LINC00342/miR-505-3p/*PGK1*基因在乳腺癌患者肿瘤转移中的表达以及对细胞生物学特性的影响

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摘要 长链非编码RNA LINC00342已被证实在多种癌症中参与重要生物学功能。然而, LINC00342在乳腺癌中的作用和机制尚不清楚。在该研究中, 选取28例乳腺癌患者肿瘤组织和癌旁正常组织, 乳腺癌细胞和正常乳腺上皮细胞, 用qRT-PCR检测LINC00342、miR-505-3p和磷酸甘油酸激酶1(*PGK1*) mRNA的表达情况。Western blot检测PGK1蛋白的表达情况。将乳腺癌细胞MCF-7分为NC组(空白)、si-LINC00342组(转染si-LINC00342)、si-NC组(转染si-NC)、miR-505-3p组(转染miR-505-3p模拟物)、miR-NC组(转染miR-NC)、si-PGK1组(转染si-PGK1)、si-NC组(转染si-NC)、si-LINC00342+pcDNA-PGK1组(同时转染si-LINC00342与pcDNA-PGK1)和si-LINC00342+pcDNA组(同时转染si-LINC00342与pcDNA)。利用Western blot检测PGK1、迁移侵袭标志物基质金属蛋白酶MMP2和MMP9的表达情况, MTT法检测细胞增殖能力, Transwell法检测细胞迁移和侵袭数量。双荧光素酶活性实验检测miR-505-3p与LINC00342、PGK1之间的靶向结合。结果显示, LINC00342、*PGK1* mRNA和PGK1蛋白在乳腺癌组织和细胞中显著上调, miR-505-3p显著下调。干扰LINC00342、过表达miR-505-3p或干扰PGK1均能抑制乳腺癌细胞增殖、迁移和侵袭, 以及MMP2和MMP9蛋白表达。此外, LINC00342可直接靶向miR-505-3p调控PGK1表达, 高表达PGK1可逆转LINC00342低表达对MCF-7增殖、迁移和侵袭的抑制效果。该研究提示, 干扰LINC00342表达可能通过靶向miR-505-3p调控PGK1的表达, 抑制乳腺癌细胞的增殖、迁移和侵袭。

关键词 乳腺癌; 增殖; 转移; LINC00342; miR-505-3p; PGK1

The Expression of LINC00342/miR-505-3p/*PGK1* Gene Expression in Breast Cancer Patients with Tumor Metastasis and Its Influence on Cell Biological Characteristics

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Abstract Long non-coding RNA LINC00342 has been shown to be involved in important biological functions in a variety of cancers. However, the role and mechanism of LINC00342 in breast cancer remain unclear. This study selected 28 paired tumor tissue and adjacent normal tissue from breast cancer patients, as well as breast cancer cells and normal breast epithelial cells. The expressions of LINC00342, miR-505-3p, *PGK1* (phosphoglycerate kinase 1) mRNA were detected by qRT-PCR. The protein expression of PGK1 was detecetd by Western blot. The breast cancer MCF-7 cells were divided into NC group (blank), si-LINC00342 group (transfected with si-LINC00342), si-NC group (transfected with si-NC), miR-505-3p group (transfected with miR-505-3p mimic), miR-

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NC group (transfected with miR-NC), si-PGK1 group (transfected with si-PGK1), si-NC group (transfected with si-NC), si-LINC00342+pcDNA-PGK1 group (simultaneous transfection with si-LINC00342 and pcDNA-PGK1), si-LINC00342+pcDNA group (simultaneous transfection with si-LINC00342 and pcDNA). Western blot was used to detect the expression of PGK1, MMP2 (matrix metalloprotease 2), and MMP9 protein. The MTT method was implemented to monitor the cell proliferation ability, and the Transwell method was employed to assess the number of cell migration and invasion. The dual luciferase activity assay detects the targeted binding between miR-505-3p, LINC00342 and PGK1. The results showed that LINC00342, *PGK1* mRNA, and PGK1 protein were significantly up-regulated in breast cancer tissues and cells, while miR-505-3p was significantly down-regulated. LINC00342 interference, miR-505-3p overexpression or PGK1 knockdown could inhibit the proliferation, migration, invasion, and the expression of MMP2 and MMP9 proteins in breast cancer cells. In addition, LINC00342 can directly target miR-505-3p to regulate the expression of PGK1, and high expression of PGK1 can reverse the inhibitory effect of low expression of LINC00342 on the proliferation, migration, and invasion of MCF-7. This study suggests that interference with the expression of LINC00342 may regulate the expression of PGK1 by targeting miR-505-3p to inhibit the proliferation, migration, and invasion of breast cancer cells.

Keywords breast cancer; proliferation; metastasis; LINC00342; miR-505-3p; PGK1

乳腺癌是全世界女性中诊断率最高的癌症[1]。长链非编码RNA(long non-coding RNA, lncRNA)和微小RNA(microRNA, miRNA/miR)是近年来受到研究人员广泛关注的两组非编码RNA[2]。lncRNA长度超过200个核苷酸, 已在人类癌症的发病机制中显示出广泛的生物学功能。miRNA是含大约22个核苷酸的小RNA转录物, 通过mRNA降解或翻译抑制在转录后调节基因表达[3]。据报道, lncRNA作为竞争性内源RNA(competing endogenous RNA, ceRNA)发 挥作用, 竞争miRNA以在转录后水平上调其靶基因的表达[4]。LINC00342是一种位于染色体2q11.1上的新型lncRNA, 在胃癌[5]、婴儿血管瘤[6]和结直肠癌[7]中上调。LIU等[6]报道, LINC00342在婴儿血管瘤中起致癌作用, LINC00342的过表达可加速血管瘤衍生的内皮细胞的增殖并减少其凋亡。另一项研究发现, LINC00342在结肠腺癌组织中过表达, 其高表达水平与结肠腺癌患者的不良预后有关, 而敲低LINC00342可抑制结肠腺癌细胞生物学活性[7]。然而, LINC00342的表达和功能在乳腺癌中仍然未知。

miR-505-3p在乳腺癌组织和细胞系中低表达, 且miR-505-3p低表达组患者的生存率低于miR-505-3p高表达组患者[8]。此外, miR-505-3p可抑制MDA-MB-231增殖、迁移及侵袭, 促进凋亡[9]。磷酸甘油酸激酶1(phosphoglycerate kinase 1, *PGK1*) mRNA水平升高已被证明与乳腺癌、肝癌、肺癌、胃癌患者等的总生存期短或不良预后相关[10]。资料显示, PGK1的mRNA和蛋白表达水平在各种临床病理类型的乳腺癌中均上调[11-12]。在功能上, PGK1表达的敲低抑制了乳腺癌细胞的侵袭并逆转了上皮间质转化过程[13]。但LINC00342在乳腺癌中的表达情况和详细机制是否与miR-505-3p/PGK1有关, 目前仍不清楚。本研究针对LINC00342/miR-505-3p/PGK1在乳腺癌患者肿瘤转移中的表达以及对细胞生物学特性的影响进行探讨, 以期在分子水平上丰富乳腺癌相关信息。

1 材料与方法

28例乳腺癌患者癌组织、肿瘤转移组织和相匹配的癌旁组织来自江西医学高等专科学校第一附属医院经组织学确诊的乳腺癌患者。所有患者无其他既往肿瘤, 先前未接受过任何治疗, 存在肿瘤转移。新鲜的癌组织及其匹配的正常组织经手术切除后立即收集并储存在−80 °C以提取RNA和蛋白。所有方案均经江西医学高等专科学校第一附属医院伦理委员会审查和批准(伦理号: 伦JC2022041)。所有患者在临床手术前均签署知情同意书。

乳腺癌细胞系(MCF-7、T47D、BT549)、正常乳腺上皮细胞MCF-10A购自深圳市豪地华拓生物科技有限公司; 靶向LINC00342和PGK1的小干扰RNA(si-LINC00342、si-PGK1), 各自阴性对照si-NC、miR-505-3p模拟物和抑制剂(anti-miR-505-3p), 各自阴性对照miR-NC/anti-miR-NC, PGK1的过表达

载体pcDNA-PGK1, 过表达空载体pcDNA购自广州锐博生物技术有限公司; 噻唑蓝(methyl thiazolyl tetrazolium, MTT)购自美国Sigma-Aldrich公司; PGK1抗体、基质金属蛋白酶(matrix metalloprotease, MMP)2抗体、MMP9抗体、山羊抗兔二抗购自美国Abcam公司; ECL Western blot底物、TaqMan逆转录试剂盒购自ThermoFisher Scientific公司; psiCHECK载体、双荧光素酶报告基因检测系统购自美国Promega公司; SYBR Green Master Mix购自美国Bio-Rad公司。

1.1 细胞培养与分组

MCF-7、T47D、BT549、MCF-10A细胞系均用补充有10%胎牛血清的Dulbecco改良Eagle培养基(Dulbecco's Modified Eagle's Medium, DMEM)于37 °C、5% CO_2条件下培养。将乳腺癌细胞MCF-7分为NC组(空白)、si-LINC00342组(转染si-LINC00342)、si-NC组(转染si-NC)、miR-505-3p组(转染miR-505-3p模拟物)、miR-NC组(转染miR-NC)、si-PGK1组(转染si-PGK1)、si-NC组(转染si-NC)、si-LINC00342+pcDNA-PGK1组(同时转染si-LINC00342与pcDNA-PGK1)、si-LINC00342+pcDNA组(同时转染si-LINC00342与pcDNA)。si-LINC00342+anti-miR-NC组(同时转染si-LINC00342和anti-miR-NC)和si-LINC00342+anti-miR-505-3p组(同时转染si-LINC00342和anti-miR-505-3p)。转染时, 根据制造商Invitrogen的操作手册, 通过Lipofectamine 2000进行上述转染。在转染后48 h收获细胞, 用于以下研究。

1.2 qRT-PCR检测LINC00342、miR-505-3p、*PGK1* mRNA的表达情况

使用Trizol试剂从乳腺癌组织、细胞系中分离总RNA。使用NanoDrop ND-2000分光光度计检测RNA浓度和完整性。使用TaqMan逆转录试剂盒逆转录总RNA(1 μg)。逆转录产物稀释10倍, 制成实时PCR模板。SYBR Green Master Mix试剂盒进行PCR反应。引物在附表1中显示。内源对照为*GAPDH*(mRNA)或*U6*(miRNA)。选择$2^{-\Delta\Delta Ct}$方法量化LINC00342、miR-505-3p、*PGK1* mRNA表达。

1.3 Western blot检测PGK1、MMP2和MMP9蛋白的表达情况

使用RIPA裂解缓冲液提取乳腺癌组织、转移组织或细胞系的总蛋白。总蛋白(50 μg)上样到SDS-PAGE上, 电泳后转移到PVDF膜上。用5%脱脂牛奶37 °C封闭30 min后, 将膜与一抗[(PGK1抗体稀释比例1:1 000、MMP2抗体稀释比例1:1 000、MMP9抗体稀释比例1:1 000和β-肌动蛋白(β-actin)抗体稀释比例1:1 000)]在4 °C下孵育过夜。膜用Tris-吐温20缓冲液洗涤3次, 然后与二抗(稀释比例1:10 000)在室温下孵育1 h, 然后用Tris-吐温20缓冲液洗涤3次。最后, 蛋白ECL Western blot底物覆盖, Quantity One软件分析蛋白条带。

1.4 MTT法检测细胞增殖能力

将转染乳腺癌细胞MCF-7(4×10^3个)接种到96孔板中37 °C孵育48 h后, 将MTT加入每个板中并继续孵育2 h。去除上清液并加入二甲亚砜以溶解产物。最后, 通过酶标仪测量波长在490 nm处的吸光度(*D*)值。

1.5 Transwell法检测细胞迁移和侵袭数量

将转染乳腺癌细胞MCF-7重新悬浮在无血清DMEM中, 并放入具有8 μm孔径Transwell室的上部进行迁移测定, 或放入涂有Matrigel的上室中进行侵袭测定。同时, 在下室加入补充10%胎牛血清的DMEM溶液, 37 °C、5% CO_2孵育48 h后, 将迁移或侵袭的细胞移至底面, 用4%多聚甲醛室温固定30 min, 并用0.1%结晶紫染色30 min。最后, 使用Olympus显微镜对随机选择的三个视野进行成像和计数细胞数。

1.6 双荧光素酶活性检测

通过starbase预测发现LINC00342和miR-505-3p、*PGK1*和miR-505-3p之间存在潜在结合位点, 并在结合位点内进行突变以进行双荧光素酶报告基因检测。将LINC00342或PGK1-3′UTR-野生型(WT)/突变型(MUT)克隆并插入到psiCHECK载体中。将乳腺癌细胞MCF-7以1×10^5个的数量接种在24孔板中。24 h后, 将miR-505-3p模拟物或miR-NC与LINC00342-WT/MUT载体或PGK1-3′UTR-WT/MUT载体共转染入MCF-7细胞。使用双荧光素酶报告基因检测系统测定48 h后的荧光素酶活性。另外, 在乳腺癌细胞MCF-7中同时转染si-LINC00342与anti-miR-NC, si-LINC00342与anti-miR-505-3p, 分别记为si-LINC00342+anti-miR-NC组、si-LINC00342+anti-miR-505-3p组, 48 h后根据1.3和1.4所述方法检测miR-505-3p、PGK1蛋白表达情况。

1.7 统计学分析

数据通过SPSS 22.0软件进行分析, 并以平均值±标准差($\bar{x}\pm s$)表示。通过独立样本*t*检验进行两组间差异分析, 单因素方差分析进行多组间分析, SNK-*q*检验进行组间多重分析。$P<0.05$为差异有统计学意义。

2 结果

2.1 乳腺癌患者肿瘤转移组织中LINC00342、miR-505-3p、PGK1的表达情况

28例乳腺癌患者癌组织中LINC00342、miR-505-3p、PGK1 *mRNA*和PGK1蛋白表达量分别比癌旁组织增加约1.36倍、减少约32%、增加约0.89倍和增加约0.71倍; 且乳腺癌患者的肿瘤转移组织中LINC00342、miR-505-3p、*PGK1* mRNA和PGK1蛋白表达量分别比癌组织增加约0.25倍、减少约54%、增加约0.24倍和增加约0.33倍(P<0.05)(图1)。

2.2 在乳腺癌细胞系中, LINC00342、miR-505-3p、PGK1的表达情况

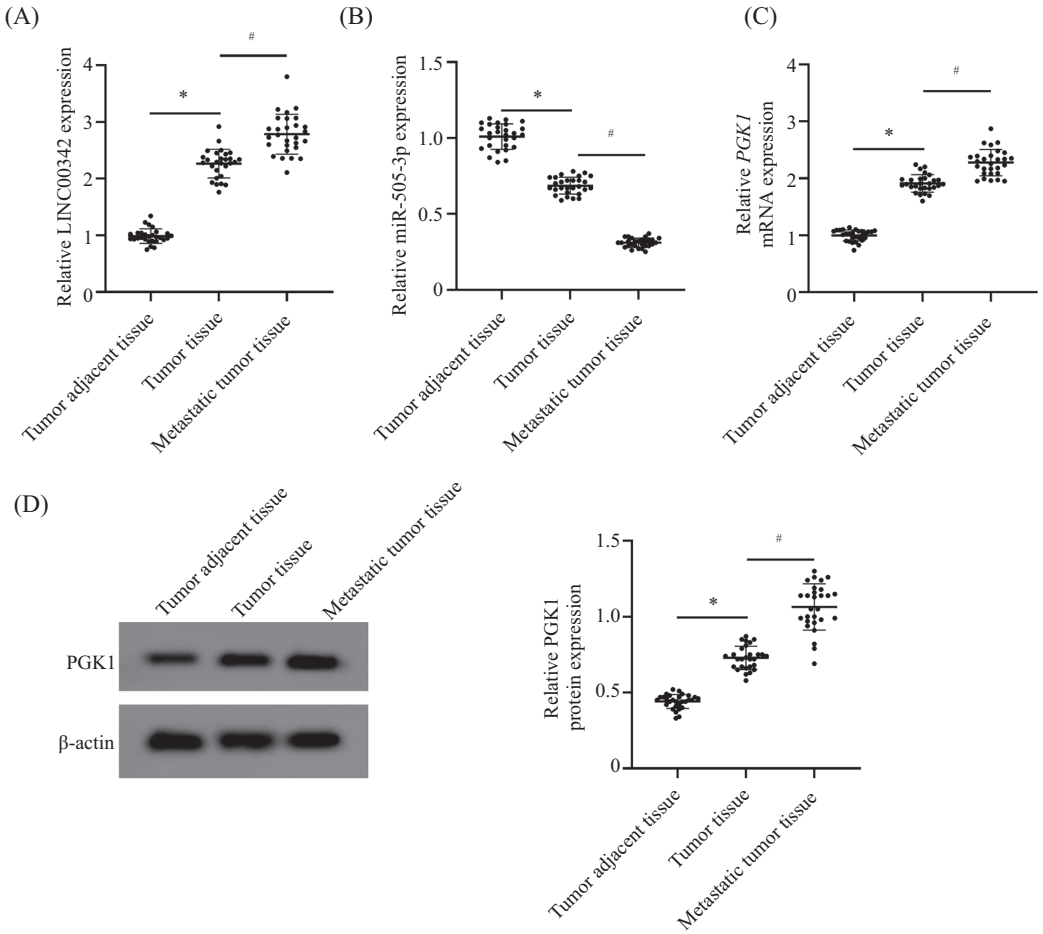
乳腺癌细胞系MCF-7、T47D、BT549中LINC00342表达量均高于正常乳腺上皮细胞MCF-10A, miR-505-3p表达量低于MCF-10A细胞, *PGK1* mRNA和PGK1蛋白表达量高于MCF-10A细胞(P<0.05)(图2)。选择差异最显著的MCF-7细胞进行后续实验。

2.3 LINC00342低表达对MCF-7乳腺癌细胞增殖、迁移和侵袭的影响

在MCF-7细胞中低表达LINC00342, si-LINC00342组LINC00342、MMP2、MMP9蛋白的表达量均比NC组低, 细胞增殖能力、迁移和侵袭数量也均比NC组低(P<0.05), 但这些指标在si-NC组与NC组间无明显波动(P>0.05)(图3)。

2.4 miR-505-3p高表达对MCF-7乳腺癌细胞增殖、迁移和侵袭的影响

在MCF-7细胞中, 与miR-NC组相比, miR-505-3p组的miR-505-3p表达量增加, MMP2、MMP9蛋白

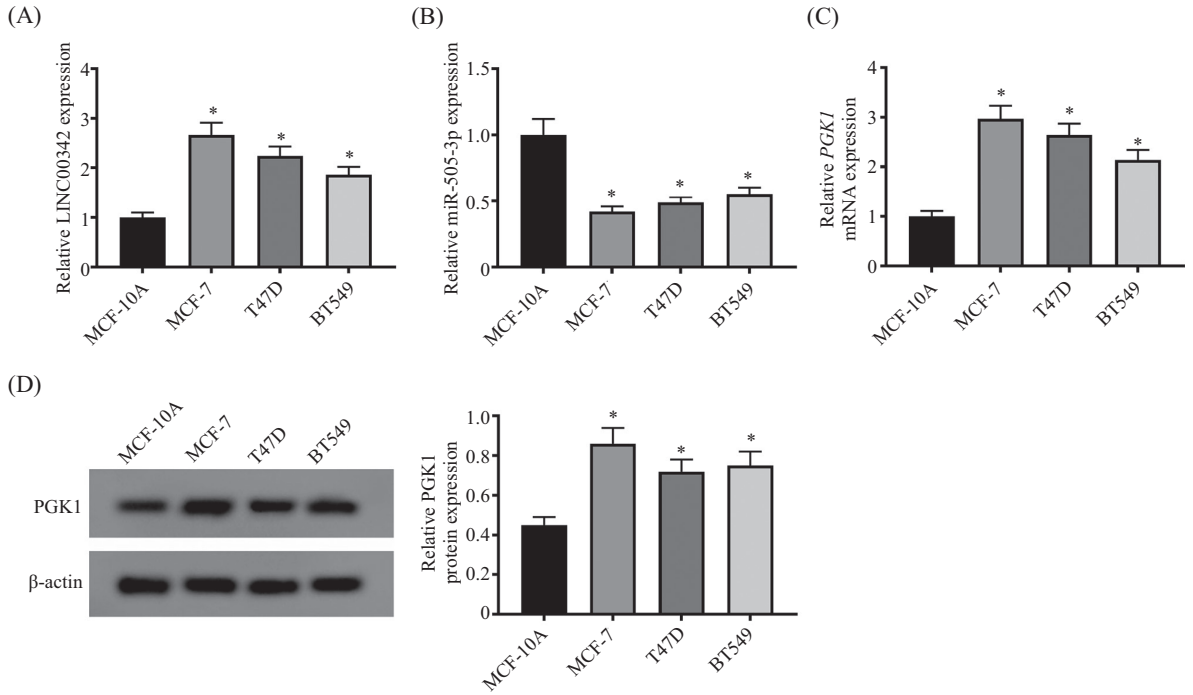


A: qRT-PCR检测LINC00342表达; B: qRT-PCR检测miR-505-3p表达; C: qRT-PCR检测*PGK1* mRNA表达; D: Western blot检测PGK1蛋白的表达情况。*P<0.05, 与癌旁组织比较; #P<0.05, 与癌组织比较。

A: the expression of LINC00342 was detected by qRT-PCR; B: the expression of miR-505-3p was detected by qRT-PCR; C: *PGK1* mRNA expression was detected by qRT-PCR; D: the expression of PGK1 protein was detected by Western blot. *P<0.05 compared with tumor adjacent tissue; #P<0.05 compared with tumor tissue.

图1 在乳腺癌患者肿瘤转移组织中LINC00342、miR-505-3p、PGK1表达情况

Fig.1 Expression of LINC00342, miR-505-3p and PGK1 in metastatic tissues of breast cancer patients

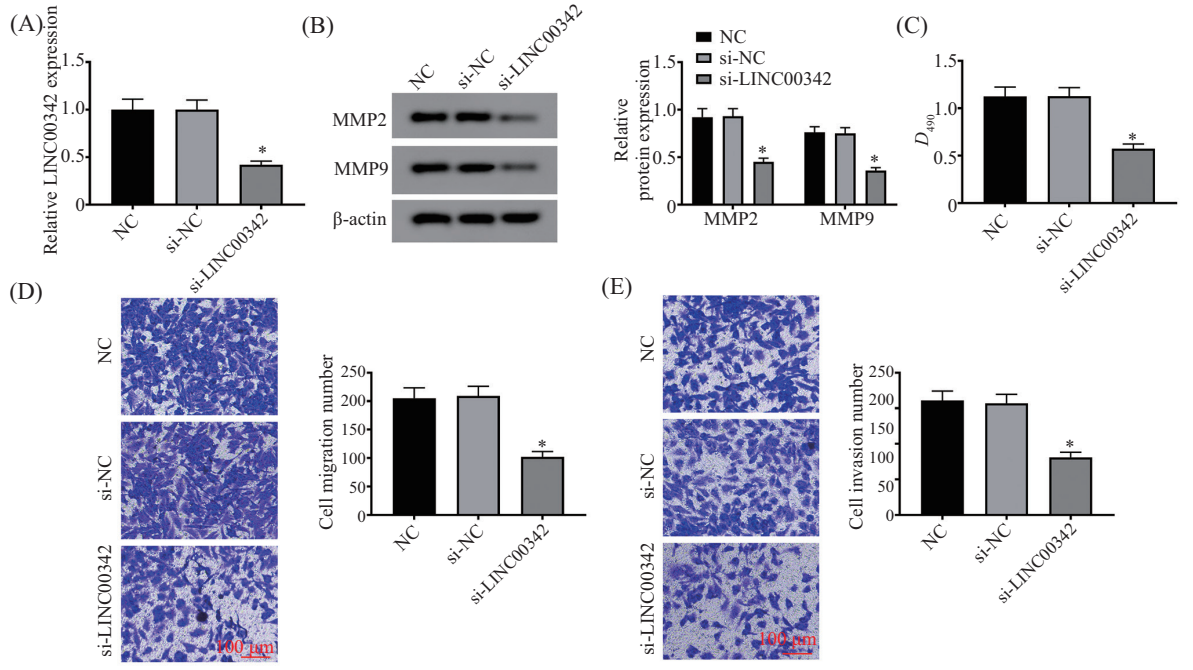


A: qRT-PCR检测LINC00342表达; B: qRT-PCR检测miR-505-3p表达; C: qRT-PCR检测*PGK1* mRNA表达; D: Western blot检测PGK1蛋白的表达情况。*P<0.05, 与MCF-10A组比较。

A: the expression of LINC00342 was detected by qRT-PCR; B: the expression of miR-505-3p was detected by qRT-PCR; C: *PGK1* mRNA expression was detected by qRT-PCR; D: the expression of PGK1 protein was detected by Western blot. *P<0.05 compared with MCF-10A group.

图2 在乳腺癌细胞系中LINC00342、miR-505-3p、PGK1的表达情况

Fig.2 Expression of LINC00342, miR-505-3p and PGK1 in breast cancer cell lines

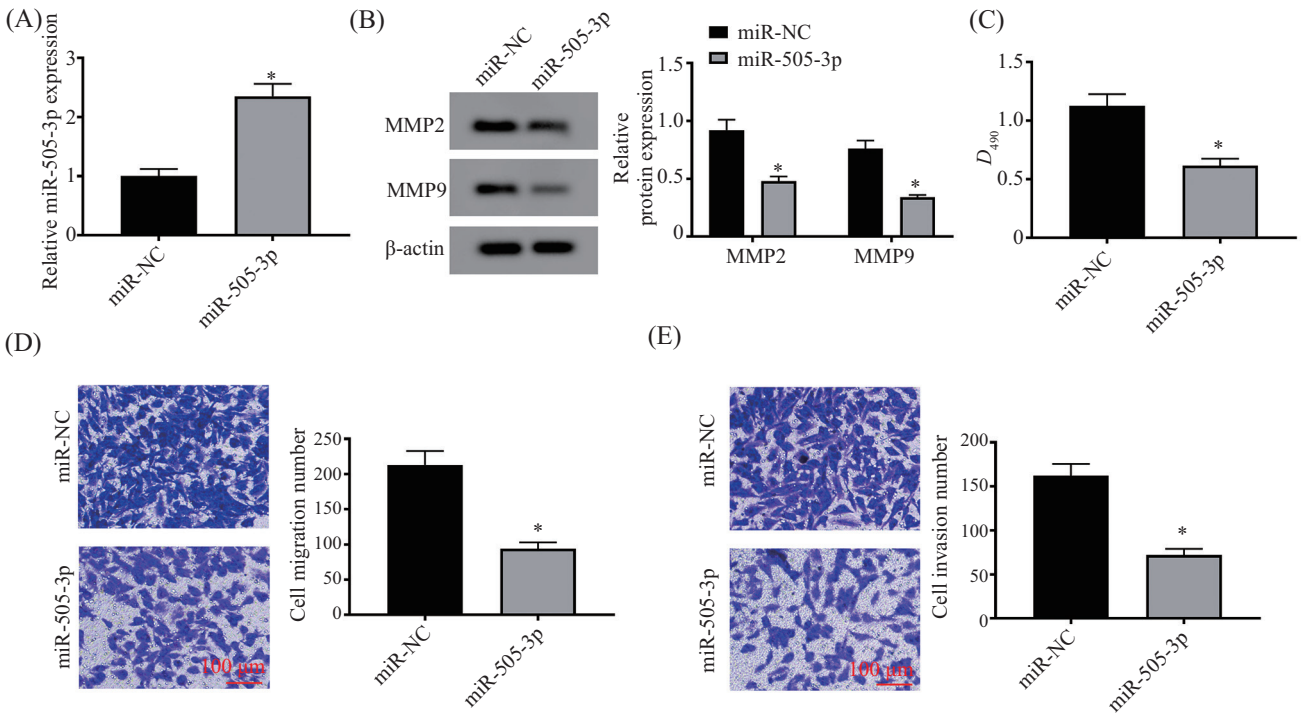


A: qRT-PCR检测LINC00342表达; B: Western blot检测MMP2和MMP9蛋白的表达; C: MTT法检测细胞增殖能力; D、E: Transwell法检测细胞迁移和侵袭数量。*P<0.05, 与NC组比较。

A: the expression of LINC00342 was detected by qRT-PCR; B: the expressions of MMP2 and MMP9 proteins were detected by Western blot; C: MTT assay was used to detect cell proliferation; D,E: Transwell method was used to detect the number of cell migration and invasion. *P<0.05 compared with NC group.

图3 干扰LINC00342表达对MCF-7乳腺癌细胞增殖、迁移和侵袭的影响

Fig.3 The effect of knockdown of LINC00342 on proliferation, migration and invasion of MCF-7 breast cancer cells



A: qRT-PCR检测miR-505-3p表达; B: Western blot检测MMP2和MMP9蛋白的表达; C: MTT法检测细胞增殖能力; D、E: Transwell法检测细胞迁移和侵袭数量。*P<0.05, 与miR-NC组比较。

A: the expression of miR-505-3p was detected by qRT-PCR; B: the protein expressions of MMP2 and MMP9 were detected by Western blot; C: MTT assay was used to detect cell proliferation; D,E: Transwell was used to detect the number of cell migration and invasion. *P<0.05 compared with miR-NC group.

图4　miR-505-3p高表达对MCF-7乳腺癌细胞增殖、迁移和侵袭的影响

Fig.4　The effect of overexpression of miR-505-3p on proliferation, migration and invasion of MCF-7 breast cancer cells

的表达量、增殖能力、迁移和侵袭数量降低(P<0.05)(图4)。

2.5　PGK1低表达对MCF-7乳腺癌细胞增殖、迁移和侵袭的影响

si-PGK1组MCF-7细胞中PGK1、MMP2、MMP9蛋白的表达量低于si-NC组, 且增殖能力、迁移和侵袭数量也低于si-NC组(P<0.05)(图5)。

2.6　LINC00342靶向miR-505-3p调控PGK1的表达

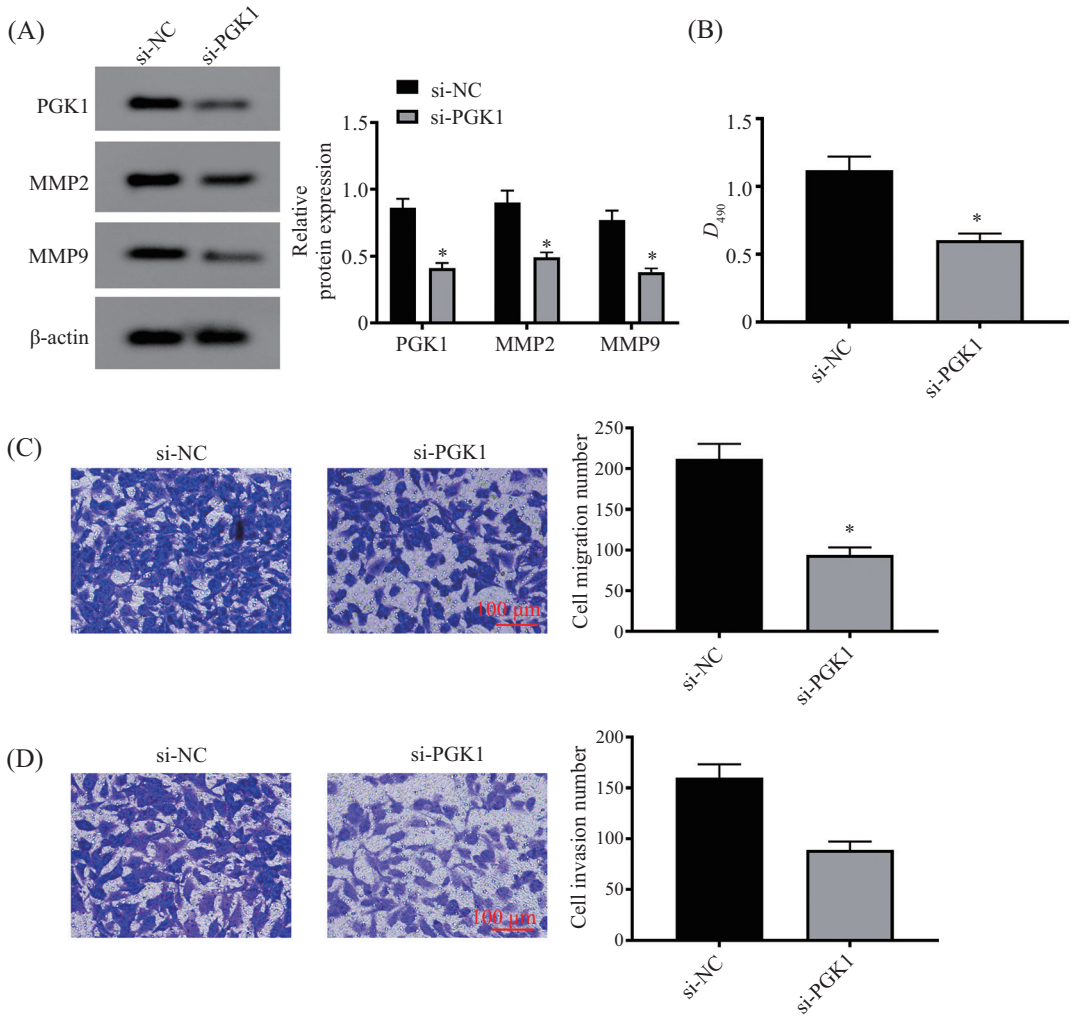
Starbase预测出miR-505-3p和LINC00342、*PGK1*之间的结合序列, 构建的LINC00342或PGK1-3′UTR-WT/MUT报告质粒序列见图6A和图6B。miR-505-3p组共转染LINC00342-WT或PGK1-3′UTR-WT的MCF-7细胞荧光素酶活性均比miR-NC组减弱(P<0.05), 但共转染LINC00342-MUT或PGK1-3′UTR-MUT时MCF-7细胞荧光素酶活性无明显变化(P=0.844和P=0.591)。si-LINC00342组MCF-7细胞中miR-505-3p表达量高于si-NC组, PGK1蛋白表达量低于si-NC组(P<0.05); si-LINC00342+anti-miR-505-3p组MCF-7细胞中miR-505-3p表达量低于si-LINC00342+anti-miR-NC组, PGK1蛋白表达量高于si-LINC00342+anti-miR-NC组(P<0.05)(图6)。

2.7　高表达PGK1可以逆转LINC00342低表达对MCF-7增殖、迁移和侵袭的影响

si-LINC00342+pcDNA-PGK1组MCF-7细胞中PGK1、MMP2、MMP9蛋白的表达量均比si-LINC00342+pcDNA组增加, 且增殖能力、迁移和侵袭数量也比si-LINC00342+pcDNA组升高(P<0.05)(图7)。

3　讨论

LINC00342是一种新发现的lncRNA, 已有诸多研究调查了LINC00342在人类恶性肿瘤中的作用。例如, LINC00342通过靶向miR-203a-3p来促进非小细胞肺癌细胞的增殖、集落形成、迁移和侵袭[14]。然而, LINC00342在乳腺癌中的潜在功能仍不清楚。在本研究中, 我们发现乳腺癌患者LINC00342表达水平明显较高, 此外, LINC00342在乳腺癌肿瘤转移组织和乳腺癌细胞系中高表达, 这也代表了乳腺癌



A: Western blot检测PGK1、MMP2和MMP9蛋白的表达; B: MTT法检测细胞增殖能力; C、D: Transwell法检测细胞迁移和侵袭数量。*P<0.05, 与si-NC组比较。

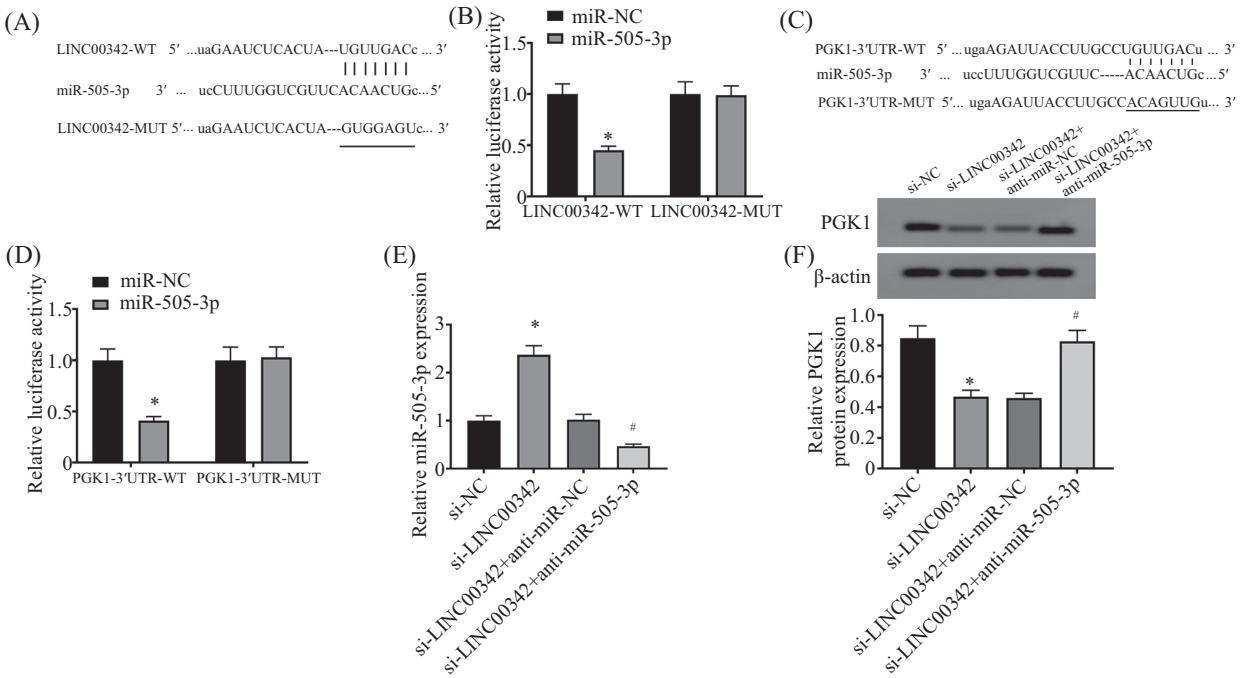
A: the protein expressions of PGK1, MMP2 and MMP9 were detected by Western blot; B: MTT assay was used to detect cell proliferation; C,D: Transwell method was used to detect the number of cell migration and invasion. *P<0.05 compared with si-NC group.

图5 PGK1低表达对MCF-7乳腺癌细胞增殖、迁移和侵袭的影响

Fig.5 The effect of knockdown of LINC00342 on proliferation, migration and invasion of MCF-7 breast cancer cells

患者的不良预后。我们的研究进一步表明, 低表达LINC00342抑制乳腺癌细胞的增殖、迁移和侵袭。这些发现表明, LINC00342在乳腺癌中充当癌基因, 与前述报告类似, 表明LINC00342可能是乳腺癌治疗的新靶点。

lncRNA通常通过吸附miRNA以降低其表达水平来发挥关键的调节功能, 例如在胰腺癌组织和细胞中上调的lncRNA ZEB1-AS1通过海绵miR-505-3p上调TRIB2表达, 促进胰腺癌的发生[15]。在本研究中, 通过生物信息学预测和荧光素酶报告基因检测, 我们确定LINC00342通过与miR-505-3p部分位点结合而充当miR-505-3p的ceRNA。之前的研究报道, miR-505-3p表达量在骨转移性前列腺癌组织中降低, miR-505-3p表达与患者的不良临床病理特征呈负相关, 并且miR-505-3p下调可预测前列腺癌的无骨转移生存率, miR-505-3p的上调会抑制前列腺癌细胞的侵袭和迁移[16]。TANG等[17]证实, miR-505在非小细胞肺癌组织和细胞系中下调, 与患者肿瘤淋巴结转移、分期和远处转移呈负相关。此外, miR-505-3p在胶质瘤中的表达量降低, 这与胶质瘤患者的不良临床结果和预后有关, miR-505-3p的过表达抑制了胶质瘤细胞的增殖、迁移和侵袭[18]。在本研究中, 我们在乳腺癌患者转移组织、癌组织和细胞系中检测到了低水平的miR-505-3p。功能实验表明, 高表达miR-505-3p后, 乳腺癌细胞的增殖、迁移和侵袭能力均减弱, 与ZHAO等[8]报道的miR-505-3p沉默显



A: starbase预测LINC00342和miR-505-3p的靶向结合区域, 下划线表示LINC00342的突变序列(MUT)。B: 双荧光素酶活性实验检测LINC00342和miR-505-3p的靶向结合能力。C: starbase预测*PGK1*和miR-505-3p的靶向结合区域, 下划线表示突变序列。D: 双荧光素酶活性实验检测PGK1和miR-505-3p的靶向结合能力。*P<0.05, 与miR-NC组比较。E: qRT-PCR检测miR-505-3p表达。F: Western blot检测PGK1蛋白的表达。*P<0.05, 与si-NC组比较; #P<0.05, 与si-LINC00342+anti-miR-NC组比较。

A: starbase predicted the targeted binding region of LINC00342 and miR-505-3p, the underline indicates the mutation sequence of LINC00342 (MUT). B: dual-luciferese reporter assay was used to detect the targeting binding ability of LINC00342 and miR-505-3p. C: starbase predicted the targeted binding region of PGK1 and miR-505-3p, the underline indicates the mutation sequence. D: dual-luciferese reporter assay was used to detect the targeting binding ability of PGK1 and miR-505-3p. *P<0.05 compared with miR-NC group. E: the expression of miR-505-3p was detected by qRT-PCR. F: the expression of PGK1 protein was detected by Western blot. *P<0.05 compared with si-NC group; #P<0.05 compared with si-LINC00342+anti-miR-NC group.

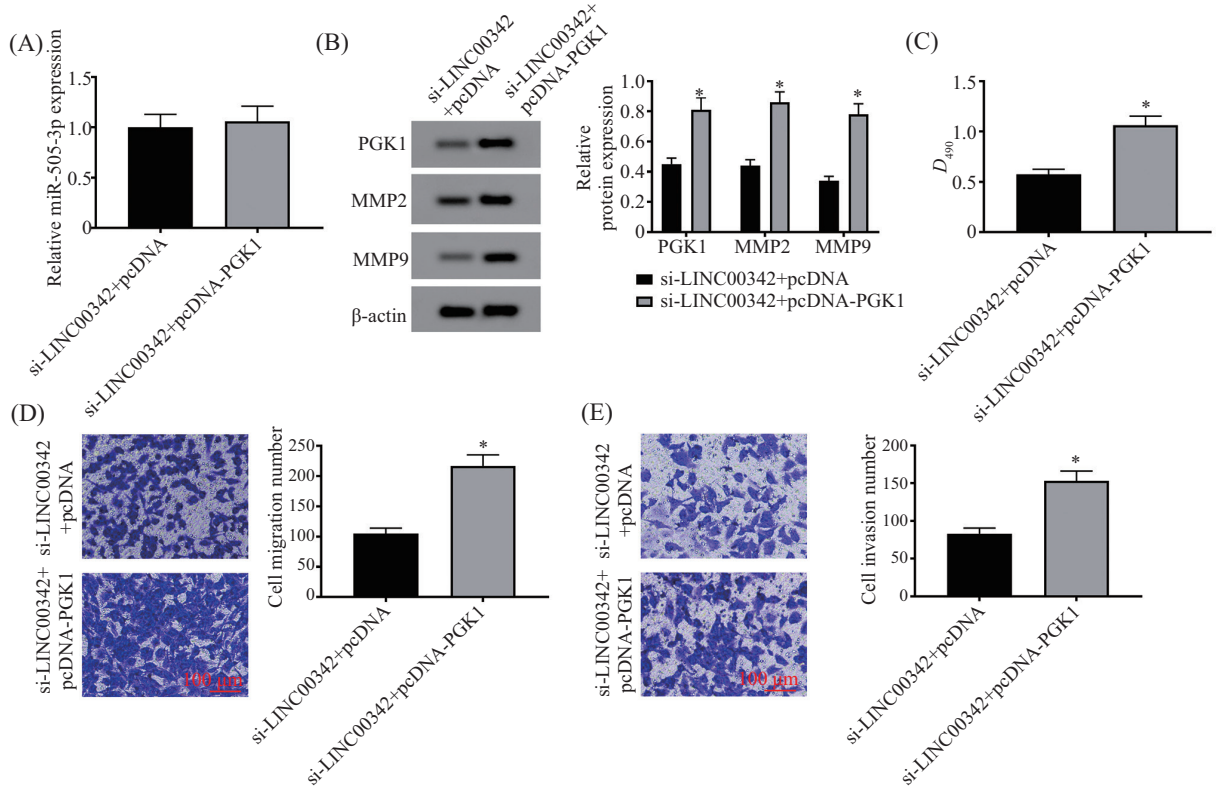
图6 LINC00342靶向miR-505-3p调控PGK1的表达

Fig.6 Regulation of PGK1 expression by LINC00342 targeting miR-505-3p

著促进乳腺癌细胞增殖和侵袭符合, 再次证实了乳腺癌中miR-505-3p的肿瘤抑制功能。

PGK1是糖酵解途径中第一个产生ATP的关键酶。PGK1不仅是一种代谢酶, 也是一种蛋白激酶, 通过磷酸化一些重要的底物来介导肿瘤的生长、迁移和侵袭[19-20]。这与本研究的结果相一致, 即乳腺癌组织、肿瘤转移组织或细胞系(MCF-7、T47D、BT549)中PGK1表达水平上调, 低表达PGK1导致乳腺癌细胞增殖、迁移和侵袭抑制。先前的研究表明, PGK1参与了miR-16-1-3p在乳腺癌细胞增殖、迁移、侵袭和乳腺癌肺转移中的调控[21]。miR-450b-3p[22]、miR-548c-5p[23]和miR-6869-5p[24]已被证明可以直接靶向*PGK1* 3′UTR并抑制PGK1的表达。本研究发现, PGK1表达受miR-505-3p靶向调控。此外, LINC00342通过靶向miR-545-5p/冠层成纤维细胞生长因子信号调节剂2(canopy fibroblast growth factor signaling regulator 2, CNPY2)轴, 在胃癌中发挥潜在的致癌作用[5]。LINC00342通过靶向miR-19a-3p/NPEPL1轴, 促进结直肠癌的生长和转移[7]。在本研究中, LINC00342可以通过靶向miR-505-3p来调控PGK1的表达。同时, 高表达PGK1可以逆转LINC00342低表达对MCF-7增殖、迁移和侵袭的抑制效果。这些结果显示, 低表达LINC00342抑制乳腺癌进展的功能可能是通过靶向miR-505-3p调控PGK1来实现的。

值得注意的是, 肿瘤侵袭转移的发生最后需要对肿瘤细胞外基质进行破坏和降解, MMP2和MMP9作为降解细胞外基质的重要酶类在肿瘤侵袭转移中发挥重要作用。研究发现, 低表达PGK1能抑制MMP2和MMP9表达参与肝癌细胞迁移[25]。在本研究中, 低表达LINC00342、过表达miR-505-3p、低表达PGK1能抑制乳腺癌细胞MMP2和MMP9蛋白的表达。同时, 高表达PGK1可以逆转LINC00342低表达对MCF-7细胞中MMP2和MMP9蛋白的抑制效果。



A: qRT-PCR检测miR-505-3p表达; B: Western blot检测PGK1、MMP2和MMP9蛋白的表达; C: MTT法检测细胞增殖能力; D、E: Transwell法检测细胞迁移和侵袭数量。*P<0.05, 与si-LINC00342+pcDNA组比较。

A: the expression of miR-505-3p was detected by qRT-PCR; B: the protein expressions of PGK1, MMP2 and MMP9 were detected by Western blot; C: MTT assay was used to detect cell proliferation; D,E: Transwell was used to detect the number of cell migration and invasion. *P<0.05 compared with si-LINC00342+pcDNA group.

图7 高表达PGK1可以逆转LINC00342低表达对MCF-7增殖、迁移和侵袭的影响

Fig.7 Overexpression of PGK1 could reverse the effect of knokdown of LINC00342 on proliferation, migration and invasion of MCF-7

这提示, LINC00342对乳腺癌迁移侵袭的抑制作用与LINC00342/PGK1/MMPs通路有关。

综上所述, 本研究首次揭示LINC00342在乳腺癌组织、肿瘤转移组织和细胞系中表达量增加, 低表达LINC00342通过靶向miR-505-3p调控PGK1的表达, 从而抑制乳腺癌细胞的增殖、迁移和侵袭, 这可能为乳腺癌患者及转移患者提供新的治疗思路。

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附表1 qRT-PCR引物序列

Supplemental table 1 qRT-PCR primer sequences

名称 Name	序列(5′→3′) Sequence (5′→3′)
LINC00342 forward primer	GGA GGG ACC CAG ATA A
LINC00342 reverse primer	TTG TTC CTT CCG TTT T
PGK1 forward primer	AAC CAG AGG ATT AAG GCT GC
PGK1 reverse primer	GCC TAC ACA GTC CTT CAA GA
GAPDH forward primer	ACA ACT TTG GTA TCG TGG AAG G
GAPDH reverse primer	GCC ATC ACG CCA CAG TTT C
miR-505-3p forward primer	CGC GGA TCC CAG ACT CCC AGC AAT CAC
miR-505-3p reverse primer	CCG GAA TTC GCA GTA TTC CCC ACC ATT T
U6 forward primer	TGC GGG TGC TCG CTT CGG CAG C
U6 reverse primer	GTG CAG GGT CCG AGG T