun R, *et al.* Long non-coding RNA ZEB2-AS1 promotes the proliferation, metastasis and epithelial mesenchymal transition in triple-negative breast cancer by epigenetically activating ZEB2 [J]. J Cell Mol Med, 2019, doi: 10.1111/jcmm.14213. [Epub ahead of print]
- [22] Mu QJ, Li HL, Yao Y, *et al.* Chromodomain helicase/ATPase DNA-binding protein 1-like gene (CHD1L) expression and implications for invasion and metastasis of breast cancer [J]. PLoS One, 2015, **10**(11): e0143030
- [23] Li H, Zhang B, Liu Y, *et al.* EBP50 inhibits the migration and invasion of human breast cancer cells via LIMK/cofilin and the PI3K/Akt/mTOR/MMP signaling pathway [J]. Med Oncol, 2014, **31**(9): 162
- [24] Fouad H, Salem H, Ellakwa DE, *et al.* MMP-2 and MMP-9 as prognostic markers for the early detection of urinary bladder cancer [J]. J Biochem Mol Toxicol, 2019, **33**(4): e22275