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·基础研究·

lncRNA HOTTIP通过miR-637/KLK4轴促进肺癌SPC-A-1细胞的恶性生物学行为

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[摘 要] **目的:**探讨lncRNA HOTTIP对肺癌细胞增殖、凋亡及EMT的影响及其作用机制。**方法:**利用qPCR检测lncRNA HOTTIP、miR-637和KLK4在肺癌SPC-A-1、正常肺上皮BEAS-2B细胞中的表达量;siRNA干扰SPC-A-1细胞中lncRNA HOTTIP的表达后,分别通过CCK-8、Transwell、流式细胞术和WB法检测SPC-A-1细胞增殖、侵袭、凋亡和EMT能力的变化。miRanda软件和双荧光素酶报告基因实验分析lncRNA HOTTIP和miR-637之间的靶向关系,RNA pull-down实验检测lncRNA HOTTIP和miR-637的吸附作用,检测lncRNA HOTTIP通过miR-637对SPC-A-1细胞增殖、侵袭、凋亡和EMT的调控。利用TargetScan软件分析miR-637与KLK4的相关性,双荧光素酶报告基因实验检测miR-637与KLK4 mRNA之间的相互作用;检测miR-637通过KLK4 mRNA对SPC-A-1细胞增殖、侵袭、凋亡和EMT的调控。下调lncRNA HOTTIP和miR-637表达后,利用qPCR和WB检测KLK4 mRNA和蛋白表达水平的变化。**结果:**与BEAS-2B细胞比,在SPC-A-1细胞中lncRNA HOTTIP呈高表达(P<0.01),miR-637呈低表达(P<0.01),KLK4呈高表达(P<0.01)。下调lncRNA HOTTIP后,SPC-A-1细胞增殖、侵袭与EMT能力显著减弱,细胞凋亡率显著上升(P<0.01);lncRNA HOTTIP与miR-637具有靶向关系;下调miR-637表达后,SPC-A-1细胞增殖、侵袭与EMT能力显著上升,细胞凋亡率显著降低(P<0.01)。miR-637与KLK4 3'UTR特异性结合。miR-637通过KLK4显著促进了SPC-A-1细胞增殖、侵袭与EMT,细胞凋亡率显著上升(P<0.01)。下调lncRNA HOTTIP使KLK4表达显著降低,而下调miR-637可促进KLK4表达(P<0.05)。**结论:**上调lncRNA HOTTIP可通过miR-637/KLK4轴促进肺癌SPC-A-1细胞的增殖、侵袭与EMT而抑制癌细胞凋亡。

[关键词] lncRNA;同源基因A远端转录本;miR-637;激肽释放酶相关肽酶4;SPC-A-1细胞;增殖;上皮-间质转化
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Up-regulation of lncRNA HOTTIP promotes the malignant biological behaviors of lung cancer SPC-A-1 cells through miR-637/KLK4 axis

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[Abstract] Objective: To investigate the effect and mechanism of lncRNA HOTTIP on proliferation, apoptosis and EMT of lung cancer cells. **Methods:** The expressions of lncRNA HOTTIP, miR-637 and KLK4 in SPC-A-1, BEAS-2B cells were detected by qPCR. After siRNA interference with the expression of lncRNA HOTTIP, the proliferation, invasion, apoptosis and EMT of SPC-A-1 cells were detected by CCK-8, Transwell, flow cytometry, and WB, respectively. The targeting relationship between lncRNA HOTTIP and miR-637 was analyzed by miRanda software and dual-luciferase reporter gene assay. RNA pull-down assay was used to detect the adsorption of lncRNA HOTTIP and miR-637, and to detect the effects of lncRNA HOTTIP regulating miR-637 on proliferation, invasion, apoptosis, and EMT of SPC-A-1 cells. The correlation between miR-637 and KLK4 was analyzed by TargetScan software, and the interaction between miR-637 and KLK4 was detected by dual-luciferase reporter gene assay. After siRNA interference with the expression of KLK4, the proliferation, invasion, apoptosis, and EMT of SPC-A-1 cells were detected. After down regulation of lncRNA HOTTIP and miR-637 expression, the levels of KLK4 mRNA and protein expression were detected by qPCR and WB. **Results:** Compared with BEAS-2B cells, the expression of lncRNA HOTTIP in SPC-A-1 cells was significantly up-regulated (P<0.01), the

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expression of miR-637 was down-regulated (P<0.01), the KLK4 expression was up-regulated (P<0.01). Down-regulation of lncRNA HOTTIP could significantly reduce the proliferation, invasion, and EMT capacity of SPC-A-1 cells, and increase the apoptosis rate (P<0.01). lncRNA HOTTIP had a targeting relationship with miR-637. Down-regulation of miR-637 expression could significantly promote the proliferation, invasion and EMT capacity of SPC-A-1 cells, and inhibit the apoptosis rate (P<0.01). miR-637 specifically bound to KLK4 3'UTR. Down-regulation of KLK4 could significantly inhibit the proliferation, invasion, and EMT capacity of SPC-A-1 cells, and increase the apoptosis rate (P<0.01). Down-regulation of lncRNA HOTTIP could significantly decrease KLK4 expression, while down-regulation of miR-637 could promote KLK4 expression (P<0.05). **Conclusion:** Up-regulation of lncRNA HOTTIP promotes proliferation, invasion, and EMT of lung cancer SPC-A-1 cells through miR-637/KLK4 axis, and inhibits the apoptosis of cancer cells.

[Key words] lncRNA; homeobox A transcript at the distal tip (HOTTIP); miR-637; kallikrein-related peptidase 4 (KLK4); SPC-A-1 cell; proliferation; epithelial-mesenchymal transition (EMT)

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肺癌病死率居高不下，其相关发病机制和治疗成为目前研究热点[1-2]。lncRNA能够参与基因组印记、组包装和染色质修饰、基因调控和激活，其表达异常与细胞发育异常及疾病的发生密切相关[3-4]。lncRNA在肺癌中表达失调，异常表达的 lncRNA能作为关键的调控因子，参与多种生物学过程，影响肺癌细胞的增殖和凋亡、肿瘤的转移、侵袭、血管生成及耐药，为肺癌临床治疗提供了新思路[4-5]。

同源基因A远端转录本（homeobox A transcript at the distal tip，HOTTIP）是一种新发现的lncRNA，位于同源箱a基因簇的5′端，能够通过影响细胞增殖、侵袭、凋亡和转移参与肿瘤的形成和进展[6]。近年来研究[7-9]发现，HOTTIP在肝癌、乳腺癌、胃癌、胰腺癌、食管鳞状细胞中表达升高，能够作为一种致癌基因发挥作用。然而，HOTTIP在人类肺癌中的生物学作用和调控机制仍不甚清楚。

本研究旨在分析lncRNA HOTTIP对肺癌SPC-A-1细胞增殖、凋亡及EMT的影响，为进一步阐明lncRNA HOTTIP在肺癌中发挥调控作用的分子机制奠定基础。

1　材料与方法

1.1　细胞系、质粒与主要试剂

肺癌SPC-A-1细胞和人正常肺上皮BEAS-2B细胞购自武汉普诺赛生命科技有限公司，DMEM培养基购自美国Gibco公司，Turbofect转染试剂购自Thermo Fisher Scientific公司，双荧光素酶活性检测试剂盒购自美国Abcam公司，CCK-8试剂盒购自上海碧云天生物科技有限公司，E-cadherin和N-cadherin及GAPDH多克隆抗体购自美国圣克鲁斯公司。由上海Genepharma生物有限公司合成lncRNA HOTTIP siRNA、miR-637抑制物（inhibitor）、miR-637模拟物（mimic）、激肽释放酶相关肽酶4（kallikrein-related peptidase 4，KLK4）siRNA等质粒。

1.2　细胞培养和转染

肺癌SPC-A-1和BEAS-2B细胞使用添加10%胎牛血清的DMEM培养基培养，置于37 ℃、5% CO_2的培养箱中，当细胞汇合度长到90%时，取状态良好的细胞用于后续实验。

将SPC-A-1细胞接种于12孔板上，在显微镜下观察细胞汇合度约70%时，向细胞中加入无血清的DMEM培养基，按照Turbofect转染试剂说明书将重组质粒、Turbofect及Opti-MEM混匀，加入待转染细胞于培养孔中，培养48 h后收集各组细胞样品进行后续实验。

1.3　CCK-8实验检测lncRNA HOTTIP对SPC-A-1细胞增殖活力的影响

将对数生长期的SPC-A-1细胞稀释后按每孔100 μl铺在96孔板中，每组设3个复孔，待细胞生长至约70%汇合时转染lncRNA HOTTIP siRNA，分为lncRNA HOTTIP siRNA组和siRNA NC组。培养24、48、72 h后，向每孔细胞中加入10 μl配制好的CCK-8溶液，于细胞培养箱中避光2 h，用酶标检测450 nm波长处细胞的光密度（D）值。并按照公式[（实验孔D值−空白孔D值）/（对照孔D值−空白孔D值）]×100%“计算细胞增殖率。

1.4　流式细胞术检测lncRNA HOTTIP对SPC-A-1细胞凋亡的影响

将对数生长期的SPC-A-1细胞稀释后铺在6孔板中，置于37 ℃、5%CO_2培养箱中静置培养，将重组质粒转染细胞48 h后，收集细胞，以2 000×g离心5 min，用PBS清洗细胞沉淀，再加入500 μl结合缓冲液重悬细胞，再向其中加入5 μl Annexin Ⅴ-FITC和5 μl PI，避光孵育20 min后，上机检测细胞凋亡。

1.5　双荧光素酶报告基因实验检测SPC-A-1细胞荧光素酶活性强度

取对数生长期的SPC-A-1细胞稀释后铺于12孔板，细胞融合度约75%时，采用Turbofect试剂将lncRNA HOTTIP WT、lncRNA HOTTIP MUT质粒分

别与miR-637 mimic、mimic NC共转染至SPC-A-1细胞，同时将KLK4 WT、KLK4 MUT分别与miR-637 mimic、mimic NC质粒共转染至SPC-A-1细胞，最后测定荧光素酶活性强度。

1.6 Transwell小室实验检测lncRNA HOTTIP对SPC-A-1细胞侵袭能力的影响

基质凝胶稀释后涂在Transwell上室中，12 h后，室温干燥，SPC-A-1细胞转染成功后，取含1×10^5个细胞的悬液铺于Transwell上室，取含10%FBS的培养基加入下室，持续孵育24 h，用4%多聚甲醛固定，PBS清洗后用结晶紫染色，细胞置于显微镜下随机选取5个视野观察并分析结果。

1.7 RNA pull-down实验检测lncRNA HOTTIP与miR-637的结合

将lncRNA HOTTIP重组质粒进行酶切和胶回收后，与体外转录混合物混匀，常温放置3 h，加入DNase Ⅰ，常温放置15 min；通过试剂盒提取RNA，取4 μg RNA在90 ℃条件下反应2 min，加入等体积RNA结合缓冲液，放置0.5 h，RNA与细胞蛋白液混匀，1 h后留取少量本底对照Input样，取50 μl磁珠加入样品中，4 ℃下过夜，洗涤3次，加入蛋白酶K缓冲液，30 min后转移上清并提取RNA，反转录为cDNA后进行qPCR。

1.8 qPCR检测KLK4 mRNA的表达量

各组细胞总RNA通过PrimeScript™Ⅳ 1st Strand cDNA Synthesis Mix逆转录试剂盒反转录成cDNA。以反转成的cDNA为模板，配制20 μl qPCR体系，每个样品按照42 ℃、2 min，37 ℃、15 min，85 ℃、5 s进行，40个循环，实验所用的引物见表1。

表1 引物序列

Tab.1 Primer sequences

Target	Sequences
lncRNA HOTTIP	F：5'-CCTA A A GCCACGCTTCTTTG-3'
lncRNA HOTTIP	R：5'-TGCAGGCTGGAGATCCTACT-3'
miR-637	F：5'-ACUGGGGGCUUUCGGGCUCUGCGU-3'
miR-637	R：5'-ACGCAGAGCCCGAAAGCCCCCAGU-3'
KLK4	F：5'-GACTCCTACACCGTGGGA-3'
KLK4	R：5'-CGCCTGATGGTGTTAGAC-3'
GAPDH	F：5'-TGCACCACCAACTGCTTAGC-3'
GAPDH	R：5'-GGCATGGACTGTGGTCATGAG-3'

1.9 WB检测KLK4蛋白的表达量

RIPA充分裂解细胞蛋白后，通过BCA蛋白检测试剂盒检测蛋白浓度。取50 μg蛋白样品进行SDS-PAGE，电泳结束后将蛋白转移到PVDF膜上。PVDF膜置于TBST缓冲液配制的5%脱脂奶粉中，封闭3 h，结束后将PVDF膜置于TBST稀释的特异性一抗中，4 ℃下过夜，TBST缓冲液中清洗5次，每次5 min，PVDF膜置于TBST稀释的二抗中，室温下2 h，PVDF膜滴加ECL反应液进行蛋白曝光。使用ImageJ软件分析蛋白的灰度值，蛋白相对表达量=所测蛋白灰度值/GAPDH灰度值。

1.10 统计学处理

以上各实验均独立重复3次。采用SPSS 17.0软件进行分析，符合正态分布的计量资料以$\bar{x}\pm s$表示，多组间比较采用单因素方差分析，其中两两比较采用LSD-t检验，以$P<0.05$或$P<0.01$表示差异具有统计学意义。

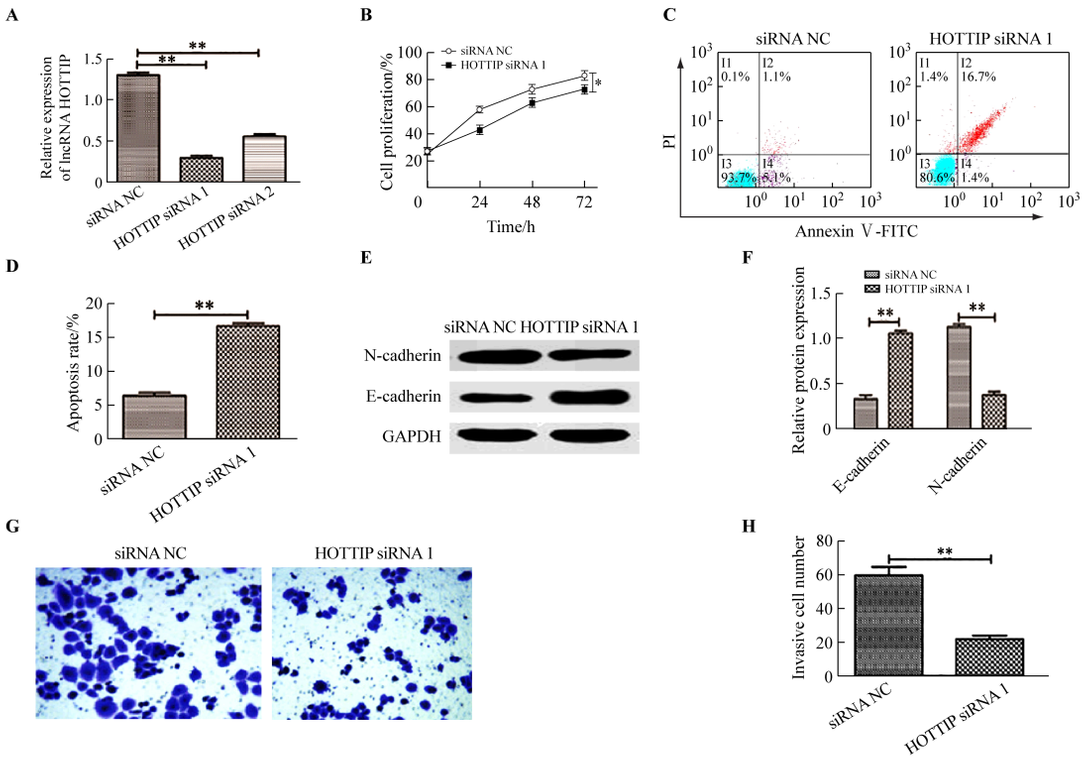
2 结 果

2.1 lncRNA HOTTIP对SPC-A-1细胞的增殖、凋亡及EMT的影响

qPCR检测结果显示，lncRNA HOTTIP在SPC-A-1细胞中的表达量显著高于BEAS-2B细胞中的表达量（2.05±0.62 *vs* 1.03±0.26，$P<0.01$）。细胞转染实验结果（图1A）显示，与siRNA NC组相比，HOTTIP siRNA1组和HOTTIP siRNA2组细胞中的HOTTIP表达量显著降低（均$P<0.01$），且在HOTTIP siRNA1组细胞中表达量最低，因此后续选择HOTTIP siRNA1进行实验。CCK-8实验结果（图1B）显示，HOTTIP siRNA1组SPC-A-1细胞增殖活力低于siRNA NC组（$P<0.05$）。流式细胞术检测结果（图1C、D）显示，与siRNA NC组相比，HOTTIP siRNA1组细胞凋亡率显著上升（$P<0.01$）。WB实验检测结果（图1E、F）显示，与siRNA NC组相比，HOTTIP siRNA1组N-cadherin蛋白表达量显著降低（$P<0.01$），E-cadherin蛋白表达量显著上升（$P<0.01$）。Transwell实验检测结果（图1G、H）显示，与siRNA NC组相比，HOTTIP siRNA1组细胞侵袭数目显著减少（$P<0.01$）。以上结果表明，下调lncRNA HOTTIP能够抑制SPC-A-1细胞的增殖、侵袭及EMT进程，促进细胞凋亡。

2.2 lncRNA HOTTIP与miR-637之间的靶向关系

生物信息数据库（miRanda）数据分析预测结果显示，lncRNA HOTTIP和miR-637之间存在结合位点（图2A）。细胞转染后qPCR检测结果显示，与转染mimic NC组相比、miR-637 mimic组细胞中的miR-637表达量有显著差异（2.21±0.86 *vs* 1.08±0.37，$P<0.01$）。双荧光素酶报告基因实验结



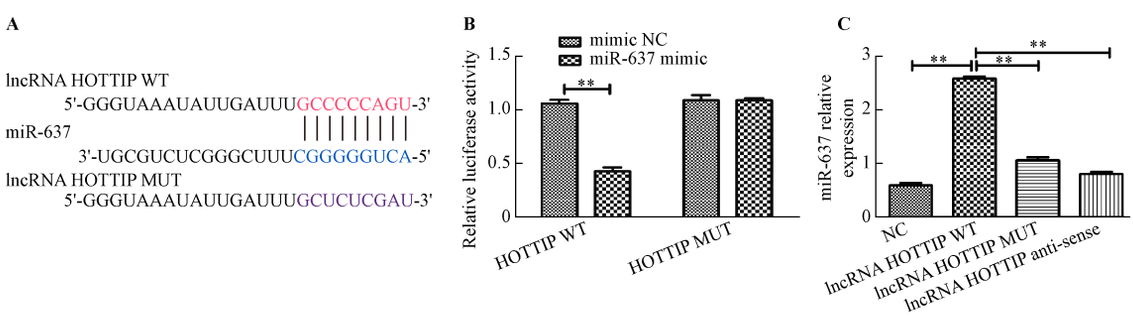
$^{**}P$<0.01

A: The relative expression of lncRNA HOTTIP was detected by qPCR; B: CCK-8 assay was used to detect the effect of down-regulated lncRNA HOTTIP on proliferation of SPC-A-1 cells; C: The apoptosis of SPC-A-1 cells was detected by flow cytometry; D: Apoptosis rate of SPC-A-1 cells; E: The effect of down-regulated lncRNA HOTTIP on EMT-related proteins in SPC-A-1 cells was detected by WB; F: Relative protein expression was detected by WB; G: The effect of down-regulated lncRNA HOTTIP on invasion of SPC-A-1 cells was detected by Transwell (×200); H: Number of invasive SPC-A-1 cells

图 1　lncRNA HOTTIP 对 SPC-A-1 细胞的增殖、凋亡及 EMT 相关蛋白表达的影响

Fig.1　Effects of lncRNA HOTTIP on proliferation, apoptosis, and the expression of EMT-related proteins in SPC-A-1 cells

果显示，lncRNA HOTTIP WT+miR-637 mimic 组的荧光素酶活性显著低于 lncRNA HOTTIP WT+mimic NC 组（P<0.01，图 2B），lncRNA HOTTIP WT+miR-637 mimic 组的荧光素酶活性与 lncRNA HOTTIP MUT+mimic NC 组无显著差异（P>0.05，图 2B）。与阴性对照组、突变组和反义链组相比，lncRNA HOTTIP WT 组能够吸附 miR-637（P<0.01，图 2C）。



$^{**}P$<0.01

A: Binding sites between lncRNA HOTTIP and miR-637 predicted by miRanda; B: The interaction between lncRNA HOTTIP and miR-637 was detected by dual-luciferase reporter gene assay; C: RNA pull-down was used to detect the adsorption between lncRNA HOTTIP and miR-637

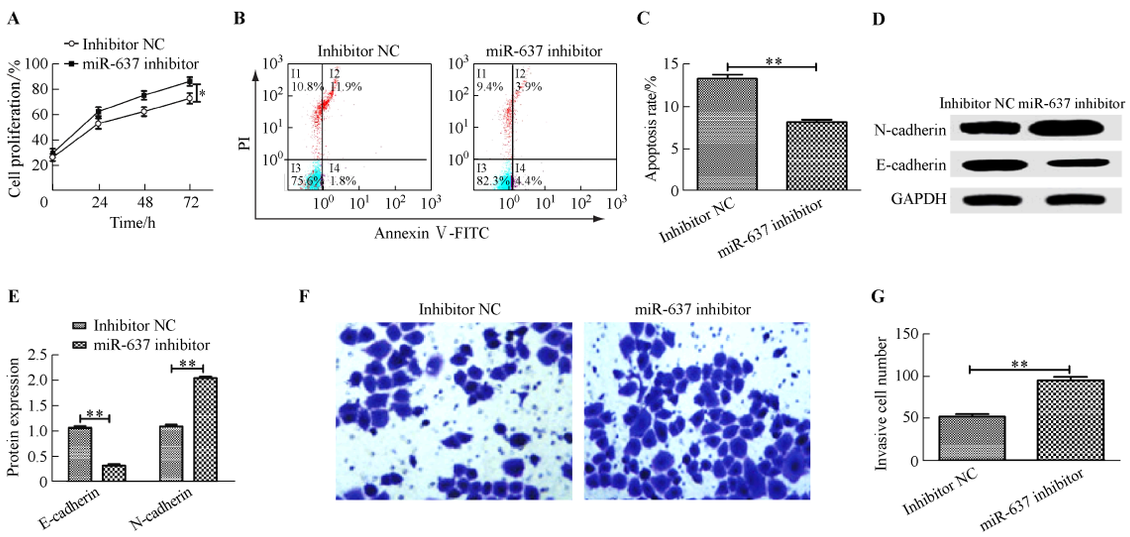
图 2　lncRNA HOTTIP 和 miR-637 之间的靶向关系

Fig.2　Targeting relationship between lncRNA HOTTIP and miR-637

2.3　miR-637 对 SPC-A-1 细胞的增殖、凋亡及 EMT 的影响

qPCR 检测结果显示，BEAS-2B 细胞中 miR-637 的表达量显著高于 SPC-A-1 细胞（1.14±0.38 *vs* 0.67±0.27，P<0.01）。与 Inhibitor NC 组相比，miR-637 inhibitor 组 SPC-A-1 细胞内 miR-637 的表达量显著下降（0.52±0.73 *vs* 1.18±0.83，P<0.01）。CCK-8 实验检测结果（图 3A）显示，与 Inhibitor NC 组相

比，miR-637 inhibitor组SPC-A-1细胞增殖活力显著升高（P<0.05）。流式细胞术检测结果（图3B、C）显示，与Inhibitor NC组相比，miR-637 inhibitor组细胞凋亡率显著下降（P<0.01）。WB实验检测结果（图3D、E）显示，与Inhibitor NC组相比，miR-637 Inhibitor组E-cadherin蛋白表达量显著降低而N-cadherin蛋白表达量显著上升（均P<0.01）。Transwell实验检测结果（图3F、G）显示，与Inhibitor NC组相比，miR-637 inhibitor组细胞侵袭数目显著增加（P<0.01）。结果显示，下调miR-637能够促进SPC-A-1细胞的增殖、侵袭及EMT，抑制细胞凋亡。



$^{*}P$<0.05, $^{**}P$<0.01

A: CCK-8 assay was used to detect the effect of down-regulated miR-637 on proliferation of SPC-A-1 cells; B: The effect of down-regulated miR-637 on SPC-A-1 cell apoptosis was detected by flow cytometry; C: Apoptosis rate of SPC-A-1 cells; D: The effect of down-regulated miR-637 on expression of EMT-related proteins in SPC-A-1 cells was detected by WB; E: Relative proteins expression; F: Transwell was used to detect the effect of down-regulated miR-637 on invasion of SPC-A-1 cells (×100); G: Number of invasive SPC-A-1 cells

图3　miR-637对SPC-A-1细胞的增殖、凋亡及EMT相关蛋白表达的影响

Fig.3　Effects of miR-637 on proliferation, apoptosis, and expression of EMT-related proteins in SPC-A-1 cells

2.4　lncRNA HOTTIP通过miR-637调控SPC-A-1细胞的增殖、凋亡、侵袭及EMT进程

CCK-8实验检测结果（图4A）显示，pcDNA-HOTTIP+Mimic NC组SPC-A-1细胞增殖活力显著高于pcDNA-3.1（+）+Mimic NC组（P<0.01），pcDNA-3.1（+）+miR-637 mimic组细胞增殖活力显著低于pcDNA-3.1（+）+Mimic NC组（P<0.05），pcDNA-HOTTIP+miR-637 mimic组细胞增殖活力显著高于pcDNA-3.1(+)+miR-637 mimic组（P<0.05）。

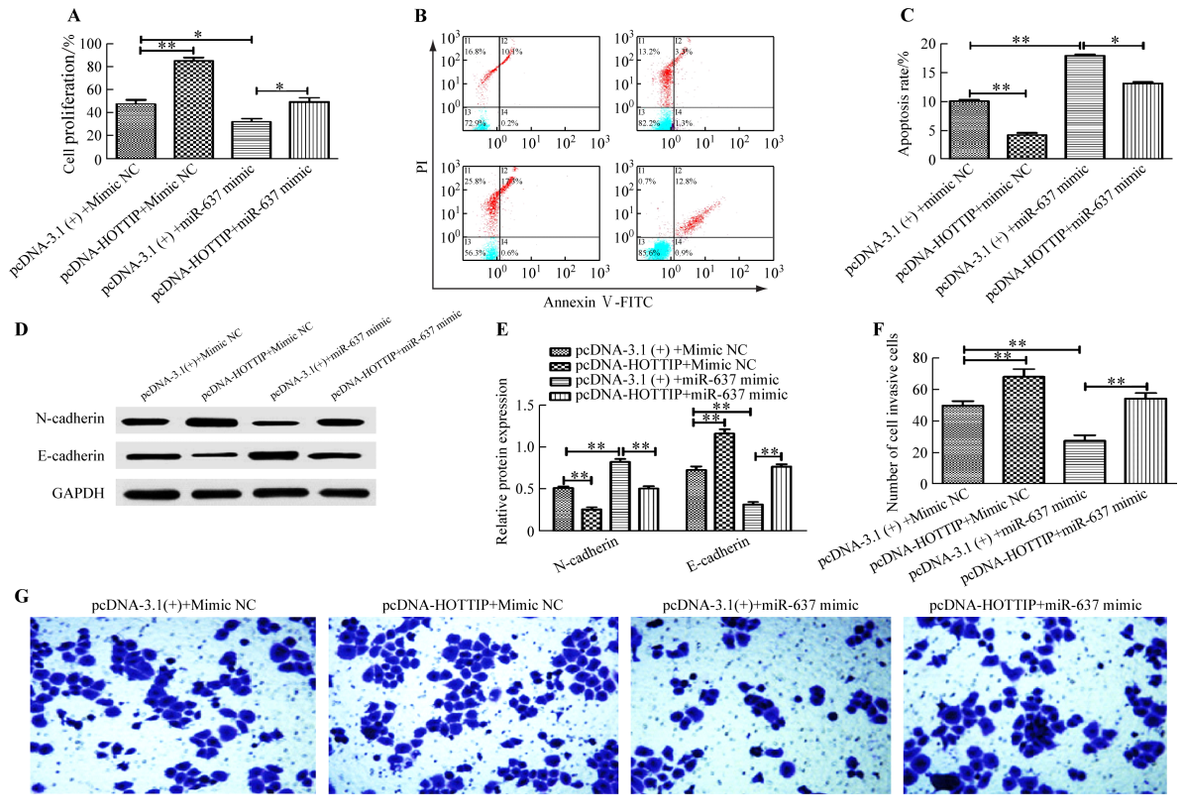
流式细胞术检测结果（图4B、C）显示，与pcDNA-3.1(+)+Mimic NC组相比，pcDNA-HOTTIP+Mimic NC组SPC-A-1细胞凋亡率显著下降（P<0.01），pcDNA-3.1（+）+miR-637 mimic组细胞凋亡率显著增高（P<0.01），与pcDNA-3.1(+)+miR-637 mimic组相比pcDNA-HOTTIP+miR-637 mimic组细胞凋亡率显著下降（P<0.05）。

WB实验检测结果（图4D、E）显示，与pcDNA-3.1（+）+Mimic NC组相比，pcDNA-HOTTIP+Mimic NC组N-cadherin蛋白表达量显著上升、E-cadherin蛋白表达量显著下降，pcDNA-3.1（+）+miR-637 mimic组E-cadherin蛋白表达量显著上升、N-cadherin蛋白表达量显著下降；与pcDNA-3.1（+）+miR-637 mimic组相比，pcDNA-HOTTIP+miR-637 mimic组N-cadherin蛋白表达量显著上升、E-cadherin蛋白表达量显著下降（均P<0.01）。

Transwell实验检测结果（图4F、G）显示，pcDNA-HOTTIP+Mimic NC组SPC-A-1细胞侵袭数目显著高于pcDNA-3.1（+）+Mimic NC组，pcDNA-3.1（+）+miR-637 mimic组细胞侵袭数目显著低于pcDNA-3.1（+）+Mimic NC组，pcDNA-HOTTIP+miR-637 mimic组细胞侵袭数目显著高于pcDNA-3.1（+）+miR-637 mimic组（均P<0.01）。

2.5　miR-637靶向结合KLK4 mRNA

TargetScan软件预测结果显示，miR-637与KLK4 mRNA存在结合位点（图5A）。双荧光素酶报告基因实验检测结果（图5B）显示，KLK4 WT+miR-637 mimic组的荧光素酶活性显著低于KLK4 WT+Mimic NC组（P<0.01），KLK4 MUT+miR-637 mimic组的荧光素酶活性与KLK4 MUT+Mimic NC组无显著差异（P>0.05）。

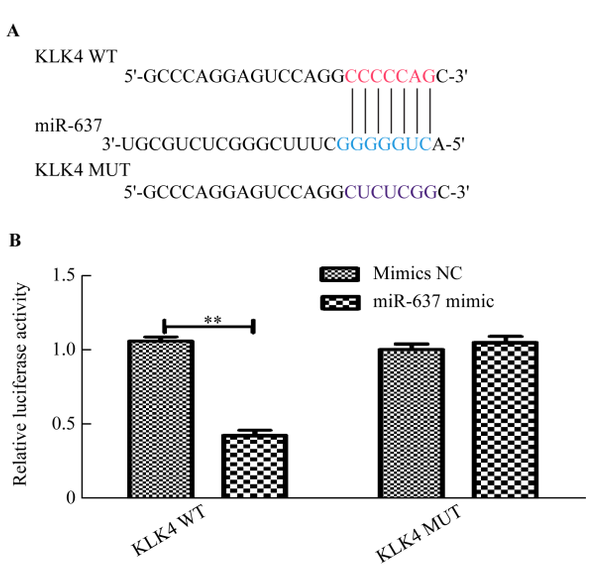


$^{*}P<0.05$, $^{**}P<0.01$

A: CCK-8 assay was used to detect the effect of lncRNA HOTTIP on proliferation of SPC-A-1 cells through miR-637; B: The effect of lncRNA HOTTIP on SPC-A-1 cell apoptosis through miR-637 was detected by flow cytometry; C: Apoptosis rate of SPC-A-1 cells; D: WB was used to detect the effect of lncRNA HOTTIP on expression of EMT-related proteins in SPC-A-1 cells through miR-637; E: Relative protein expression; F: Number of invasive SPC-A-1 cells; G: Transwell was used to detect the effect of lncRNA HOTTIP on invasion of SPC-A-1 cells through miR-637 (×100)

图 4 lncRNA HOTTIP 通过 miR-637 影响 SPC-A-1 细胞的增殖、侵袭、凋亡及 EMT 进程

Fig.4 Effects of lncRNA HOTTIP on proliferation, invasion, apoptosis, and EMT in SPC-A-1 cells through miR-637



$^{**}P<0.01$

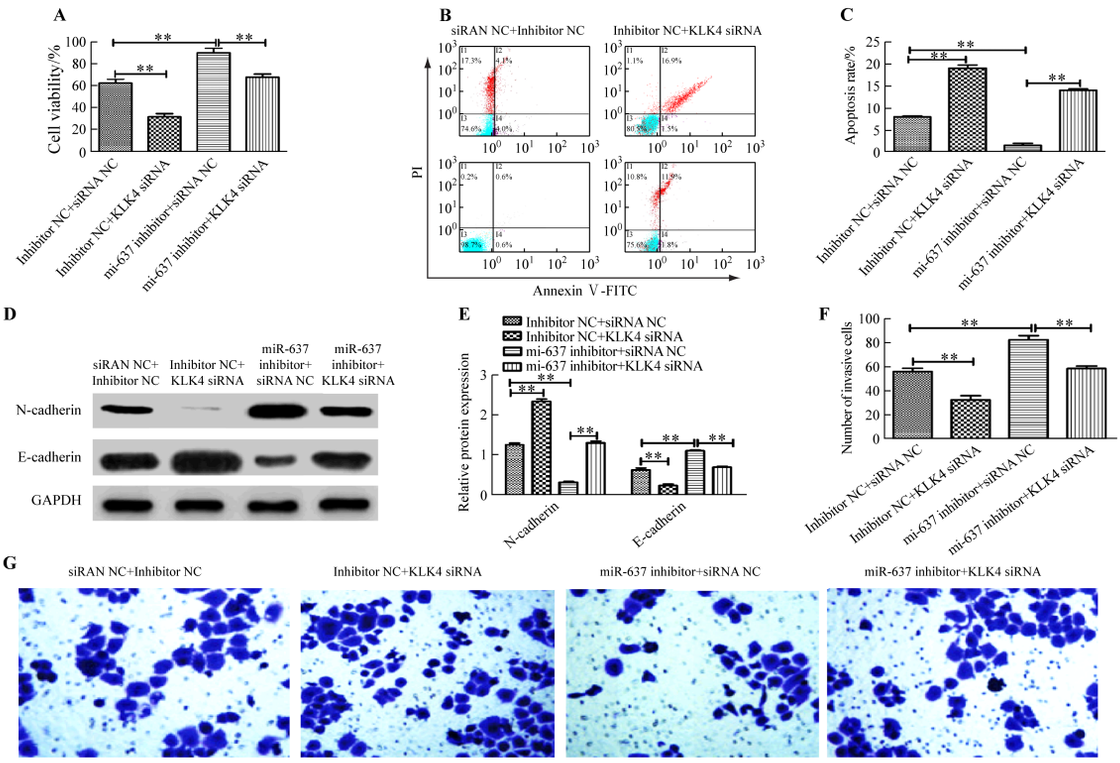
A: Binding sites between miR-637 and KLK4 mRNA predicted by TargetScan; B: Dual-luciferase reporter gene assay was used to detect the interaction between miR-637 and KLK4

图 5　miR-637 和 KLK4 mRNA 之间的靶向关系

Fig.5　Targeting relationship between miR-637 and KLK4 mRNA

2.6　miR-637 通过 KLK4 调控 SPC-A-1 细胞的增殖、凋亡及 EMT 进程

SPC-A-1 细胞中的 KLK4 表达量高于 BEAS-2B 细胞(2.48±0.73 *vs* 0.93±0.37, $P<0.01$)。KLK4 siRNA 组的 KLK4 表达量显著低于 KLK4 NC 组(0.52±0.37 *vs* 1.18±0.81, $P<0.01$)。与 inhibitor NC+siRNA NC 组相比, inhibitor NC+KLK4 siRNA 组 SPC-A-1 细胞增殖活力显著降低($P<0.01$)、细胞凋亡率显著升高($P<0.01$), 而 N-cadherin 蛋白表达量显著降低($P<0.01$), E-cadherin 蛋白表达量显著上升($P<0.01$); miR-637 inhibitor+siRNA NC 组 SPC-A-1 细胞增殖活力显著升高($P<0.01$)、细胞凋亡率显著降低($P<0.01$), E-cadherin 蛋白表达量显著降低而 N-cadherin 蛋白表达量显著上升。与 miR-637 inhibitor+siRNA NC 组相比, miR-637 inhibitor+KLK4 siRNA 组 SPC-A-1 细胞增殖活力显著降低、细胞凋亡率显著升高, N-cadherin 蛋白表达量显著降低而 E-cadherin 蛋白表达量显著上升。此部分实验结果显示, 下调 miR-637 能够通过 KLK4 促进 SPC-A-1 细胞的增殖、侵袭及 EMT 进程, 而促进细胞凋亡(均 $P<0.01$, 图 6)。



$^{**}P<0.01$

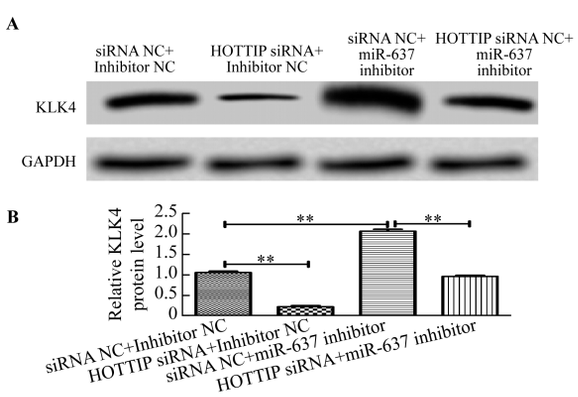
A: CCK-8 assay was used to detect the effect of down-regulated KLK4 on proliferation of SPC-A-1 cells; B: The effect of down-regulated KLK4 on SPC-A-1 cell apoptosis was detected by flow cytometry; C: Apoptosis rate of SPC-A-1 cells; D: The effect of down-regulated KLK4 on expression of EMT-related proteins in SPC-A-1 cells was detected by WB; E: Relative protein expression; F: Number of invasive SPC-A-1 cells; G: Transwell was used to detect the effect of miR-637 on invasion of SPC-A-1 cells through KLK4 (×200)

图6　KLK4对SPC-A-1细胞的增殖、侵袭、凋亡及EMT进程的影响

Fig.6　Effects of KLK4 on proliferation, invasion, apoptosis, and EMT of SPC-A-1 cells

2.7　lncRNA HOTTIP通过miR-637调控KLK4表达

qPCR检测结果显示，与对照组相比，HOTTIP siRNA+Inhibitor NC组KLK4 mRNA表达量显著下降（0.43±0.81 *vs* 1.09±0.73，$P<0.01$），siRNA NC+miR-637 inhibitor组KLK4 mRNA表达量显著增高（1.93±0.37 *vs* 0.95±0.38，$P<0.01$），HOTTIP siRNA+miR-637 inhibitor组KLK4 mRNA表达量显著低于siRNA NC+miR-637 inhibitor组（0.85±0.73 *vs* 1.36±0.72，$P<0.05$）。WB实验检测结果（图7）显示，HOTTIP siRNA+Inhibitor NC组KLK4蛋白表达量显著低于siRNA NC+Inhibitor NC组，siRNA NC+miR-637 inhibitor组KLK4蛋白表达量显著高于siRNA NC+Inhibitor NC组，HOTTIP siRNA+miR-637 inhibitor组KLK4蛋白表达量显著低于siRNA NC+miR-637 inhibitor组（均$P<0.01$）。结果表明，lncRNA HOTTIP通过miR-637调控KLK4表达。



$^{**}P<0.01$

A: The effect of lncRNA HOTTIP on KLK4 expression through miR-637 was detected by WB; B: Relative expression of KLK4 protein

图7　lncRNA HOTTIP通过miR-637影响KLK4表达

Fig.7　lncRNA HOTTIP affected KLK4 expression through miR-637

3　讨　论

lncRNA具有重要的生物学功能，与物种进化、胚胎发生和肿瘤发生密切相关[10]。大多数lncRNA通过与RNA聚合酶Ⅱ结合在转录水平上抑制基因表达，参与人类生理和病理过程[11]，因此，lncRNA表达异常可能是导致多种人类疾病的主要原因。最近发现的一种位于人类外周组织7p15.2基因区的lncRNA HOTTIP与多种疾病的发病和发展有关[12]，HOTTIP表达的增加是诊断恶性肿瘤的一个潜在的生物标志物。另一方面，肺癌诊断和治疗进展缓慢，

患者的预后仍然较差,因此从分子角度探寻一种新的治疗策略必不可少。在众多癌症中,如消化系统的胰腺癌、肝细胞癌和胃癌,肌肉骨骼系统的骨肉瘤等,HOTTIP表达均异常[13-14]。本研究发现,HOTTIP在肺癌SPC-A-1细胞中表达上调,敲低HOTTIP抑制了SPC-A-1细胞的增殖和EMT、促进了癌细胞凋亡,提示HOTTIP在SPC-A-1细胞中可能作为一个癌基因促进肿瘤的生长和转移。

一般认为,lncRNA可能作为竞争性内源RNA(competing endogenous RNA, ceRNA),以细胞类型依赖的方式调节不同miRNA的表达。miRNA与lncRNA的结合降低了miRNA水平,导致miRNA靶基因表达增加。因此,本研究探讨了HOTTIP是否可通过与miRNA相互作用而发挥ceRNA的功能。利用生物信息学软件和双荧光素酶报告基因实验证实HOTTIP与miR-637之间存在直接结合。另外发现,miR-637和KLK4 mRNA之间具有靶向和负调控关系。近些年发现miR-637和KLK4在癌症的发展过程中发挥着重要的生物学功能,如miR-637通过抑制Akt1的表达抑制人胰腺导管腺癌细胞的肿瘤发生[15];miR-637在胃癌细胞中通过靶向ERBB3抑制细胞迁移和侵袭、促进细胞凋亡[16]。KLK4可能与子宫内膜腺上皮细胞的增殖和分化有关,在子宫内膜癌的发生和发展中具有一定的作用[17]。本研究发现miR-637在SPC-A-1细胞中表达下调、KLK4表达上调,下调miR-637促进了SPC-A-1细胞的增殖和EMT、抑制了癌细胞凋亡,而且HOTTIP可以通过miR-637调控SPC-A-1细胞的增殖、凋亡和EMT。进一步研究表明,lncRNA HOTTIP通过miR-637调控了KLK4的表达。

综上所述,HOTTIP在肺癌SPC-A-1细胞中表达上调,而且通过miR-637 / KLK4轴调控SPC-A-1细胞的增殖、侵袭、凋亡和EMT进程,表明lncRNA HOTTIP在肺癌发生发展过程中起重要作用,具有成为肺癌标志物的潜力。

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