lncRNA PSMA3-AS1靶向调控miR-627-3p对肝癌Hep3B细胞增殖、迁移及侵袭的影响

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摘要 该文旨在探讨lncRNA PSMA3-AS1对肝癌细胞增殖、迁移及侵袭的影响及其可能的作用机制。采用qRT-PCR法对肝癌组织、癌旁组织、正常人肝上皮细胞THLE-3, 以及人肝癌细胞MHCC97H、Hep3B、SK-HEP-1中lncRNA PSMA3-AS1、miR-627-3p的表达量进行检测; 将si-NC、si-lncRNA PSMA3-AS1、miR-NC、miR-627-3p mimics分别转染至Hep3B细胞, si-lncRNA PSMA3-AS1与anti-miR-NC, 以及si-lncRNA PSMA3-AS1与anti-miR-627-3p共转染至Hep3B细胞; 双荧光素酶报告实验检测lncRNA PSMA3-AS1与miR-627-3p的靶向关系; MTT法检测细胞增殖; 平板克隆形成实验检测细胞克隆形成情况; Transwell实验检测细胞迁移及侵袭; 蛋白质印迹法检测MMP2、MMP9蛋白表达量。qRT-PCR实验结果显示, 与癌旁组织比较, 肝癌组织中lncRNA PSMA3-AS1的表达量升高(P<0.05), miR-627-3p的表达量降低(P<0.05), 而与THLE-3细胞比较, MHCC97H、Hep3B、SK-HEP-1细胞中lncRNA PSMA3-AS1的表达量升高(P<0.05), miR-627-3p的表达量降低(P<0.05); 双荧光素酶报告实验结果显示, lncRNA PSMA3-AS1可靶向结合miR-627-3p; 转染si-lncRNA PSMA3-AS1或转染miR-627-3p mimics后细胞活力以及MMP2、MMP9蛋白水平降低(P<0.05), 细胞克隆形成数、迁移及侵袭细胞数减少(P<0.05); 共转染si-lncRNA PSMA3-AS1和anti-miR-627-3p可恢复转染si-lncRNA PSMA3-AS1对Hep3B细胞增殖、克隆形成、迁移及侵袭的抑制作用。干扰lncRNA PSMA3-AS1表达可通过靶向调控miR-627-3p而减弱肝癌细胞的增殖、克隆形成、迁移及侵袭能力。

关键词 肝癌; lncRNA PSMA3-AS1; miR-627-3p; 细胞增殖; 迁移; 侵袭

The Effect of lncRNA PSMA3-AS1 on the Proliferation, Migration and Invasion of Liver Cancer Hep3B Cells Through Targeted Regulation of miR-627-3p

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Abstract This study aimed to investigate the effects of lncRNA PSMA3-AS1 on the proliferation, migration and invasion of liver cancer cells and its possible mechanism. The expression levels of lncRNA PSMA3-AS1 and miR-627-3p in liver cancer tissues, paracancerous tissues and normal human liver epithelial cells THLE-3, human liver cancer cells MHCC97H, Hep3B and SK-HEP-1 were detected by qRT-PCR. si-NC, si-lncRNA PSMA3-AS1, miR-NC and miR-627-3p mimics were transfected into Hep3B cells, respectively. si-lncRNA PSMA3-AS1 and anti-miR-NC, as well as si-lncRNA PSMA3-AS1 and anti-miR-627-3p were co-transfected into Hep3B cells.

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The targeting relationship between lncRNA PSMA3-AS1 and miR-627-3p were detected by dual-luciferase reporter assay. Cell proliferation was detected by MTT assay, cell clonal formation was detected by plate colony formation assay, and cell migration and invasion were detected by Transwell assay. The expression levels of MMP2 and MMP9 proteins were detected by Western blot. The results of qRT-PCR assay showed that compared with paracancerous tissues, the expression of lncRNA PSMA3-AS1 in liver cancer tissues was increased (P<0.05), while the expression of miR-627-3p was decreased (P<0.05). Compared with THLE-3 cells, the expression of lncRNA PSMA3-AS1 in MHCC97H, Hep3B and SK-HEP-1 cells was increased (P<0.05), while the expression of miR-627-3p was decreased (P<0.05). The results of dual-luciferase reporter assay showed that lncRNA PSMA3-AS1 could target and bind miR-627-3p. The results of MTT assay, plate colony formation assay, Transwell assay and Western blot assay showed that the cell viability and the protein levels of MMP2 and MMP9 were decreased after transfection of si-lncRNA PSMA3-AS1 or miR-627-3p mimics (P<0.05), and the number of colony formation, migration and invasion cells was decreased (P<0.05). The results of MTT assay, plate colony formation assay, Transwell assay and Western blot assay showed that co-transfection of si-lncRNA PSMA3-AS1 and anti-miR-627-3p could restore the inhibitory effects of transfected si-lncRNA PSMA3-AS1 on Hep3B cell proliferation, clone formation, migration and invasion. Interfering the expression of lncRNA PSMA3-AS1 can attenuate the proliferation, clonogenesis, migration and invasion of liver cancer cells by targeting miR-627-3p expression.

Keywords liver cancer; lncRNA PSMA3-AS1; miR-627-3p; cell proliferation; migration; invasion

肝癌是常见的一种恶性肿瘤, 其发病率与死亡率较高, 手术结合放化疗是其主要的治疗方法, 但由于肿瘤细胞转移能力强导致肝癌患者预后较差[1-2]。肿瘤靶向治疗成为研究重点, 因此就目前而言, 首先需要对临床肝癌的发病机制进行分析, 分析不同方法对其治疗的效果, 找出不同治疗策略的优劣之处, 为后续临床肝癌的治疗提供基础[3]。长链非编码RNA(long non-coding RNA, lncRNA)属于非编码RNA分子, 长度大于200个核苷酸, 其表达异常与肿瘤发生之间具有密切关系。lncRNA在肝癌中出现异常表达, 并可通过充当miRNA的竞争性内源RNA(competing endogenous RNA, ceRNA)分子而参与肝癌发生及发展过程[4]。研究表明lncRNA在肝癌中表达上调或下调, 并可在肝癌的治疗中作为潜在治疗靶点[5-6]。长链非编码RNA PSMA3反义RNA1(lncRNA PSMA3-AS1)在肺癌细胞中表达上调, 并可促进肺癌细胞生长及侵袭[7]。但lncRNA PSMA3-AS1与肝癌的相关研究报道相对较少。LncBase Predicted v.2预测显示, lncRNA PSMA3-AS1与miR-627-3p存在结合位点。研究表明miR-627-3p在骨肉瘤中表达下调, 并可抑制骨肉瘤细胞的增殖及转移[8]。但lncRNA PSMA3-AS1/miR-627-3p分子轴在肝癌发生及发展过程中的作用机制尚未被阐明。因此, 为进一步研究肝癌的具体发病机制, 并为肝癌患者提供新的治疗方向, 本研究探讨了lncRNA PSMA3-AS1是否可通过靶向调控miR-627-3p而在肝癌细胞的增殖和凋亡过程中发挥作用。

1 材料与方法

1.1 材料与试剂

收集2020年1月至6月本院收治的63例肝癌患者的肝癌组织及其癌旁组织样本, 置于–80 °C超低温冰箱内保存, 所有患者均经病理诊断确诊为肝癌, 其中男34例, 女29例, 年龄51~71岁, 平均年龄(62.35±5.16)岁。所有患者均签署知情同意书, 本研究经大连医科大学附属第一医院医学伦理委员会审核批准(批准号: YJ-KY-2019-011)。

正常人肝上皮细胞THLE-3, 以及人肝癌细胞MHCC97H、Hep3B、SK-HEP-1均购自美国ATCC中心; DMEM培养基、胎牛血清及MTT溶液均购自上海碧云天生物技术有限公司; Transwell小室、Matrigel基质胶购自美国BD公司; Trizol试剂及双荧光素酶活性试剂盒购自北京索莱宝科技有限公司; 反转录试剂盒与SYBR Green试剂盒购自天根生化科技(北京)有限公司; 双荧光素酶报告基因载体购自

美国Promega公司; lncRNA PSMA3-AS1小分子干扰RNA(si-lncRNA PSMA3-AS1)及其阴性对照(si-NC)、miR-627-3p寡核苷酸模拟物(miR-627-3p mimics)及其阴性对照mimic NC序列(miR-NC)、miR-627-3p特异性寡核苷酸抑制剂(anti-miR-627-3p)及其其阴性对照(anti-miR-NC)、Lipofectamine™ 3000转染试剂均购自广州锐博生物科技有限公司; lncRNA PSMA3-AS1过表达载体(pcDNA-lncRNA PSMA3-AS1)及其对照空载体(pcDNA)购自吉满生物科技(上海)有限公司; 兔抗人基质金属蛋白酶-2(matrix metalloproteinase 2, MMP2)、基质金属蛋白酶-9(matrix metalloproteinase 9, MMP9)抗体与辣根过氧化物酶(horseradish peroxidase, HRP)标记的山羊抗兔IgG二抗均购自美国CST公司。

1.2 方法

1.2.1 细胞转染及分组 细胞培养: THLE-3细胞、MHCC97H、Hep3B、SK-HEP-1细胞均用含有10%胎牛血清、100 U/mL青霉素和100 U/mL链霉素的DMEM培养基, 在恒温箱(37 °C、5% CO_2)内持续培养。

细胞转染: 采用脂质体转染法进行转染, 取对数生长期Hep3B细胞(2.5×10^5个/mL)接种于6孔板, 用不含血清的培养基分别稀释si-NC、si-lncRNA PSMA3-AS1、miR-NC、miR-627-3p mimics、si-lncRNA PSMA3-AS1和anti-miR-NC、si-lncRNA PSMA3-AS1和anti-miR-627-3p后室温静置5 min, 用不含血清的培养基稀释Lipofectamine™ 3000转染试剂, 将转染试剂与转染物分别充分混匀, 以获得转染复合物。6孔板内加入培养基和上述混合液, 培育6 h后将不含血清的培养基更换为DMEM完全培养基继续培养48 h。

实验分组: 采用脂质体转染法将si-NC、si-lncRNA PSMA3-AS1、miR-NC、miR-627-3p mimics转染至Hep3B细胞, 分别记为si-NC组、si-lncRNA PSMA3-AS1组、miR-NC组、miR-627-3p组; 采用脂质体转染法将si-lncRNA PSMA3-AS1和anti-miR-NC共转染至Hep3B细胞, 记为si-lncRNA PSMA3-AS1+anti-miR-NC组; 采用脂质体转染法将si-lncRNA PSMA3-AS1和anti-miR-627-3p共转染至Hep3B细胞, 记为si-lncRNA PSMA3-AS1+anti-miR-627-3p组; 同时将正常培养的Hep3B细胞记为空白对照组(NC组)。

1.2.2 双荧光素酶报告实验检测lncRNA PSMA3-AS1与miR-627-3p的靶向关系 LncBase Predicted v.2预测显示lncRNA PSMA3-AS1与miR-627-3p存在结合位点, 将结合位点与突变位点的片段克隆至pmirGLO载体得到野生型载体lncRNA PSMA3-AS1-WT、突变型载体lncRNA PSMA3-AS1-MUT, 采用脂质体转染法将上述载体分别与miR-NC或miR-627-3p mimics共转染至Hep3B细胞, 将共转染后的Hep3B细胞置于培养箱(37 °C、体积分数5% CO_2)内继续培养24 h后采用相关试剂盒对其相对荧光素酶活性进行检测。采用脂质体转染法将si-NC、si-lncRNA PSMA3-AS1、pcDNA、pcDNA-lncRNA PSMA3-AS1分别转染至Hep3B细胞, 培养48 h后收集细胞并采用qRT-PCR实验检测miR-627-3p的表达量。

1.2.3 qRT-PCR分析 Trizol试剂法提取各类组织和细胞(包括癌旁组织、肝癌组织、THLE-3细胞, 以及MHCC97H、Hep3B、SK-HEP-1细胞)的总RNA, 反转录合成cDNA, PCR扩增反应体系: 10 μL SYBR Green Master Mix, 正反向引物各0.8 μL, 2 μL cDNA, ddH_2O补足至20 μL; 反应参数设置为: 95 °C预变性2 min, 95 °C变性15 s, 60 °C退火30 s, 72 °C延伸30 s, 重复以上步骤40次。采用$2^{-\Delta\Delta Ct}$法计算lncRNA PSMA3-AS1、miR-627-3p的相对表达量。

1.2.4 MTT比色法检测细胞增殖 首先取Hep3B细胞进行预先处理, 完成后将其置于96孔板(3×10^3个/孔)内进行实验, 每孔加入20 μL MTT溶液, 于37 °C、5% CO_2培养箱中培养4 h后弃培养基, 避光储存并加入150 μL DMSO, 轻摇混匀后于490 nm波长处检测各孔吸光度(*D*)值。

1.2.5 平板克隆形成实验 将各组Hep3B细胞接种于6孔板(500个/孔), 于37 °C、5% CO_2培养箱中培养14天, 弃培养基, 用预冷的PBS洗涤后加入500 μL甲醇固定20 min(–20 °C), 加入400 μL 1%结晶紫染色液染色15 min(37 °C), 随后使用显微镜(200倍)对其进行观察, 记录*L*>0.1 mm(L为细胞的直径)的细胞克隆形成数。

1.2.6 Transwell检测细胞迁移和侵袭 迁移实验: 首先做好Hep3B细胞的预处理, 完成后准备Transwell小室, 将Hep3B细胞加入上室(200 μL/孔), 600 μL的10%胎牛血清培养液加入下室, 并培养24 h, 培养完毕后使用PBS冲洗, 并于37 °C使用多聚甲醛固定20 min, 用1%结晶紫染色10 min后在电子显微镜下观察迁移细胞数量。侵袭实验: 具体步骤与迁移实验相同, 但

在上室中置入Hep3B细胞前, 首先应将Matrigel进行稀释, 并将其铺满上室表面, 然后再进行后续实验。

1.2.7 蛋白质印迹法检测MMP2、MMP9蛋白水平 首先对各组细胞总蛋白进行提取, 并加入RIPA裂解液, 剂量根据实际情况调整, 提取细胞总蛋白, 对蛋白含量进行检测, 记录蛋白表达并对蛋白进行电泳分离, 分离后将其置于PVDF膜中, 该过程中需要做好PVDF膜的封闭(37 ℃、2 h)处理, 使用5%脱脂牛奶即可, 将一抗和实验溶液[包括MMP2(1:1 000)、MMP9(1:1 000)一抗与内参β-actin抗体(1:3 000)稀释液]加入, 随后将其置于4 ℃环境中孵育, 过夜后使用TBST洗涤, 完成后再次加入二抗稀释液, 稀释浓度为1:5 000, 加入二抗后室温孵育60 min, 加入ECL完成显影后使用ImageJ软件分析各蛋白条带灰度值。

1.3 统计学分析

应用SPSS 24.0软件对数据进行处理并分析, 所有数据都以$\bar{x}\pm s$的形式呈现, 两组间比较采用t检验; 多组间比较采用单因素方差分析, 进一步两两比较采用LSD-t检验; 用Pearson法进行相关性分析, P<0.05则表明有显著性差异。

2 结果

2.1 肝癌患者中lncRNA PSMA3-AS1与miR-627-3p的表达情况

采用qRT-PCR法检测肝癌组织与癌旁组织中lncRNA PSMA3-AS1、miR-627-3p的表达量, 结果显示, 与癌旁组织相比, 肝癌组织中lncRNA PSMA3-AS1的表达量升高(P<0.05), miR-627-3p的表达量降低(P<0.05)。Pearson法结果显示, lncRNA PSMA3-AS1与miR-627-3p呈负相关(r=–0.760 1, P<0.000 1)(图1)。

2.2 在肝癌细胞系中, lncRNA PSMA3-AS1表达上调, miR-627-3p表达下调

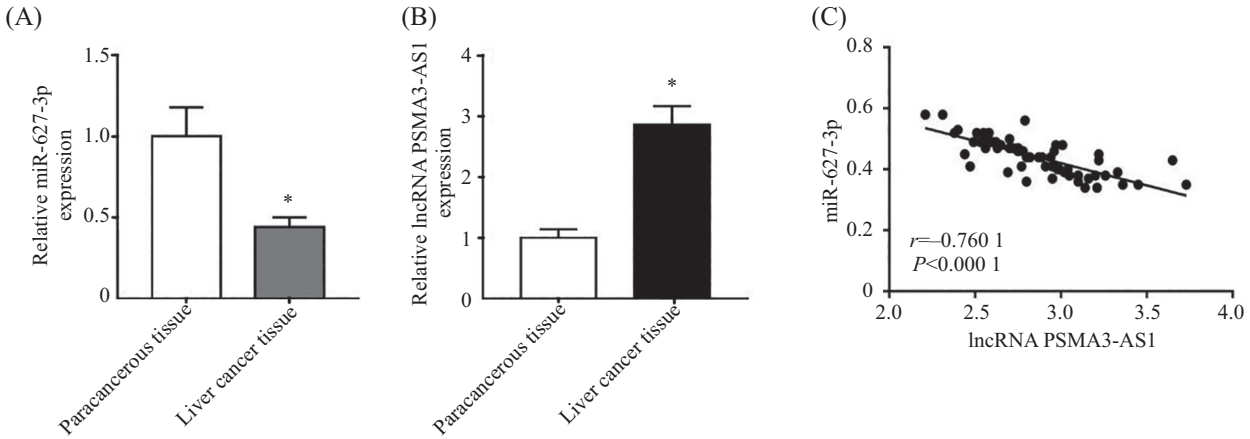
采用qRT-PCR法检测肝癌细胞系中lncRNA PSMA3-AS1、miR-627-3p的表达量。结果显示, 与THLE-3细胞比较, MHCC97H、Hep3B、SK-HEP-1细胞中lncRNA PSMA3-AS1的表达量升高(P<0.05), 表达量最高的为Hep3B细胞组; miR-627-3p表达量降低(P<0.05), 表达量最低的为Hep3B细胞组(图2)。

2.3 LncBase Predicted v.2预测lncRNA PSMA3-AS1和miR-627-3p之间的靶向关系

lncRNA PSMA3-AS1与miR-627-3p存在结合位点(图3)。miR-627-3p表达增高, lncRNA PSMA3-AS1-WT荧光素酶活性随之降低(P<0.05, 图4A)。与si-NC组相比, si-lncRNA PSMA3-AS1组miR-627-3p的表达量升高(P<0.05); 与pcDNA组比较, pcDNA-lncRNA PSMA3-AS1组miR-627-3p的表达量降低(P<0.05, 图4B)。

2.4 低表达lncRNA PSMA3-AS1抑制肝癌Hep3B细胞增殖、迁移和侵袭

采用MTT法、平板克隆形成实验与Transwell

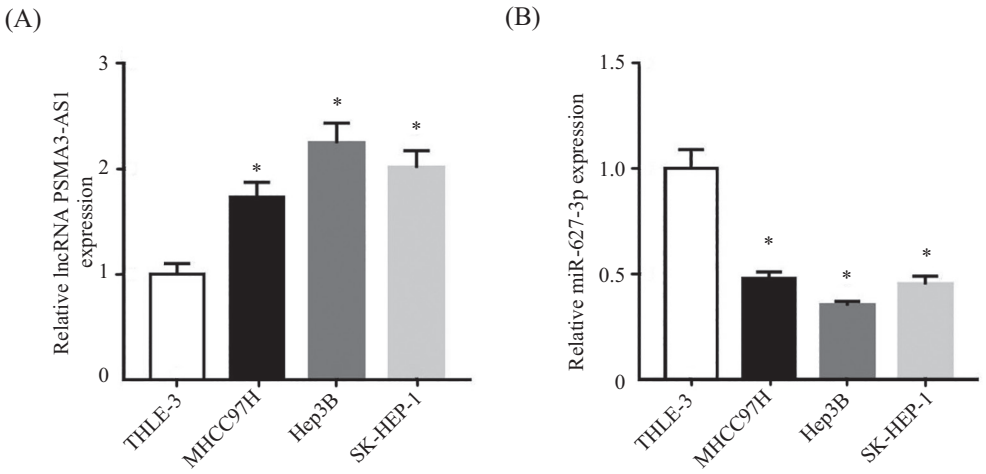


A: qRT-PCR法检测肝癌组织和癌旁组织中miR-627-3p的表达量; B: qRT-PCR法检测肝癌组织和癌旁组织中lncRNA PSMA3-AS1的表达量; C: Pearson法分析肝癌组织中lncRNA PSMA3-AS1、miR-627-3p表达量的相关性。*P<0.05, 与癌旁组织相比。

A: qRT-PCR was used to detect the expression of miR-627-3p in liver cancer tissue and paracancerous tissue; B: qRT-PCR was used to detect the expression of lncRNA PSMA3-AS1 in liver cancer tissue and paracancerous tissue; C: Pearson method was used to analyze the correlation of lncRNA PSMA3-AS1 and miR-627-3p expression in liver cancer tissue. *P<0.05 compared with paracancerous tissues.

图1 肝癌患者中lncRNA PSMA3-AS1和miR-627-3p的表达量

Fig.1 Expression of lncRNA PSMA3-AS1 and miR-627-3p in liver cancer patients



A: qRT-PCR法检测肝癌细胞系中lncRNA PSMA3-AS1的表达量; B: qRT-PCR法检测肝癌细胞系中miR-627-3p的表达量。*P<0.05, 与THLE-3细胞相比。THLE-3: 正常人肝上皮细胞。

A: qRT-PCR was used to detect the expression of lncRNA PSMA3-AS1 in liver cancer cell lines; B: qRT-PCR was used to detect the expression of miR-627-3p in liver cancer cell lines. *P<0.05 compared with THLE-3. THLE-3: normal human liver epithelial cells.

图2 lncRNA PSMA3-AS1、miR-627-3p在肝癌细胞系中的表达情况

Fig.2 Expression of lncRNA PSMA3-AS1 and miR-627-3p in liver cancer cell lines

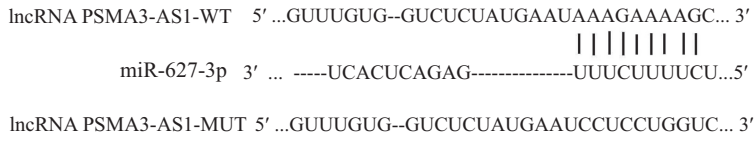
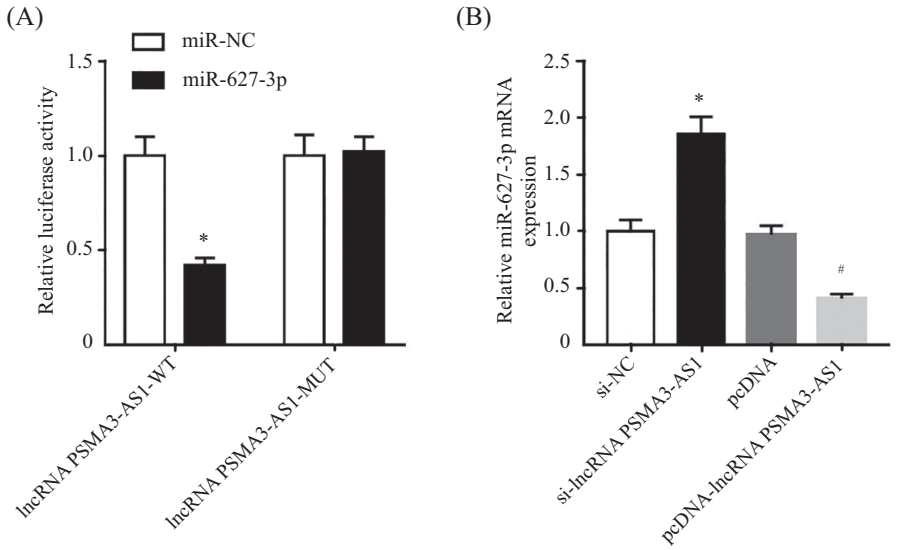


图3 LncBase Predicted v.2预测显示lncRNA PSMA3-AS1与miR-627-3p之间存在结合位点

Fig.3 The binding sites between lncRNA PSMA3-AS1 and miR-627-3p were predicted by LncBase Predicted v.2



A: 双荧光素酶活性检测; *P<0.05, 与miR-NC组比较; B: qRT-PCR检测miR-627-3p的表达; *P<0.05, 与si-NC组比较; $^{\#}P$<0.05, 与pcDNA组比较。

A: dual luciferase activity detection; *P<0.05 compared with miR-NC group; B: qRT-PCR was used to detect the expression of miR-627-3p; *P<0.05 compared with si-NC group; $^{\#}P$<0.05 compared with pcDNA group.

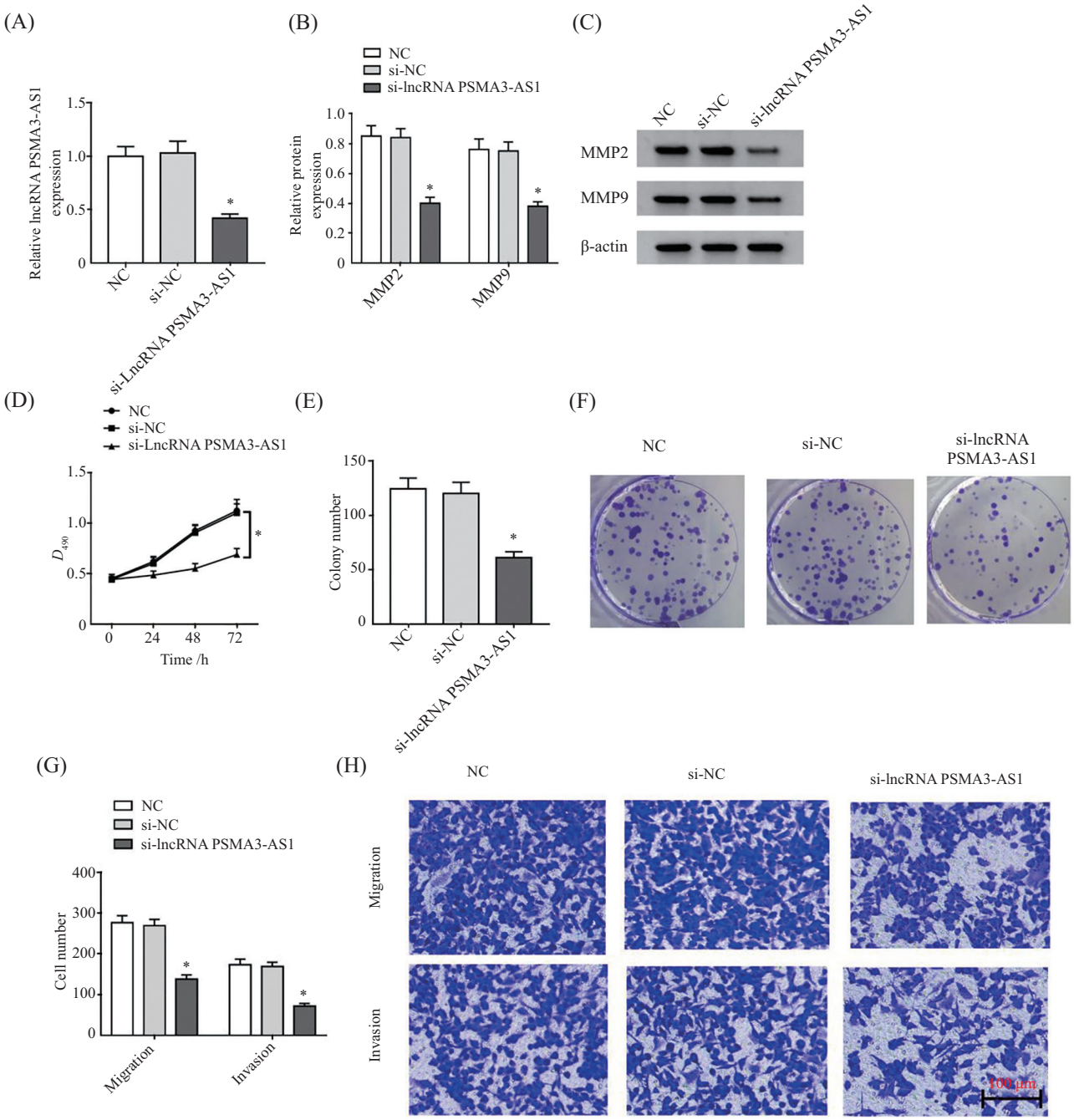
图4 lncRNA PSMA3-AS1靶向调控miR-627-3p的表达

Fig.4 lncRNA PSMA3-AS1 targeted and regulated the expression of miR-627-3p

实验分别检测细胞增殖、克隆形成、迁移及侵袭能力, 结果显示, si-lncRNA PSMA3-AS1组细胞活力、克隆形成数、迁移及侵袭细胞数低于si-NC组、NC组, MMP2、MMP9蛋白水平均显著低于si-NC组、NC组(P<0.05, 图5)。

2.5 高表达miR-627-3p抑制肝癌Hep3B细胞增殖、迁移和侵袭

采用MTT法、平板克隆形成实验与Transwell实验分别检测细胞增殖、克隆形成、迁移及侵袭能力, 结果显示, miR-627-3p组细胞活力、克隆形

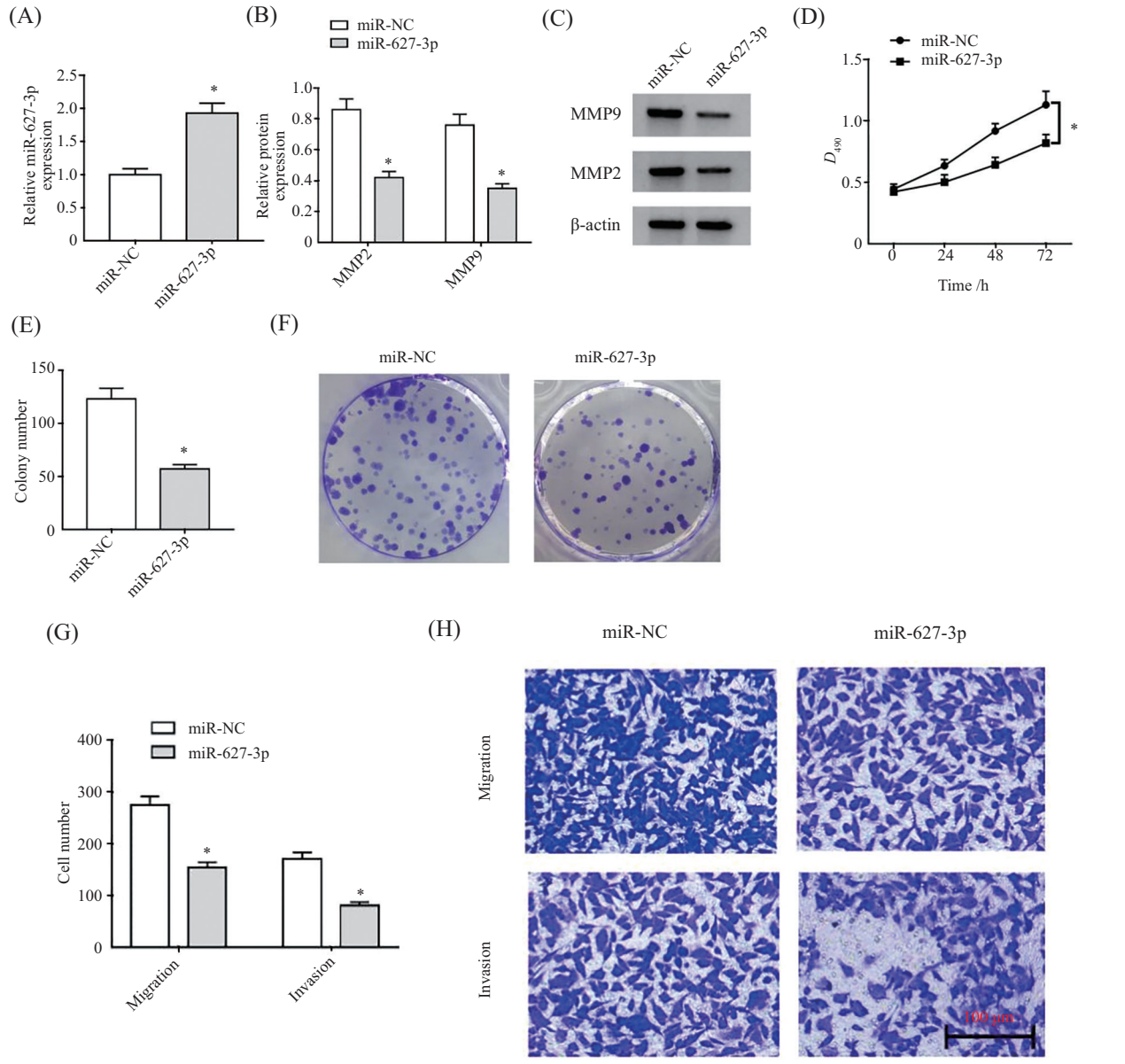


A: qRT-PCR检测lncRNA PSMA3-AS1转染结果; B、C: Western Blot检测MMP2、MMP9蛋白的表达; D: 细胞增殖活性; E、F: 细胞克隆实验; G、H: Transwell检测细胞迁移和侵袭。*P<0.05, 与si-NC组比较。

A: qRT-PCR was used to detect the results of lncRNA PSMA3-AS1 transfection; B,C: Western blot was used to detect the expression of MMP2 and MMP9 protein; D: cell proliferation activity; E,F: cell cloning experiment; G,H: Transwell was used to detect cell migration and invasion. *P<0.05 compared with si-NC group.

图5 低表达lncRNA PSMA3-AS1抑制肝癌Hep3B细胞增殖、迁移和侵袭

Fig.5 Low expression of lncRNA PSMA3-AS1 inhibited the proliferation, migration and invasion of liver cancer Hep3B cells



A: qRT-PCR检测lncRNA PSMA3-AS1转染结果; B、C: Western blot检测MMP2、MMP9蛋白的表达; D: 细胞增殖活性; E、F: 细胞克隆实验; G、H: Transwell检测细胞迁移和侵袭。*P<0.05, 与miR-NC组比。

A: qRT-PCR was used to detect the results of lncRNA PSMA3-AS1 transfection; B,C: Western blot was used to detect the expression of MMP2 and MMP9 proteins; D: cell proliferation activity; E,F: cell cloning experiment; G,H: Transwell was used to detect cell migration and invasion. *P<0.05 compared with miR-NC group.

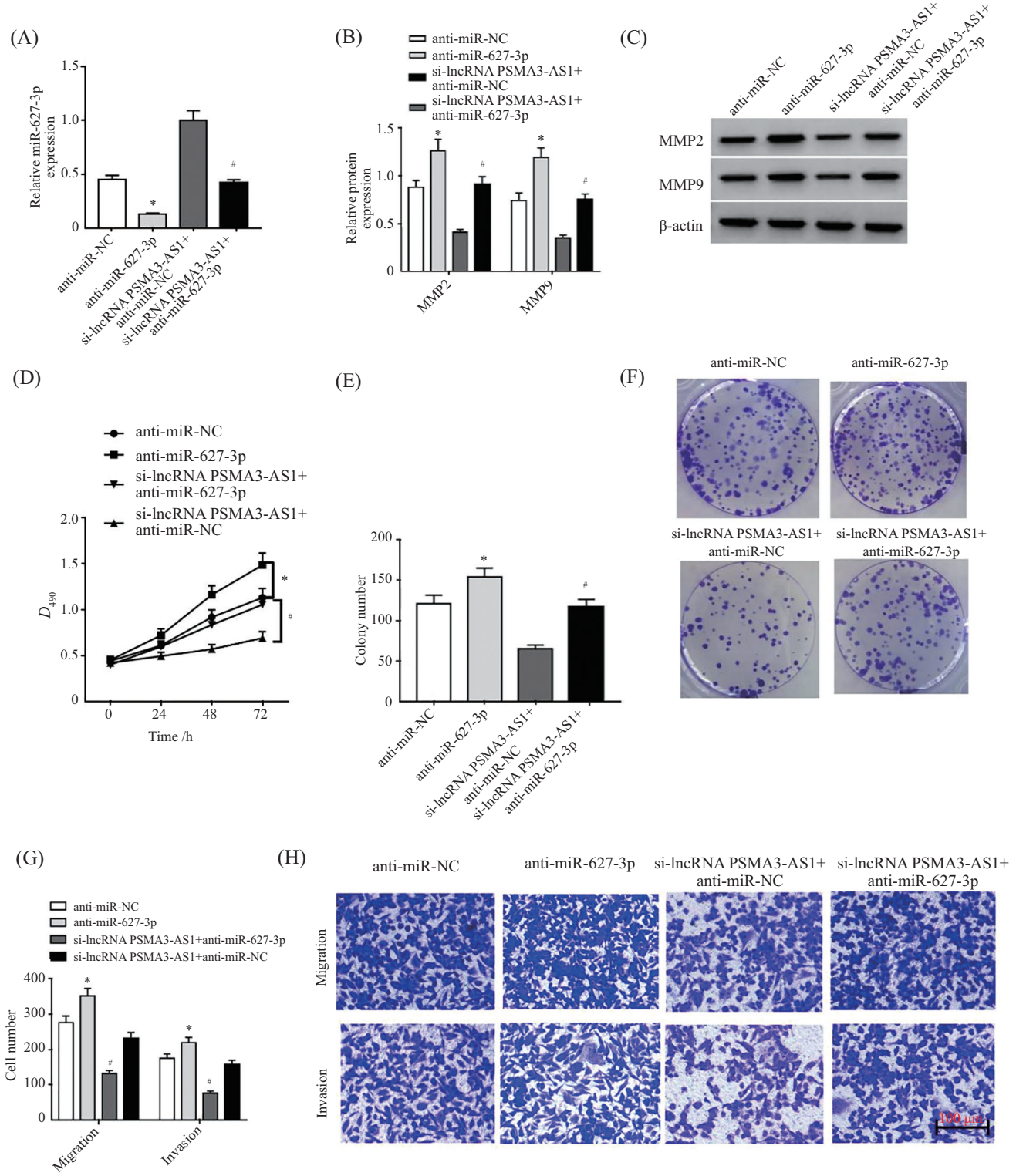
图6 高表达miR-627-3p抑制肝癌Hep3B细胞增殖、迁移和侵袭

Fig.6 High expression of miR-627-3p inhibited the proliferation, migration and invasion of liver cancer Hep3B cells

成数、迁移及侵袭细胞数低于miR-NC组, MMP2、MMP9蛋白水平显著低于miR-NC组(P<0.05, 图6)。

2.6 低表达miR-627-3p可以逆转lncRNA PSMA3-AS1低表达对Hep3B细胞增殖、迁移和侵袭的影响

采用MTT法、平板克隆形成实验与Transwell实验分别检测细胞增殖、克隆形成、迁移及侵袭能力, 结果显示, si-lncRNA PSMA3-AS1+anti-miR-627-3p组细胞活力较si-lncRNA PSMA3-AS1+anti-miR-NC组显著升高(P<0.05); 与si-lncRNA PSMA3-AS1+anti-miR-NC组相比, si-lncRNA PSMA3-AS1+anti-miR-627-3p组细胞克隆形成数、迁移及侵袭细胞数增多, MMP2、MMP9蛋白水平均表达上升(P<0.05, 图7)。



A: qRT-PCR检测lncRNA PSMA3-AS1转染结果; B、C: Western blot检测MMP2、MMP9蛋白的表达; D: 细胞增殖活性; E、F: 细胞克隆实验; G、H: Transwell检测细胞迁移和侵袭。*P<0.05, 与anti-miR-NC组比; #P<0.05, 与si-lncRNA PSMA3-AS1+anti-miR-NC比。

A: qRT-PCR was used to detect the results of lncRNA PSMA3-AS1 transfection; B,C: Western blot was used to detect the expression of MMP2 and MMP9 proteins; D: cell proliferation activity; E,F: cell cloning experiment; G,H: Transwell was used to detect cell migration and invasion. *P<0.05 compared with anti-miR-NC group; #P<0.05 compared with si-lncRNA PSMA3-AS1+anti-miR-NC.

图7　低表达miR-627-3p可以逆转lncRNA PSMA3-AS1低表达对肝癌Hep3B细胞增殖、迁移和侵袭的影响

Fig.7　Low expression of miR-627-3p could reverse the effect of low expression of lncRNA PSMA3-AS1 on the proliferation, migration and invasion of liver cancer Hep3B cells

3 讨论

lncRNA具有miRNA的反应元件, 即lncRNA可作为miRNA的ceRNA分子而参与多种肿瘤发生及发展过程, 同时参与调控细胞周期与细胞分化等多个生命活动过程, 是遗传学的研究热点之一[9-10]。lncRNA XIST[11]、lncRNA LOXL1-AS1[12]、lncRNA MIR4435-2HG[13]在肝癌组织及细胞系中表达水平升高, 并可促进肝癌细胞增殖及转移。

lncRNA PSMA3-AS1在结直肠癌中呈高表达, 并可通过作为miR-4429的ceRNA分子而促进结直肠癌细胞迁移及侵袭[14]。lncRNA PSMA3-AS1可通过作为miR-101的ceRNA分子而正向调控其靶基因*EZH2*的表达, 从而促进食管癌细胞增殖及转移[15]。lncRNA PSMA3-AS1可通过调节miR-302a-3p/RAB22A分子轴而促进胶质瘤细胞增殖、迁移及侵袭[16]。但lncRNA PSMA3-AS1在肝癌中的表达情况及其可能的作用机制尚未可知。本研究结果显示, 肝癌组织与细胞系中lncRNA PSMA3-AS1的表达量升高, 干扰lncRNA PSMA3-AS1表达可降低肝癌细胞活力, 减少细胞克隆形成数, 提示干扰lncRNA PSMA3-AS1表达可抑制肝癌细胞增殖及克隆形成。MMP2、MMP9作为基质金属蛋白酶, 其可对细胞的转移和迁移进行调控, 已有研究发现, MMP2、MMP9蛋白水平的升高, 可通过降解细胞外基质从而促进细胞转移[17]。而本研究通过对肝癌细胞的靶点进行分析, 结果发现将lncRNA PSMA3-AS1表达进行抑制/干扰后, 肝癌迁移及侵袭细胞数减少, MMP2、MMP9表达下调, 提示干扰lncRNA PSMA3-AS1表达可抑制肝癌细胞迁移及侵袭, 其作用机制与抑制细胞外基质降解有关。

本研究初步证实lncRNA PSMA3-AS1可作为miR-627-3p的ceRNA分子, 并可负向调控miR-627-3p的表达。研究表明miR-627-3p在肺癌细胞中表达下调, 上调其表达可抑制肺癌细胞增殖及侵袭[18]。miR-627-3p在结直肠癌[19]、非小细胞肺癌[20]中表达水平降低, 并可促进细胞增殖。本研究发现, miR-627-3p在肝癌组织和细胞系中表达水平降低, 上调其表达可抑制肝癌细胞增殖及迁移, 而抑制其表达可减弱干扰lncRNA PSMA3-AS1表达对肝癌细胞增殖、迁移等生物学行为的作用。这提示lncRNA PSMA3-AS1可通过靶向调控miR-627-3p的表达水平而促进肝癌细胞分化和增殖, 从而参与肝癌发生发展进程。

综上所述, 肝癌组织与细胞系中lncRNA PSMA3-AS1表达上调, miR-627-3p表达下调, 干扰lncRNA PSMA3-AS1表达可通过促进miR-627-3p表达而抑制肝癌细胞增殖、克隆形成、迁移及侵袭, 在今后肝癌的治疗中, lncRNA PSMA3-AS1/miR-627-3p可能作为肝癌治疗靶点, 为进一步阐明肝癌发病机制奠定基础。但lncRNA PSMA3-AS1具有多个miRNA的反应元件, 其是否可通过靶向调控其他miRNA而参与肝癌发生及发展过程尚需进一步探究。

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