·论 著·

CircSMARCA5通过miR-4295/PTEN轴调控糖酵解抑制胃癌细胞增殖和侵袭

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［摘要］ **背景与目的：**胃癌是常见的消化系统恶性肿瘤。作者既往研究发现circSMARCA5在胃癌中表达降低并能够抑制胃癌进展，但其具体机制目前仍不清楚。本研究探究circSMARCA5抑制胃癌细胞增殖和侵袭的分子机制。**方法：**采用细胞计数试剂盒-8（cell counting kit-8，CCK-8）和transwell实验检测过表达circSMARCA5对胃癌细胞增殖和侵袭能力的影响。通过检测细胞外酸化率、葡萄糖摄取水平和乳酸生成量，分析过表达circSMARCA5对胃癌细胞糖酵解的影响。采用实时荧光定量聚合酶链式反应（real-time fluorescent quantitative polymerase chain reaction，RTFQ-PCR）检测circSMARCA5、miR-4295和*PTEN*的基因表达，采用蛋白质印迹法（Western blot）检测GLUT1和LDHA的蛋白水平。建立BACB/c裸小鼠皮下移植瘤模型，观察过表达circSMARCA5对移植瘤生长的影响，利用免疫组织化学方法检测两组皮下瘤中GLUT1、LDHA的表达水平及Ki-67增殖指数。通过双荧光素酶报告基因实验、Pearson相关性分析和RNA免疫沉淀（RNA immunoprecipitation，RIP）实验检测circSMARCA5与miR-4295、miR-4295及PTEN的靶向调控关系。**结果：**过表达circSMARCA5能够抑制胃癌细胞增殖和侵袭。CircSMARCA5过表达组细胞的糖酵解速率、糖酵解能力值、葡萄糖摄取水平和乳酸生成量均低于对照组。此外，过表达circSMARCA5能够抑制裸小鼠皮下移植瘤的生长。进一步研究发现，circSMARCA5可发挥分子海绵作用下调miR-4295表达，而miR-4295通过与PTEN mRNA的3′-UTR结合抑制PTEN表达。在circSMARCA5过表达组细胞中上调miR-4295或下调PTEN表达可部分逆转circSMARCA5对胃癌细胞增殖、侵袭和糖酵解的影响。**结论：**CircSMARCA5通过竞争性结合miR-4295上调PTEN表达，调控细胞糖酵解，从而抑制胃癌细胞的增殖和侵袭。

［**关键词**］胃肿瘤；环状RNA；miR-4295；PTEN；糖酵解；侵袭
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CircSMARCA5 inhibits glycolysis and suppresses proliferation and invasion of gastric cancer cells through miR-4295/PTEN axis

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［**Abstract**］ **Background and purpose:** Gastric cancer is one of the most common malignant tumors of digestive system. Previous study demonstrated that circSMARCA5 was downregulated and could function as a tumor suppressor in gastric cancer.

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However, the molecular mechanism has not yet been documented. This study aimed to investigate the effects of circSMARCA5 on the proliferation and invasion of gastric cancer cells and their molecular mechanisms. **Methods:** Cell counting kit-8 (CCK-8) assays and transwell assays were performed to examine the cell proliferative and invasive abilities, respectively. The effect of circSMARCA5 on gastric cancer cell glycolysis was assessed by detecting the extracellular acidification rate, glucose uptake level and lactate production. Real-time fluorescent quantitative polymerase chain reaction (RTFQ-PCR) assay was performed to detect the expression levels of circSMARCA5, miR-4295 and PTEN. The levels of GLUT1 and LDHA were measured by Western blot assay. The transplanted xenograft model in nude mice was established, and the effects of circSMARCA5 on the tumor growth were observed. Immunohistochemistry assays were performed to examine the expression levels of GLUT1, LDHA and Ki-67 proliferation index in xenograft tumors. Dual luciferase reporter gene assays, Pearson's correlation analysis and RNA immunoprecipitation (RIP) assays were used to confirm the targeting relationship of circSMARCA5 and miR-4295 as well as miR-4295 and PTEN. **Results:** CircSMARCA5 overexpression inhibited the proliferation and invasion of gastric cancer cells. Compared to the control group, the glycolysis rate, glycolysis capacity, glucose uptake and lactate production in the circSMARCA5-overexpresing group were significantly decreased. In addition, nude mouse transplanted xenograft assays showed that the volume and the weight of the tumors in the circSMARCA5-overexpressing group were lower compared with the control group. Further studies indicated that circSMARCA5 acted as a molecular sponge to inhibit the expression of miR-4295. Besides, miR-4295 could inhibit the expression of PTEN by binding with the 3'-UTR of PTEN mRNA. Rescue experiments by upregulating miR-4295 or downregulating PTEN expression in the circSMARCA5-overexpressing gastric cancer cells were performed, and the results showed that upregulation of miR-4295 or PTEN knockdown could abolish the inhibitory effects of circSMARCA5 overexpression on cell proliferation, invasion and glycolysis. **Conclusion:** CircSMARCA5 suppresses the proliferation and invasion of gastric cancer cells by targeting miR-4295, increasing the expression level of PTEN and subsequently regulating glycolysis.

［**Key words**］ Stomach neoplasms; Circular RNA; miR-4295; PTEN; Glycolysis; Invasion

胃癌是全世界发病率第五位、死亡率第四位的恶性肿瘤，每年新发病例数近109万，死亡病例数约77万[1]。复发和转移是胃癌患者的首要死亡原因，然而目前对此仍缺乏有效的治疗手段。因此，探索胃癌复发转移机制、寻找有效治疗靶点对改善患者预后具有重要意义。

环状RNA（circular RNA，circRNA）广泛存在于真核生物中，具有高度稳定性、组织特异性和进化保守性等特征，能在多层面调控机体的生理和病理过程[2]，且circRNA与多种肿瘤的增殖、侵袭相关。高表达的circ-RanGAP1 通过负调控miR-877-3p，促进胃癌细胞增殖和侵袭[3]。CircTLK1能结合miR-136-5p，促进结肠癌细胞的增殖和侵袭[4]。

在前期研究中，作者发现circSMARCA5在胃癌组织中呈低表达，与胃癌患者的TNM分期、神经脉管侵犯和淋巴结转移具有显著负相关性，过表达circSMARCA5能抑制胃癌细胞增殖和侵袭[5]，但其具体分子机制仍不清楚。本文通过研究circSMARCA5对胃癌细胞糖代谢重编程的影响，探讨其潜在的分子机制，以期为胃癌提供新的治疗靶点。

1　材料和方法

1.1　细胞培养与实验试剂

人正常胃黏膜上皮细胞系GES-1及人胃癌细胞系MKN45、MKN74、AGS购自中国科学院典型培养物保藏委员会细胞库。RPMI-1640培养基、10%胎牛血清、胰蛋白酶、青-链霉素和磷酸盐缓冲生理盐水（phosphate-buffered saline，PBS）均购自美国Gibco公司，Lipofectamine^{TM} 3000和TRIzol试剂购自美国Invitrogen公司，反转录试剂盒和实时荧光定量聚合酶链式反应（real-time fluorescent quantitative polymerase chain reaction，RTFQ-PCR）试剂盒购自日本TaKaRa公司，慢病毒circSMARCA5过表达载体（LV-circSMARCA5）和慢病毒阴性对照（LV-NC）购自上海吉凯基因医学科技股份有限公司，miR-4295模拟物（miR-4295 mimics）和miR-4295模拟物阴性对照（miR-4295 NC）购自广州锐博生物技术有限公司，细胞计数试剂盒-8（cell counting kit-8，CCK-8）试剂购自日本同仁化学研究所，transwell小室购自美国BD公司，Seahorse XF糖酵

解压力测试试剂盒购自美国Agilent Technologies公司，GLUT1、LDHA和Ki-67抗体、葡萄糖摄取试剂盒和乳酸检测试剂盒均购自英国Abcam公司，PTEN、β-tubulin抗体和辣根过氧化物酶偶联二抗购自美国Cell Signaling Technology公司。

1.2 临床标本和实验动物

收集2016年1月—2016年12月于皖南医学院第一附属医院行手术切除的60例胃癌患者的癌组织及癌旁黏膜组织（距肿瘤＞5 cm），其中男性44例，女性16例，年龄36～78岁。所有患者术前均未接受化疗、放疗或免疫治疗。本研究经皖南医学院第一附属医院伦理委员会批准，所有患者均签署知情同意书。BALB/c裸小鼠8只，雄性，5～6周龄，购自南京市江宁区青龙山动物繁殖场。动物许可证号：SCXK（苏）2017-0001。

1.3 实验方法

1.3.1 细胞培养与转染

利用含10%胎牛血清、青-链霉素的RPMI-1640培养基，将细胞置于37 ℃、CO_2体积分数为5%的培养箱中培养。待细胞贴壁生长融合度达80%时，加入胰蛋白酶消化并传代。取对数生长期的AGS细胞，消化后接种于6孔板中培养过夜，加入过表达circSMARCA5慢病毒载体（LV-circSMARCA5）及其对照病毒（LV-NC）转染细胞，并用嘌呤霉素筛选1周，构建稳定转染细胞株。通过RTFQ-PCR检测circSMARCA5表达水平。在过表达circSMARCA5的稳定转染细胞株中，利用Lipofectamine™ 3000转染miR-4295 mimics或si-PTEN，48 h后检测miR-4295和PTEN的表达水平。

1.3.2 RTFQ-PCR检测

利用TRIzol试剂提取组织和细胞总RNA，应用反转录试剂盒将RNA反转录成cDNA。以cDNA为模板，按照RTFQ-PCR试剂盒说明书进行PCR反应，利用$2^{-\Delta\Delta Ct}$法计算circSMARCA5、GLUT1、LDHA、miR-4295和PTEN的相对表达量。CircSMARCA5、miR-4295、GLUT1、LDHA、PTEN、β-actin和U6的引物序列由广州锐博生物技术有限公司设计并合成。circSMARCA5有义链为5′-CTCCAAGATGGGCGAAAG-3′，反义链为5′-TGTGTTGCTCCATGTCTAATCA-3′；miR-4295有义链为5′-GGAAGATCTAGGATCACAGTTAACTCAGAA-3′，反义链为5′-CGGGGTACCGCACAATCCAAAACAAGAA-3′；GLUT1有义链为5′-GGCCAAGAGTGTGCTAAA GAA-3′，反义链为5′-ACAGCGTTGATGCCAG ACAG-3′；LDHA有义链为5′-ATGGCAAC TCTAAAGGATCAGC-3′,反义链为5′-CCAA CCCCAACAACTGTAATCT-3′；PTEN有义链为5′-GAGGGATAAAACACCATG-3′，反向引物序列为5′-AGGGGTAGGATGTGAACCAGTA-3′；β-actin有义链为5′-TGACGTGGACATCC GCAAAG-3′，反向引物序列为5′-CTGGAA GGTGGACAGCGAGG-3′；U6有义链为5′-TGGACTCTGTTCGCTCAGGT-3′，反义链为5′-TGCCTCCTTCCGTACCACAT-3′。

1.3.3 CCK-8实验

将处于对数生长期的细胞按照1×10^3个/孔的密度接种于96孔板，加入100 μL培养基分别培养24、48、72及96 h后加入10 μL CCK-8试剂，置于细胞培养箱中避光温育2 h，检测450 nm波长处各孔的吸光度。实验重复3次。

1.3.4 Transwell实验

胰酶消化LV-NC和LV-circSMARCA5两组细胞后，用无血清培养基制备2×10^5个/mL的单细胞悬液，取250 μL细胞悬液加入预铺基质胶的transwell上室，在下室加入500 μL含10%胎牛血清的RPMI-1640培养基，培养24 h后，用4%多聚甲醇固定20 min，1%结晶紫染色10 min，在显微镜下观察并拍照。实验重复3次。

1.3.5 细胞糖酵解能力检测

取处于对数生长期的各组细胞，以2×10^5个细胞/mL的密度接种于Seahorse 96孔板，待细胞融合度达约80%，通过设定程序加入葡萄糖（10 mmol/L）、寡霉素（1 μmol/L）和2-脱氧葡萄糖（50 mmol/L），利用Seahorse XF细胞能量代谢分析仪检测细胞外酸化速率，并计算糖酵解速率及糖酵解能力值。取2 μL细胞培养上清液，依据葡萄糖和乳酸检测试剂盒说明书，利用GENios Plus酶标仪检测各组细胞葡萄糖摄取水平

和乳酸生成量。实验重复3次。

1.3.6 蛋白质印迹法（Western blot）检测

收集各组细胞，提取总蛋白，用二辛可宁酸（bicinchoninic acid，BCA）试剂盒测定蛋白浓度，取50 μg蛋白进行10%十二烷基硫酸钠聚丙烯酰胺凝胶电泳分离，将分离的蛋白电转移至聚偏二氟乙烯膜上。用5%脱脂奶粉封闭2 h，采用吐温-20三羟甲基氨基甲烷缓冲生理盐水（tris-buffered saline Tween，TBST）洗膜3次，每次10 min，加入稀释的GLUT1、LDHA、PTEN或β-tubulin一抗，4 ℃温育过夜。TBST洗膜3次，加入辣根过氧化物酶偶联二抗，室温温育2 h，TBST洗膜3次，用ECL化学发光试剂显色。

1.3.7 裸小鼠皮下移植瘤实验

将BACB/c裸小鼠随机分为LV-NC和LV-circSMARCA5两组，每组4只。用胰酶消化过表达circSMARCA5的AGS细胞及其对照细胞，加入无血清RPMI-1640培养基调整细胞密度至1×10^8/mL，用1 mL注射器向每只裸小鼠肩胛部皮下注射100 μL细胞悬液。从第6天开始，每3天测量肿瘤长、短径，待最大体积达约1 000 mm^3时，处死小鼠，取出肿瘤测量并称重，计算肿瘤体积，绘制移植瘤生长曲线。肿瘤体积按照如下公式计算：体积（mm^3）= 0.5×长径（mm）×短径^2（mm^2）。

1.3.8 免疫组织化学方法检测

收集各组皮下瘤的组织标本，常规用4%甲醛溶液固定，经脱水、浸蜡包埋、切片后使用山羊血清温育30 min封闭，4 ℃温育一抗过夜。次日用PBS清洗3次，室温温育二抗30 min，PBS清洗3次。滴加辣根过氧化物酶溶液，室温温育30 min，PBS清洗3次，滴加DAB显色液，当显微镜下可见染色区域发黄即可使用蒸馏水冲洗，苏木精复染 1 min。使用梯度乙醇进行脱水处理，二甲苯浸泡，最后滴加中性树胶，盖玻片封片，显微镜下观察并拍照。通过染色强度和阳性细胞比例进行免疫组织化学评分。染色强度评分标准为0分（阴性）、1分（弱阳性）、2分（中等强度）、3分（强阳性）。阳性细胞比例的评分标准为0分（＜10%）、1分（10%~49%）、2分（50%~69%）、3分（＞70%）。将染色强度评分与阳性细胞比例评分相乘计算总分。

1.3.9 双荧光素酶报告基因实验

将野生型或突变型circSMARCA5和PTEN 3′-UTR序列连接到双荧光酶报告载体中，将各野生型或突变型载体分别与miR-NC、miR-4295 mimics共转染。48 h后用双荧光素酶报告基因测定系统检测各组细胞的荧光素酶活性。

1.3.10 RNA免疫沉淀（RNA immunoprecipitation，RIP）实验

按照RIP试剂盒操作步骤验证circSMARCA5与miR-4295的靶向关系。裂解细胞后，收集裂解液进行实验，利用RTFQ-PCR检测circSMARCA5的富集程度。

1.3.11 统计学处理

采用SPSS 21.0软件进行统计学分析。计量资料以$\bar{x}\pm s$表示，正态分布的两组计量资料比较采用独立样本t检验，多组间比较采用单因素方差分析，相关性分析采用Pearson相关分析。$P<0.05$为差异有统计学意义。

2 结 果

2.1 过表达circSMARCA5能够抑制胃癌细胞的增殖和侵袭

RTFQ-PCR结果显示，circSMARCA5在胃癌细胞系中表达较正常胃黏膜上皮细胞明显降低（图1A）。LV-circSMARCA5组AGS细胞中circSMARCA5的表达水平较LV-NC组明显升高，表明过表达circSMARCA5的稳定转染细胞株构建成功（图1B）。CCK-8实验和transwell实验结果表明，LV-circSMARCA5组细胞的增殖和侵袭能力较LV-NC组降低（均$P<0.05$，图1C和D）。以上结果表明，过表达circSMARCA5能够抑制胃癌细胞的增殖和侵袭。

2.2 过表达circSMARCA5能够抑制胃癌细胞糖酵解

代谢异常是肿瘤细胞的基本特征之一，circRNA已被证实能够调控肿瘤细胞糖代谢重编程[6]。为了探索circSMARCA5是否参

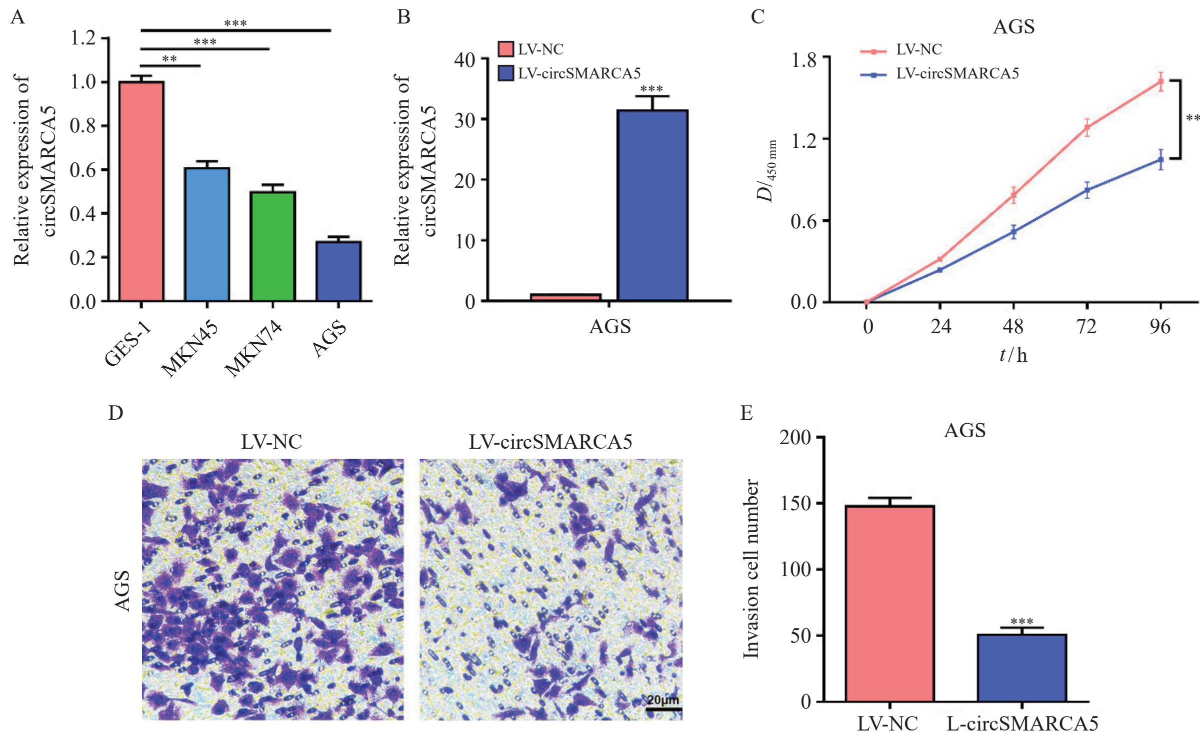

图1 过表达circSMARCA5能够抑制胃癌细胞的增殖和侵袭

Fig. 1 CircSMARCA5 overexpression suppresses the proliferation and invasion of gastric cancer cells

A: The expression levels of circSMARCA5 in three human gastric cancer cell lines and the normal human gastric mucosal epithelial cell line GES-1; B: RTFQ-PCR analysis results of circSMARCA5 in AGS cells after transfection of lentivirus overexpressing circSMARCA5; C: The proliferation of AGS cells after circSMARCA5 overexpression was detected by CCK-8 assays; D: The invasion of AGS cells after circSMARCA5 overexpression was examined by transwell assays; E: Invasion cell number of LV-NC and LV-circAMARCA5. **: $P<0.01$, compared with each other; ***: $P<0.001$, compared with each other.

与调控胃癌细胞糖酵解过程，本研究采用RTFQ-PCR检测葡萄糖转运蛋白1（glucose transporter 1，GLUT1）和乳酸脱氢酶A（lactate dehydrogenase，LDHA）的表达情况，发现过表达circSMARCA5后GLUT1和LDHA的基因表达下降（图2A）。Western blot检测结果进一步证实过表达circSMARCA5可降低GLUT1和LDHA的蛋白水平（图2B）。相较于LV-NC组，LV-circSMARCA5组细胞的葡萄糖摄取水平和乳酸生成量显著下降，差异有统计学意义（图2C）。Seahorse XF细胞能量代谢分析结果显示，与对照组相比，LV-circSMARCA5组细胞外酸化速率降低，表明过表达circSMARCA5可抑制细胞糖酵解活性（图2D）。LV-circSMARCA5组的细胞糖酵解速率及糖酵解能力值低于LV-NC组（均$P<0.05$，图2E）。以上结果表明，过表达circSMARCA5能够抑制胃癌细胞糖酵解。

2.3 过表达circSMARCA5能够抑制裸小鼠皮下移植瘤生长

裸小鼠皮下移植瘤实验结果表明，LV-circSMARCA5组的裸小鼠皮下移植瘤体积和重量显著低于LV-NC组，差异有统计学意义（$P<0.001$，图3A～C）。免疫组织化学检测结果显示，LV-circSMARCA5组皮下瘤组织的GLUT1、LDHA和Ki-67表达显著低于LV-NC组（均$P<0.05$，图3D）。以上结果表明，过表达circSMARCA5能够抑制裸小鼠皮下移植瘤生长。

2.4 CircSMARCA5靶向调控miR-4295表达

ENCORI数据库预测结果提示，circSMARCA5与miR-4295存在互补结合的核苷酸序列（图4A）。据此，我们设计了circSMARCA5与miR-4295结合位点的突变序列，并进行双荧光素酶报告基因实验。结果显示，miR-4295 mimics+circSMARCA5-WT共转

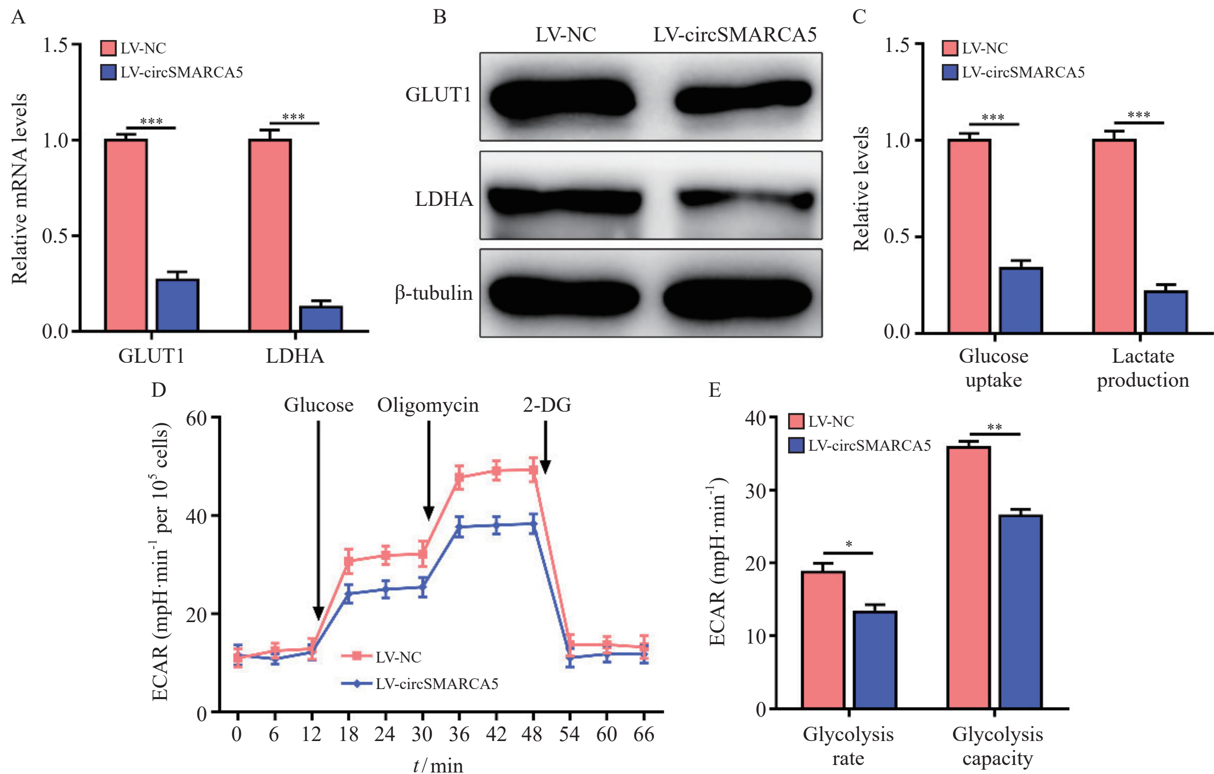

图2 过表达circSMARCA5能够抑制胃癌细胞糖酵解

Fig. 2 CircSMARCA5 overexpression inhibits glycolysis of the gastric cancer cells

A: The mRNA levels of GLUT1 and LDHA were examined by RTFQ-PCR in AGS cells with circSMARCA5 overexpression; B: The protein levels of GLUT1 and LDHA were assessed by Western blot in AGS cells with circSMARCA5 overexpression; C: The cellular glucose uptake and lactate production were detected in AGS cells with circSMARCA5 overexpression; D: Extracellular acidification rate (ECAR) was measured by Seahorse XF assays in AGS cells; E: ECAR data showed that upregulating circSMARCA5 significantly inhibited the glycolysis rate and glycolysis capacity in AGS cells. *: $P<0.05$, compared with each other; **: $P<0.01$, compared with each other; ***: $P<0.001$, compared with each other.

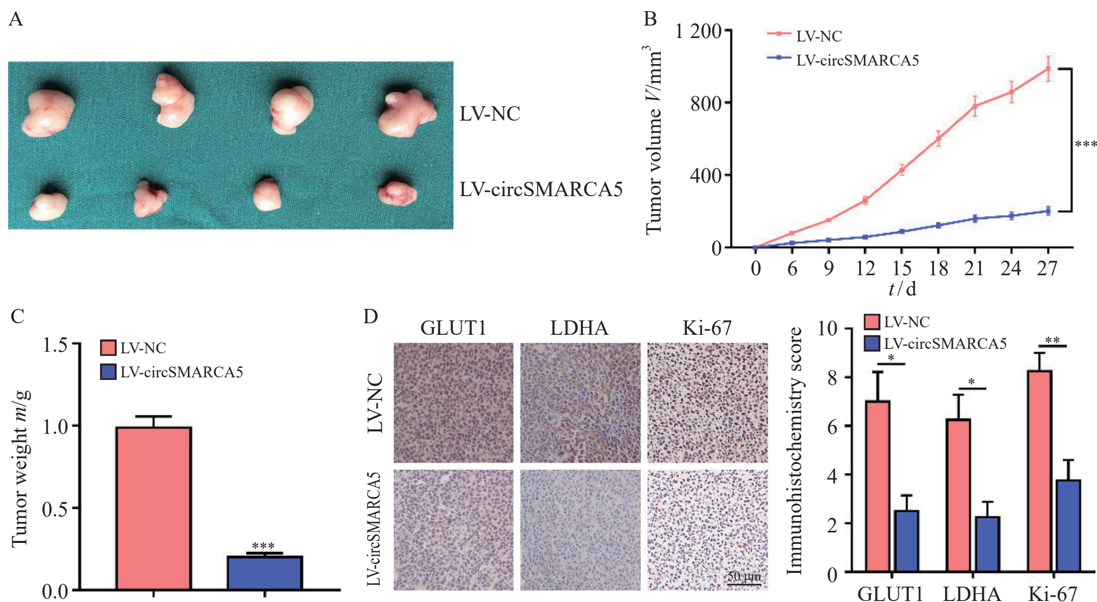

图3 过表达circSMARCA5能够抑制裸小鼠皮下移植瘤生长

Fig. 3 CircSMARCA5 overexpression suppresses xenograft tumor growth *in vivo*

A: Xenograft tumors were photographed; B: Growth curves of xenograft tumors were measured by tumor volume; C: Tumor weight was recorded; D: The expression levels of GLUT1, LDHA and Ki-67 proliferation index in xenograft tumors were analyzed by using immunohistochemistry. *: $P<0.05$, compared with each other; **: $P<0.01$, compared with each other; ***: $P<0.001$, compared with each other.

染组的荧光素酶活性显著降低（P<0.001），而miR-4295 NC+circSMARCA5-MUT组和miR-4295 mimics+circSMARCA5-MUT组的荧光素酶活性无明显改变（P>0.05，图4B）。收集60例胃癌患者癌及癌旁正常胃黏膜组织，患者的临床病理学资料见表1。RTFQ-PCR结果显示，胃癌组织中miR-4295的表达水平较正常胃黏膜组织升高（图4C）。Pearson相关性分析表明胃癌组织中circSMARCA5与miR-4295的表达水平呈负相关（r=−0.571，P<0.01，图4D）。RIP实验结果显示，与对照组相比，AGO2富集程度在LV-circSMARCA5组中更高（图4E）。RIP和荧光原位杂交（flurescence *in situ* hybridization，FISH）实验结果进一步证实了circSMARCA5与miR-4295可直接结合（图4F和G）。RTFQ-PCR结果显示，与LV-NC组相比，LV-circSMARCA5组细胞中的miR-4295表达水平出现显著下降（P<0.01，图4H）。以上结果表明，在胃癌细胞中circSMARCA5可与miR-4295结合，并靶向调控miR-4295表达。

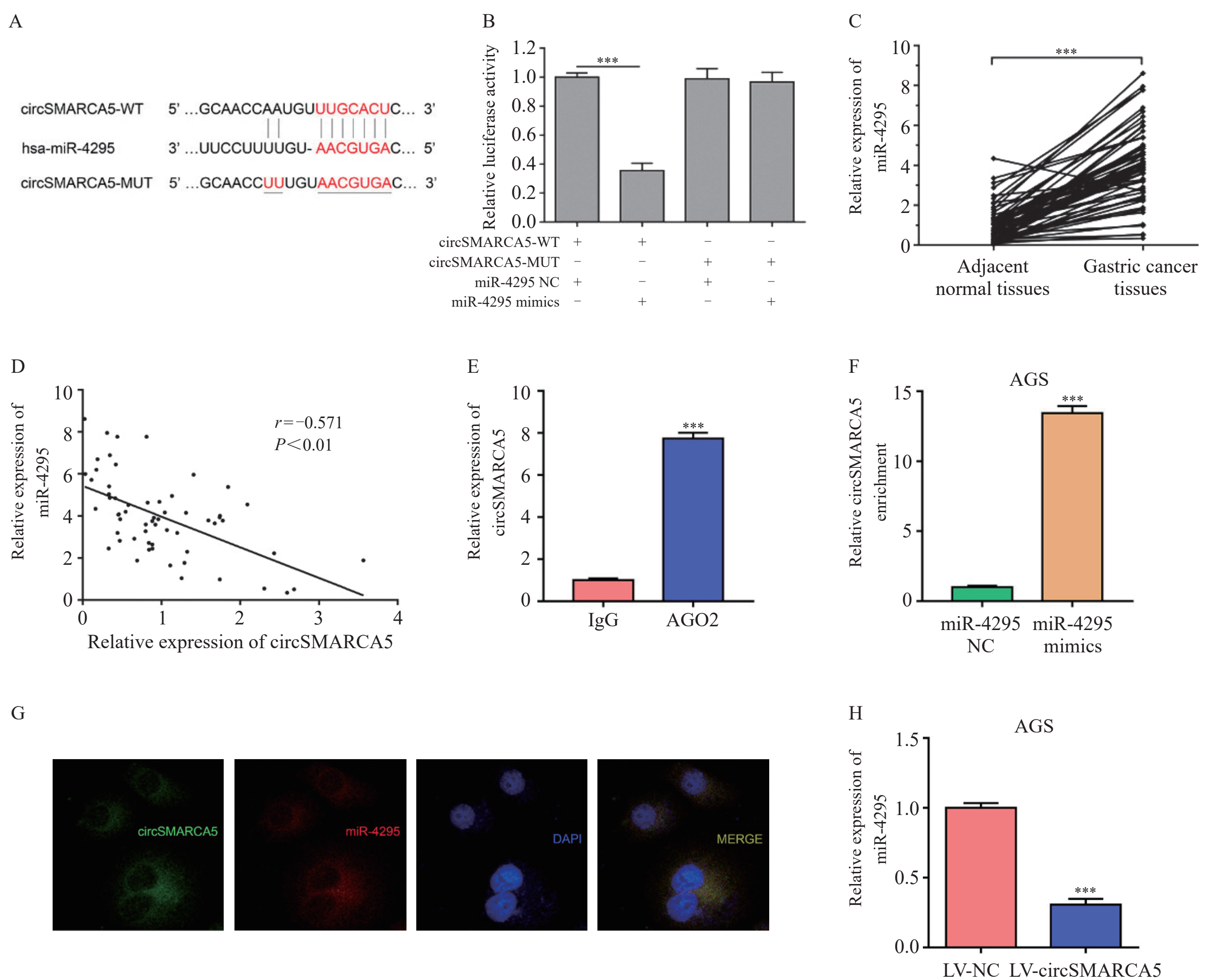

图4 CircSMARCA5靶向调控miR-4295表达

Fig. 4 CircSMARCA5 directly targets miR-4295

A: Putative binding sequence between circSMARCA5 and miR-4295; B: Luciferase activity was determined by dual luciferase reporter assays; C: The expression levels of miR-4295 in gastric cancer tissues and adjacent normal tissues were detected by RTFQ-PCR; D: Correlation analysis showed a negative correlation between circSMARCA5 and miR-4295 expression in gastric cancer tissues; E: RIP assays for AGO2 was conducted to detect the levels of endogenous circSMARCA5; F: Enrichment of circSMARCA5 in AGS cells transfected with miR-4295 NC or miR-4295 mimics; G: FISH results showed the colocalization of circSMARCA5 and miR-4295 in cytoplasm of gastric cancer cells; H: The expression of miR-4295 was detected by RTFQ-PCR in AGS cells with circSMARCA5 overexpression. ***: P<0.001, compared with each other.

表1 60例胃癌患者的临床病理学资料

Tab. 1 The clinicopathological features of 60 gastric cancer patients

Clinicopathological features	Case *n* (%)	Clinicopathological features	Case *n* (%)
Age/year		Lymph node metastasis	
＜60	21 (35.0)	Negative	22 (36.7)
≥60	39 (65.0)	Positive	38 (63.3)
Gender		Vascular invasion	
Female	16 (26.7)	No	29 (48.3)
Male	44 (73.3)	Yes	31 (51.7)
Tumor size *D*/cm		TNM staging	
＜5	41 (68.3)	Ⅰ/Ⅱ	24 (40.0)
≥5	19 (31.7)	Ⅲ	36 (60.0)
Differentiation			
Well/Moderate	28 (46.7)		
Poor	32 (53.3)		

2.5 上调miR-4295表达可部分逆转过表达circSMARCA5对胃癌细胞增殖和侵袭的影响

在circSMARCA5过表达组的AGS细胞中上调miR-4295表达水平，进行回复实验。结果显示，过表达circSMARCA5对胃癌细胞增殖、侵袭和糖酵解活性的抑制作用可被miR-4295 mimics部分挽救（图5）。

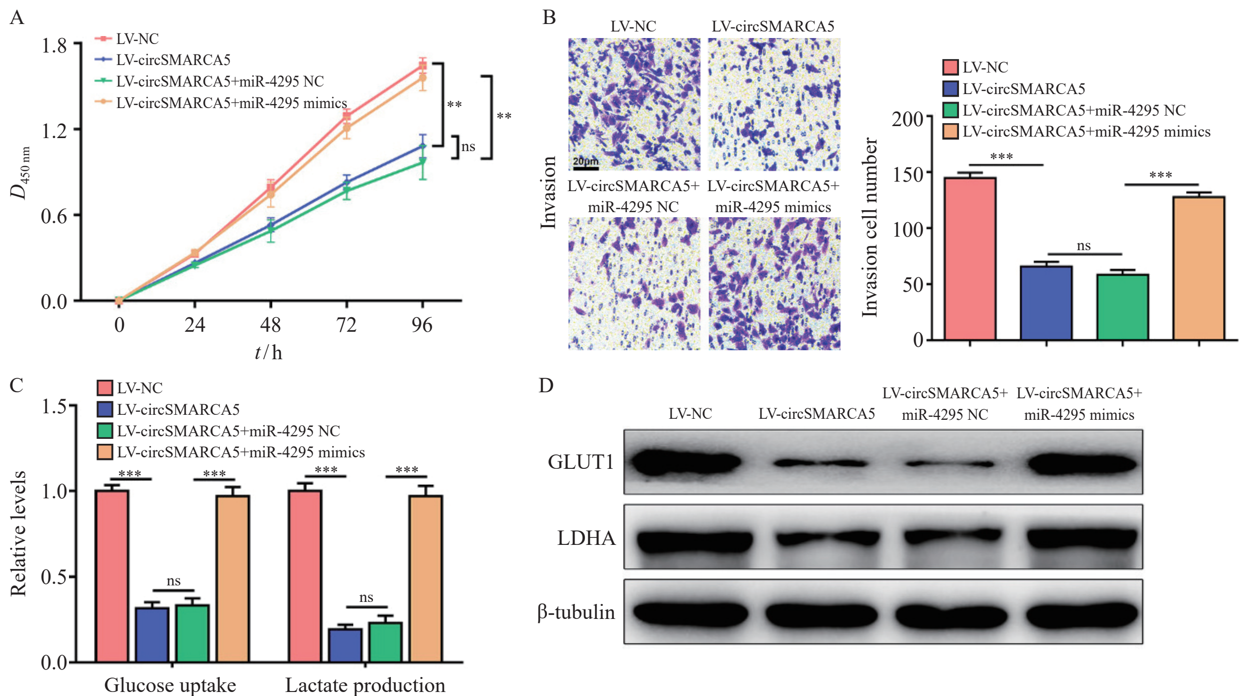

图5 CircSMARCA5通过靶向调控miR-4295表达抑制胃癌细胞增殖和侵袭

Fig. 5 CircSMARCA5 inhibits the proliferation and invasion of gastric cancer cells through targeting miR-4295

A: CCK-8 assay was performed to examine the cell proliferation of circSMARCA5-overexpressing cells with miR-4295 upregulation; B: Transwell assays were conducted to assess the invasive capability of circSMARCA5-overexpressing cells with miR-4295 upregulation; C: The cellular glucose uptake and lactate production were detected in circSMARCA5-overexpressing cells with miR-4295 upregulation; D: Western blot analysis showed the expression levels of GLUT1 and LDHA. **: $P<0.01$, compared with each other; ***: $P<0.001$, compared with each other; ns: No significance.

2.6　PTEN是miR-4295的下游靶基因

生物信息学预测结果提示miR-4295与PTEN存在互补结合的核苷酸序列，据此设计了miR-4295与PTEN结合位点的突变序列（图6A-B）。双荧光素酶报告基因实验结果显示：miR-4295 mimics+PTEN-WT共转染组的荧光素酶活性显著降低（$P<0.001$），而miR-4295 NC+ PTEN-MUT组和miR-4295 mimics+PTEN-MUT组的荧光素酶活性无明显改变（$P>0.05$，图6C）。Pearson相关性分析结果表明胃癌组织中miR-4295与PTEN的表达水平成负相关（$r=-0.421$，$P=0.001$，图6D）。RTFQ-PCR结果显示，miR-4295 mimics组胃癌细胞中PTEN mRNA表达水平显著低于miR-4295 NC组（$P<0.001$，图6E）。以上结果表明在胃癌细胞中miR-4295可与PTEN直接结合并下调PTEN的表达。

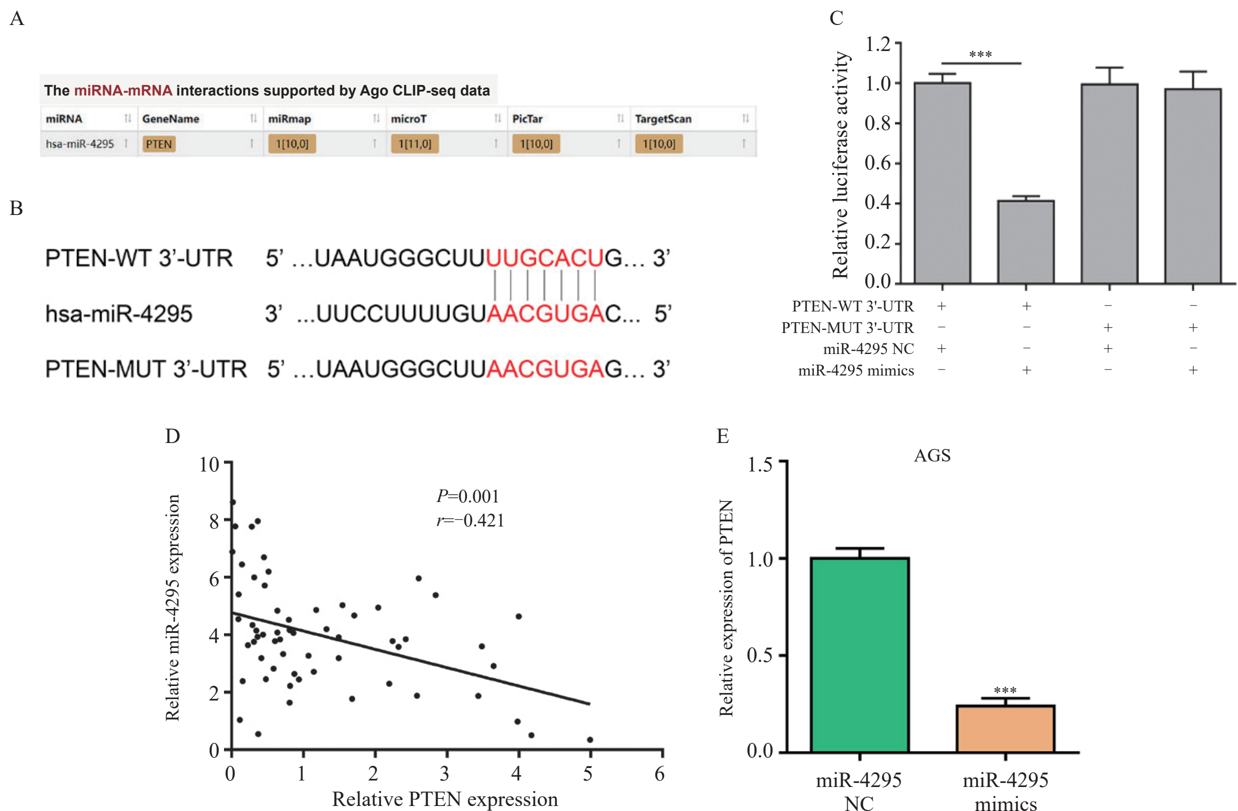

图6　*PTEN*是miR-4295的下游靶基因

Fig. 6　*PTEN* was a direct target gene of miR-4295

A: ENCORI database prediction indicated that PTEN was a potential direct target gene of miR-4295; B: Putative binding sequence between miR-4295 and PTEN; C: Luciferase activity was determined using dual luciferase reporter assays; D: A negative correlation between miR-4295 and PTEN expression level was demonstrated in gastric cancer tissues; E: The expression level of PTEN was detected by RTFQ-PCR in AGS cells with miR-4295 overexpression. ***$P<0.001$.

2.7　敲低PTEN表达可部分逆转过表达circSMARCA5对胃癌细胞增殖和侵袭的影响

在circSMARCA5过表达组的AGS细胞中敲低PTEN表达水平，进行回复实验。结果显示，过表达circSMARCA5对胃癌细胞增殖、侵袭和糖酵解活性的抑制作用可被si-PTEN部分挽救（图7A~D）。本研究机制模式图见图7E。

3　讨　　论

circRNA已被证实广泛参与了肿瘤的发生、发展过程[7]，并可作为肿瘤诊断、预后的分子标志物以及潜在的治疗靶点[8-10]。既往研究[11-12]发现，circSMARCA5在多种肿瘤中发挥

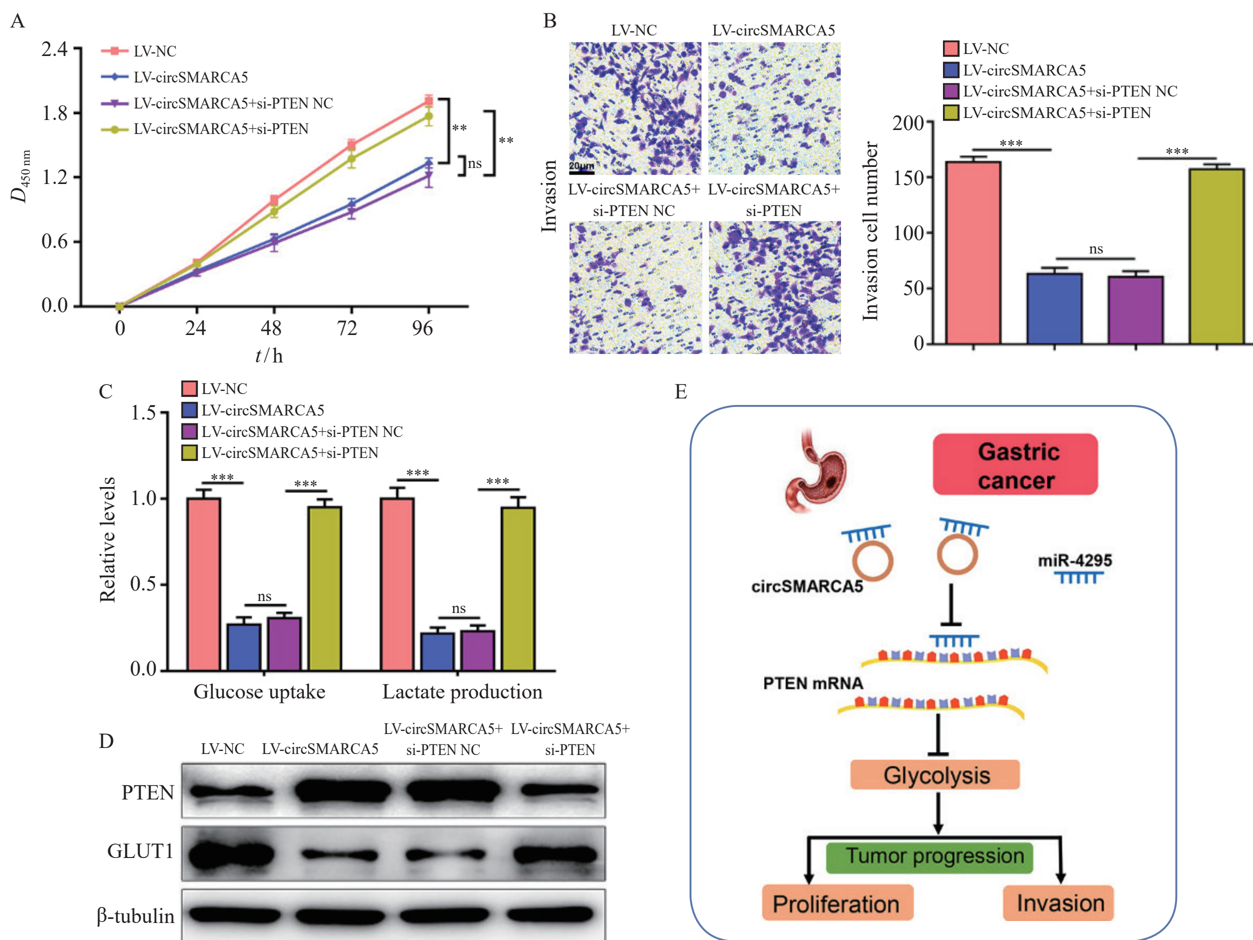

图7 CircSMARCA5通过调控miR-4295/PTEN轴抑制胃癌细胞增殖和侵袭

Fig. 7 CircSMARCA5 inhibits the proliferation and invasion of gastric cancer cells through miR-4295/PTEN axis

A: CCK-8 assays were performed to examine the cell proliferation of circSMARCA5-overexpressing cells with PTEN knockdown; B: Transwell assays were conducted to assess the invasive capability of circSMARCA5-overexpressing cells with PTEN knockdown; C: The cellular glucose uptake and lactate production were detected in circSMARCA5-overexpressing cells with PTEN knockdown; D: Western blot analysis showing the expression levels of PTEN and GLUT1; E: Schematic diagram demonstrating the molecular mechanisms underlying circSMARCA5 in gastric cancer. **: $P<0.01$, compared with each other; ***: $P<0.001$, compared with each other; ns: No significance.

了抑癌作用。circSMARCA5在肝癌组织中表达明显降低，上调circSMARCA5表达可抑制肝癌的侵袭转移[11]。在骨肉瘤中，circSMARCA5通过调控SRSF1表达，抑制肿瘤的侵袭转移[12]。circSMARCA5在胃癌中表达降低并能抑制胃癌细胞的增殖和侵袭[5]，本文在此基础上进一步探讨circSMARCA5调控胃癌增殖和侵袭的具体分子机制。

在氧含量正常的情况下，利用糖酵解是肿瘤细胞主要的能量来源，这种现象被称为Warburg效应[13]。Warburg效应为肿瘤细胞的快速增殖提供了足够的能量和营养，并且与肿瘤的侵袭转移存在着密切联系[14-15]。CircRNA可通过调节Warburg效应，影响肿瘤细胞的增殖和侵袭[6, 16]。本研究发现circSMARCA5能够显著降低胃癌细胞的葡萄糖摄取和乳酸生成量，下调葡萄糖转运蛋白GLUT1和代谢酶LDHA的表达水平，从而抑制细胞糖酵解。

为了进一步研究circSMARCA5影响胃癌增殖和侵袭的分子机制，本研究通过ENCORI数据库预测miR-4295可与circSMARCA5特异性结合。既往文献报道miR-4295在胃癌、胰腺癌和膀胱癌等多种肿瘤中表达升高，与TNM分期及淋巴结转移具有明显的正相关性[17-19]，并可调控肿瘤细胞代谢重编程[20]，进而促进肿瘤进展。本研究结果发现，miR-4295在胃癌组织中表达较癌旁组织

明显升高，并与circSMARCA5的表达水平呈负相关，FISH和双荧光素酶报告基因实验结果进一步证实了circSMARCA5可与miR-4295直接结合。

生物信息学预测miR-4295与PTEN mRNA的3′-UTR存在相互结合位点。PTEN是一个重要的代谢调控因子[21]，能够负调控PI3K/AKT信号转导通路，降低肿瘤细胞糖酵解，从而抑制肿瘤进展[22]。在本研究中，我们通过双荧光素酶报告基因实验进一步证实miR-4295与PTEN的相互结合，在胃癌组织中两者的表达呈负相关，并且在胃癌细胞中过表达miR-4295能够降低PTEN的表达。此外，回复实验结果表明，在circSMARCA5过表达组细胞中上调miR-4295或者下调PTEN表达可部分地逆转过表达circSMARCA5对胃癌细胞增殖、侵袭和糖酵解的抑制作用。

综上所述，circSMARCA5能够抑制胃癌细胞的增殖和侵袭，其分子机制可能是通过调控miR-4295/PTEN轴抑制胃癌细胞糖代谢重编程。

利益冲突声明：所有作者均声明不存在利益冲突。

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