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· 基础研究 ·

长链非编码 RNA 909 靶向调控 miR-194-5p/DACH1 轴对胰腺癌细胞增殖、迁移和侵袭的影响

邓家琦¹, 钱保林², 张丽云³, 李明星¹

(西南医科大学附属医院 1. 超声医学科 2. 肝胆外科 3. 健康管理部, 四川 泸州 646000)

摘要

背景与目的: 长链非编码 RNA 909 (LINC00909) 是一个新发现的长度约为 2 kb 的长链非编码 RNA (lncRNA), 在胶质瘤和白血病中被报道发挥癌基因的作用。然而 LINC00909 在胰腺癌中的作用鲜有报道。本研究旨在探讨 LINC00909 在胰腺癌中的表达及其对患者预后的影响, 以及 LINC00909 与其调控网络对胰腺癌细胞生物学行为的影响。

方法: 通过 Ualcan 在线分析 LINC00909 在 TCGA 数据库中 24 种肿瘤组织中的表达情况。通过 LinkedOmics 在线分析 LINC00909 在 176 例胰腺癌中的表达及预后情况。利用 lncRNA-miRNA 结合数据库 (starBase) 及 3 个 miRNA-mRNA 结合数据库 (miRmap、miRanda 和 TargetScan), 预测 LINC00909 的靶 microRNA (miRNA) 以及靶 miRNA 下游的 mRNA, 并构建 LINC00909-miRNA-mRNA 网络。用 R 语言 “limma” 包对癌旁和癌组织的 miRNA 进行表达差异对比分析。利用 R 语言 “survival” 包、“survminer” 包及 “ggstat - splot” 包, 对所得到的基因进行生存分析及相关性分析。在胰腺癌 PANC-1 细胞上, 用敲减实验 (转染 LINC00909 siRNA) 分析 LINC00909 对 PANC-1 细胞增殖、迁移及侵袭的影响; 用回复实验 (同时转染 LINC00909 siRNA 与靶 miRNA 抑制剂) 及验证 LINC00909 与靶 miRNA 之间的靶向关系。用双荧光素酶报告基因实验验证靶 miRNA 与 LINC00909 以及下游 mRNA 之间的互作关系。

结果: Ualcan 分析表明 LINC00909 在胰腺癌组织中低表达。LinkedOmics 分析表明 LINC00909 低表达患者的预后差 ($P < 0.05$)。通过 starBase 数据库获得 28 个与 LINC00909 结合的 miRNA, 进一步通过对 TCGA 胰腺癌 miRNA 表达数据分析发现, 只有 miR-194-5p 在胰腺癌中表达明显上调。通过 miRmap、miRanda 和 TargetScan 数据库获得 114 个与 miR-194-5p 结合的潜在 mRNA。进一步对 114 个 mRNA 进行表达相关性分析及生存分析, 获得 4 个 mRNA (DACH1、SOCS2、STX16 及 SNAP91), 最终获得 LINC00909-miR-194-5p-DACH1/SOCS2/STX16/SNAP91 的 ceRNA 网络。敲减实验显示, LINC00909 低表达组 PANC-1 细胞的增殖活力增加、划痕愈合率、迁移与侵袭细胞数均较对照组明显升高 (均 $P < 0.05$)。回复实验表明, 回复组 DACH1 mRNA 的表达水平升高, 且细胞的增殖活力、划痕愈合率、迁移与侵袭细胞数均较 LINC00909 低表达组明显降低 (均 $P < 0.05$)。双荧光素酶报告基因实验表明, miR-194-5p 模拟物能够抑制含有其结合位点的 LINC00909 和 DACH1 质粒的荧光素酶活性 (均 $P < 0.05$)。

结论: LINC00909 在胰腺癌组织中低表达且发挥抑癌作用, 其可能通过靶向调控 miR-194-5p/DACH1 轴来抑制 PANC-1 细胞的增殖、迁移及侵袭能力。

关键词

胰腺肿瘤; RNA, 长链非编码; 微 RNAs; 细胞增殖; 肿瘤浸润
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作者简介: 邓家琦, 西南医科大学附属医院住院医师, 主要从事介入超声 (肝脏、甲状腺) 方面的研究。

通信作者: 李明星, Email: lmx526@sina.com

Effect of long non-coding RNA 909 on proliferation, migration, and invasion of pancreatic cancer cells by targeting regulation of miR-194-5p/DACH1 axis

DENG Jiaqi¹, QIAN Baolin², ZHANG Liyun³, LI Mingxing¹

(1. Department of Ultrasound Medicine 2. Department of Hepatobiliary Surgery 3. Health Management Department, the Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan 646000, China)

Abstract

Background and Aims: Long non-coding RNA 909 (LINC00909) is a newly discovered long non-coding RNA (lncRNA) with a length of approximately 2 kb, which has been reported to function as an oncogene in gliomas and leukemias. However, the role of LINC00909 in pancreatic cancer has rarely been reported. This study was designated to investigate the expression of LINC00909 in pancreatic cancer and its influence on prognosis of the patients, as well as the impacts of LINC00909 and its regulatory network on the biological behaviors in pancreatic cancer cells.

Methods: The expressions of LINC00909 in 24 different types of cancers in TCGA datasets were analyzed through UALCAN. LinkedOmics was used to analyze the expressions of LINC00909 and prognosis in 176 pancreatic cancer patients were analyzed through LinkedOmics database. By using the lncRNA-miRNA binding database (starBase) and three miRNA-mRNA binding databases (miRmap, miRanda, and TargetScan), the potential target microRNAs (miRNAs) of LINC00909 and the mRNAs downstream to these miRNAs were predicted, and the LINC00909-miRNA-mRNA network was constructed. The differentially expressed miRNAs between pancreatic cancer and adjacent non-tumorous pancreatic tissues were identified by the "limma" package of R language. The survival analysis and correlation analysis were conducted by using "survival", "survminer", and "ggstatsplot" packages in R language. In pancreatic cancer PANC-1 cells, the influences of LINC00909 on cell proliferation, and migration/invasion abilities were investigated by a knockdown experiment (LINC00909 siRNA transfection), and the targeted relationship between LINC00909 and the potential miRNAs were verified by a rescue experiment (simultaneous transfection of LINC00909 siRNA and miRNA inhibitor). The interactions of the target miRNAs and with LINC00909 and their downstream mRNAs were verified by dual luciferase reporter assay.

Results: UALCAN analysis revealed that LINC00909 was lowly expressed in pancreatic cancer tissues. Low expression of LINC00909 predicted poor prognosis based on data from LinkedOmics databases ($P < 0.05$). A total of 28 miRNAs potentially binding to LINC00909 were obtained through the starBase. Further analysis of the miRNA expression data in pancreatic cancer from TCGA found that only miR-194-5p was significantly up-regulated in pancreatic cancer. Using the target prediction databases miRmap, miRanda and TargetScan databases, 114 potential downstream target mRNAs of miR-194-5p were obtained. After further expression correlation analysis and survival analysis of the 114 mRNAs, 4 mRNAs (DACH1, SOCS2, STX16 and SNAP91) were identified. Finally, the ceRNA network of LINC00909-miR-194-5p-DACH1/SOCS2/STX16/SNAP91 was obtained. Results of knockdown experiment showed that the proliferation activity, scratch healing rate and the numbers of migrated and invaded cells were significantly higher in LINC00909 low expression group than those in control group (all $P < 0.05$). Results of rescue experiment showed that the expression level of DACH1 mRNA was significantly increased, while and the cell proliferation activity, scratch healing rate, and the numbers of migrated and invaded cells were significantly decreased in rescue group compared with those in LINC00909 low expression group (all $P < 0.05$). Results of dual luciferase reporter assay showed that miR-194-5p mimics significantly suppressed the luciferase activity of the LINC00909 and DACH1

reporter plasmid containing binding sites (both $P < 0.05$).

Conclusion: Expression of LINC00909 is decreased in pancreatic cancer tissue and it exerts a tumor suppressor function. It may inhibit the proliferation, migration, and invasion of pancreatic cells by targeting and regulating the miR-194-5p/DACH1 axis.

Key words

Pancreatic Neoplasms; RNA, Long Noncoding; MicroRNAs; Cell Proliferation; Neoplasm Invasiveness

CLC number: R735.9

胰腺癌是一种恶性程度很高的消化系统恶性肿瘤，其主要病理类型为胰腺腺癌（pancreatic adenocarcinoma, PAAD），病死率居我国恶性肿瘤第5位，预后较差，患者生存率低^[1-2]。近年来，该病的发病率及病死率均呈逐年上升的趋势，给人民的健康安全造成严重威胁^[3]。目前还没有很好的胰腺癌早期诊断手段，外科手术切除是胰腺癌最有效的治疗手段，但因术后远处转移和局部复发，其5年生存率仍低于20%^[4]。因此，寻找有效的胰腺癌早期诊断和治疗靶点至关重要。长链非编码RNA（long non-coding RNA, lncRNA）是一类长度>200个核苷酸长度的RNA片段，其缺乏开放阅读框架，不具备编码蛋白的能力，参与转录、转录后和表观遗传学基因调控，在机体的正常发育及肿瘤发生发展中具有重要作用^[5-7]。lncRNA可作为一种竞争性内源RNA（competing endogenous RNA, ceRNA）吸附微小RNA（microRNA, miRNA），参与靶基因的表达调控，在肿瘤的发生发展中发挥重要的作用^[8-10]。长链非编码RNA 909（LINC00909）是一个新发现的长度约为2 kb的lncRNA，位于18号染色体q22.3上，在胶质瘤、结直肠癌和白血病中被报道发挥癌基因的作用^[11-14]。然而，关于LINC00909在胰腺癌中的作用鲜有报道，因此本研究从LINC00909作为ceRNA发挥生物学功能这一角度出发，通过生物信息学分析构建LINC00909 ceRNA网络，并通过细胞学实验验证LINC00909对胰腺癌细胞增殖、迁移及侵袭的影响，为进一步研究LINC00909在胰腺癌发生发展的作用机制、诊断及预后提供了新的线索和思路。

1 材料与方法

1.1 细胞和主要试剂

胰腺正常细胞系HPDE及SW1990细胞购自广

州吉妮欧生物公司，PANC-1、BXP3及ASPC1细胞购自中科院上海细胞研究所。针对LINC00909的小干扰RNA片段（si-LINC00909-1#, si-LINC00909-2#）、miR-194-5p模拟物（mimics）、miR-194-5p抑制物（inhibitor）和阴性对照（NC）购自上海吉玛生物科技有限公司，脂质体Lipofectamine 2000购自美国Thermo公司，DMEM高糖培养基、胎牛血清和胰蛋白酶购自美国Gibco公司，青霉素和链霉素购自美国Sigma公司，活细胞计数试剂盒（CCK-8）购自碧云天生物科技公司，TRIzol试剂购自美国Invitrogen公司，逆转录试剂盒PrimeScript RT Master Mix和实时定量PCR（qPCR）试剂盒One Step SYBR PrimeScript购自宝生物工程（大连）有限公司。

1.2 表达及预后分析

从TCGA数据库下载并整理胰腺癌转录组的miRNA及mRNA/lncRNA表达谱数据及临床数据。利用R语言limma包对癌旁和癌组织的miRNA进行表达差异对比分析。利用R语言“survival”包、“survminer”包及“ggstatsplot”包，对所得到的基因进行生存分析，分析其与胰腺癌患者预后的相关性。利用lncRNA-miRNA结合数据库（starBase）及3个miRNA-mRNA结合数据库（miRmap、miRanda和TargetScan），预测LINC00909的靶miRNA以及靶miRNA下游的mRNA，并构建LINC00909-miRNA-mRNA网络。

1.3 细胞培养和转染

复苏PANC-1及SW1990细胞于含10%胎牛血清和100 U/mL青霉素-链霉素的DMEM高糖培养基中，复苏HPDE、BXP3及ASPC1细胞于含10%胎牛血清和100 U/mL青霉素-链霉素的1640培养基中，于37℃、5% CO₂恒温培养箱中培养，待细胞融合度达到约80%时，用0.25%胰蛋白酶消化传代。按照1×10⁵个/孔的密度将细胞接种于6孔板内，待

细胞融合度达约50%~60%时,采用脂质体向PANC-1细胞转染 si-LINC00909、miR-194-5p mimics、miR-194-5p inhibitor及NC。

1.4 CCK-8实验

按照 5×10^3 个/孔的密度将各组细胞接种于96孔板,分别于转染后0、24、48、72 h加入10 μ L CCK-8溶液,恒温培养4 h后用酶标仪检测450 nm处的吸光度,以空白孔为参照,评估细胞的增殖情况。

1.5 划痕实验

将细胞接种于6孔板中常规培养后形成单层,用200 μ L无菌移液器枪头在单层细胞中央划出一条直线,于37 $^{\circ}$ C、5% CO₂恒温培养箱中培养,并在划痕0、24 h后用显微镜拍摄细胞伤口图像,测量细胞迁移距离并计算划痕愈合率。

1.6 Transwell实验

Matrigel在冰上融化后按1:3的比例用无血清培养基进行稀释,用预冷的枪头吸取25 μ L稀释的Matrigel加入Transwell小室的上室,37 $^{\circ}$ C静置30 min,使Matrigel聚合成胶。同时收集各组已转染的PANC-1细胞胰酶消化后计数,并接种于含Matrigel的Transwell小室上进行侵袭实验,将细胞悬液接种于不含Matrigel的Transwell小室上进行迁移实验,500 μ L含10% FBS的培养基加入下室,24 h后终止培养,用PBS将上室的细胞洗掉,4%多聚甲醛固定15 min,并用0.1%结晶紫染色。然后在显微镜下对细胞进行拍照并计数。

1.7 总RNA提取和qPCR

采用TRIzol提取细胞的总RNA,使用PrimeScript RT Master Mix逆转录合成cDNA,随后将2 μ L cDNA溶液加入qPCR反应体系,采用One Step SYBR PrimeScript于ABI 7500 RT-PCR系统进行qPCR。以GAPDH为内参,采用 $2^{-\Delta\Delta Ct}$ 表示LINC00909和DACH1的相对表达量。LINC00909上游引物:5'-TGT GCA AGG CGA GAA TGC TA-3',下游引物:5'-AAA CAG GTC ACC AAG CCA CA-3'; DACH1上游引物:5'-ATG TGG AAC AAG TTC GCA TCC-3',下游引物:5'-TGC AGT CAT TGT AGA GGG TCT-3'; GAPDH上游引物:5'-GGA GCG AGA TCC CTC CAA AAT-3';下游引物:5'-GGC TGT TGT CAT ACT TCT CAT GG-3'。

1.8 荧光素酶报告实验

采用DIANA Tools程序在线预测LINC00909和DACH1与miR-194-5p的结合位点,合成含有miR-194-5p结合位点的LINC00909和DACH1并克隆至荧光素酶报告载体pmirGLO。采用Lipofectamine 2000将LINC00909或DACH1载体与miR-194-5p模拟物或阴性对照共转染,48 h后收集细胞并使用双荧光素酶报告分析系统测定相对荧光素酶活性。

1.9 统计学处理

采用SPSS 21.0版统计学软件进行数据分析。本研究数据以均数 \pm 标准差($\bar{x} \pm s$)表示,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。以 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 胰腺癌组织中LINC00909的表达及预后情况

通过UALCAN在线分析来自TCGA数据库的24种肿瘤组织LINC00909表达水平,发现胰腺癌组织LINC00909表达水低于正常组织表达(图1A)。进一步通过LinkedOmics在线分析LINC00909在176例胰腺癌中的表达及预后情况,发现LINC00909表达随胰腺癌病理分级的上升而明显降低,差异有统计学意义($P < 0.05$)(图1B)。同时生存分析显示,LINC00909低表达患者的预后差,差异有统计学意义($P < 0.05$)(图1C)。与胰腺正常细胞系HPDE相比,LINC00909在4个胰腺癌细胞系中均下调表达($P < 0.05$)(图1D)。

2.2 靶向LINC00909的miRNA分析

通过starBase数据库预测与LINC00909结合的miRNA,获得28个与LINC00909结合的miRNA,分别是miR-105-5p、miR-122-5p、miR-130a-5p、miR-135a-5p、miR-135b-5p、miR-148a-3p、miR-148b-3p、miR-152-3p、miR-155-5p、miR-194-5p、miR-23a-3p、miR-23b-3p、miR-23c、miR-24-3p、miR-339-3p、miR-448、miR-511-3p、miR-519a-3p、miR-519b-3p、miR-519c-3p、miR-552-3p、miR-5586-5p、miR-580-3p、miR-625-5p、miR-6509-5p、miR-7853-5p、miR-874-3p及miR-892c-5p。进一步通过对TCGA胰腺癌miRNA表达数据分析发现,只有miR-194-5p在胰腺癌中明显上调表达(图2)。因此提示miR-194-5p可能是LINC00909的潜在靶miRNA。

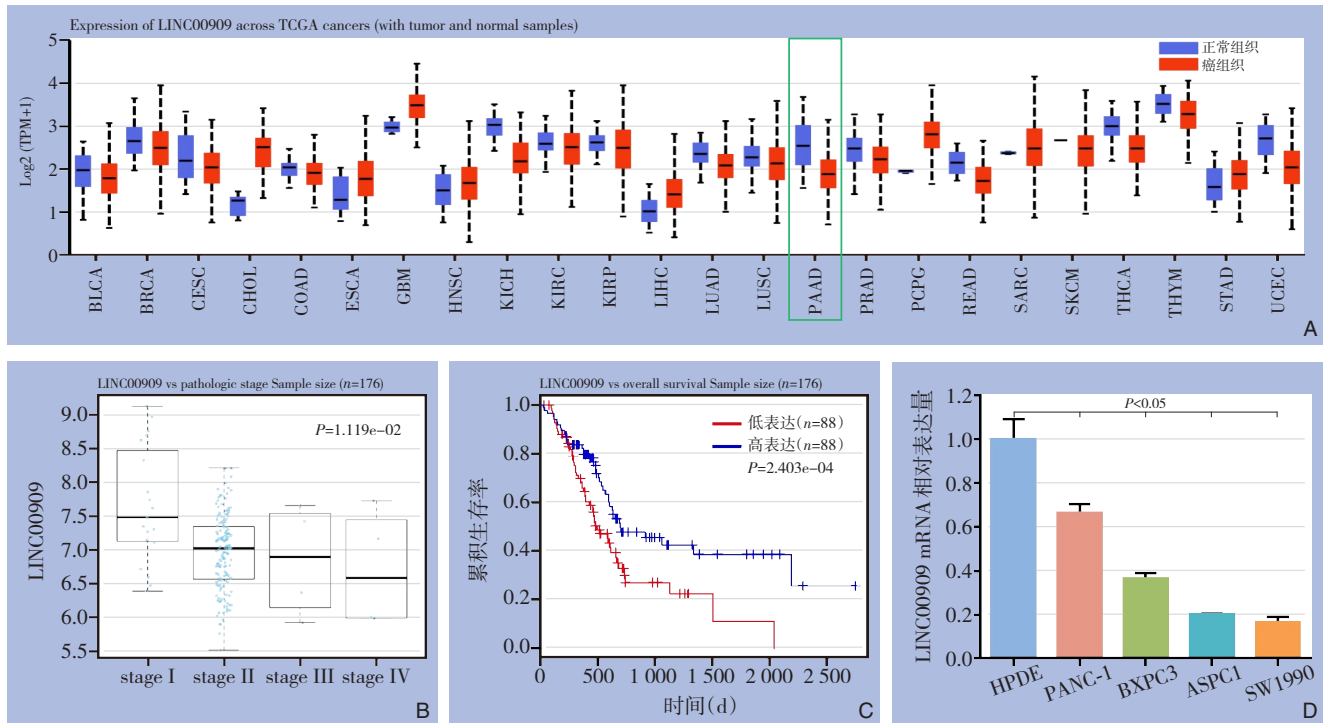


图 1 胰腺癌组织中 LINC00909 的表达及预后情况 A: LINC00909 在 24 种肿瘤中的表达; B: LINC00909 在胰腺癌病理分级中的表达; C: 生存曲线; D: 细胞表达谱

Figure 1 The expression of LINC00909 and prognosis in pancreatic cancer tissue A: Expressions of LINC00909 in 24 types of tumors; B: Expressions of LINC00909 in pancreatic cancer with different pathological grades; C: Survival curves; D: Cell expression profiles

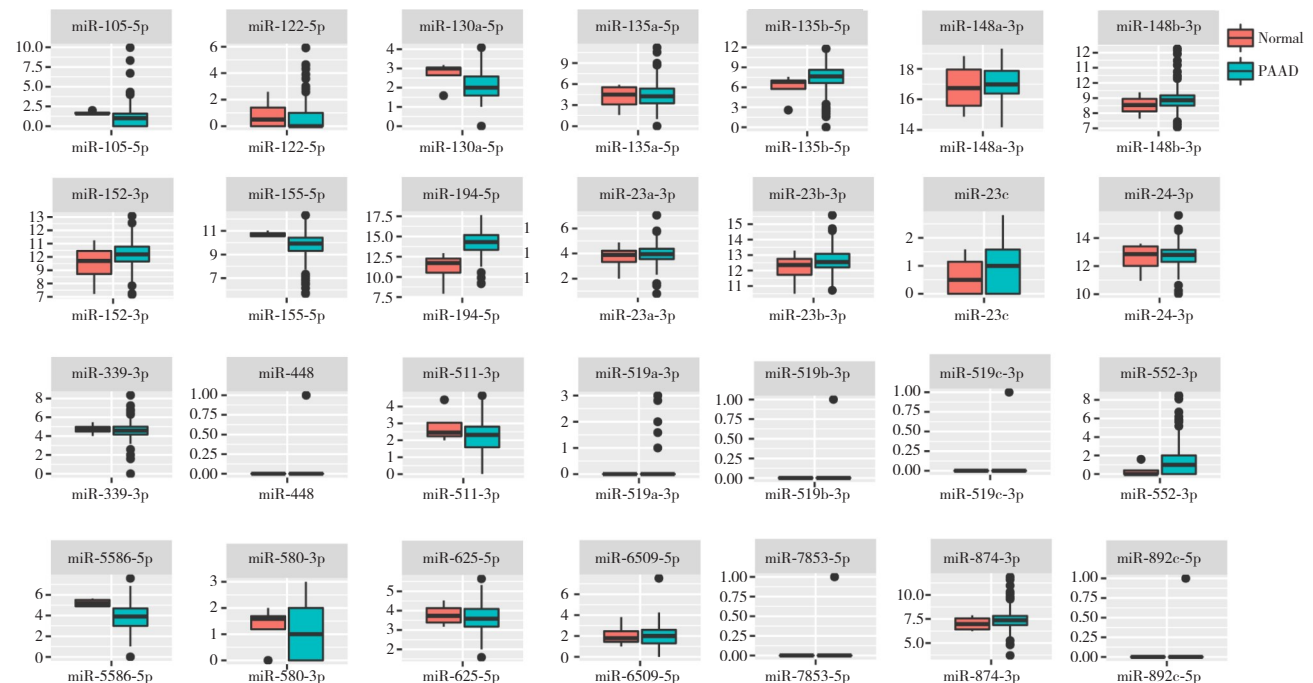


图 2 与 LINC00909 结合的 miRNA 在胰腺癌中的表达情况

Figure 2 The expressions of miRNA binding to LINC00909 in pancreatic cancer

2.3 与 miR-194-5p 结合的潜在 mRNA 分析

通过 3 个 miRNA-mRNA 相互作用预测数据库分析, 获得 114 个潜在 mRNA。进一步通过表达相关性分析及生存分析, 对 114 个潜在 mRNA 进行筛选, 最终获得 4 个 mRNA, 分别是 DACH1、SOCS2、STX16 及 SNAP91 (图 3A)。表达相关性分析表明,

4 个基因与 LINC00909 表达呈明显正相关 (图 3B)。生存分析结果表明, 4 个基因与胰腺癌不良预后有关, 且基因低表达, 患者预后差, 与 LINC00909 预后结果一致 (图 3C)。最终建了 LINC00909-miR-194-5p-DACH1/SOCS2/STX16/SNAP91 的 ceRNA 网络 (图 3D)。

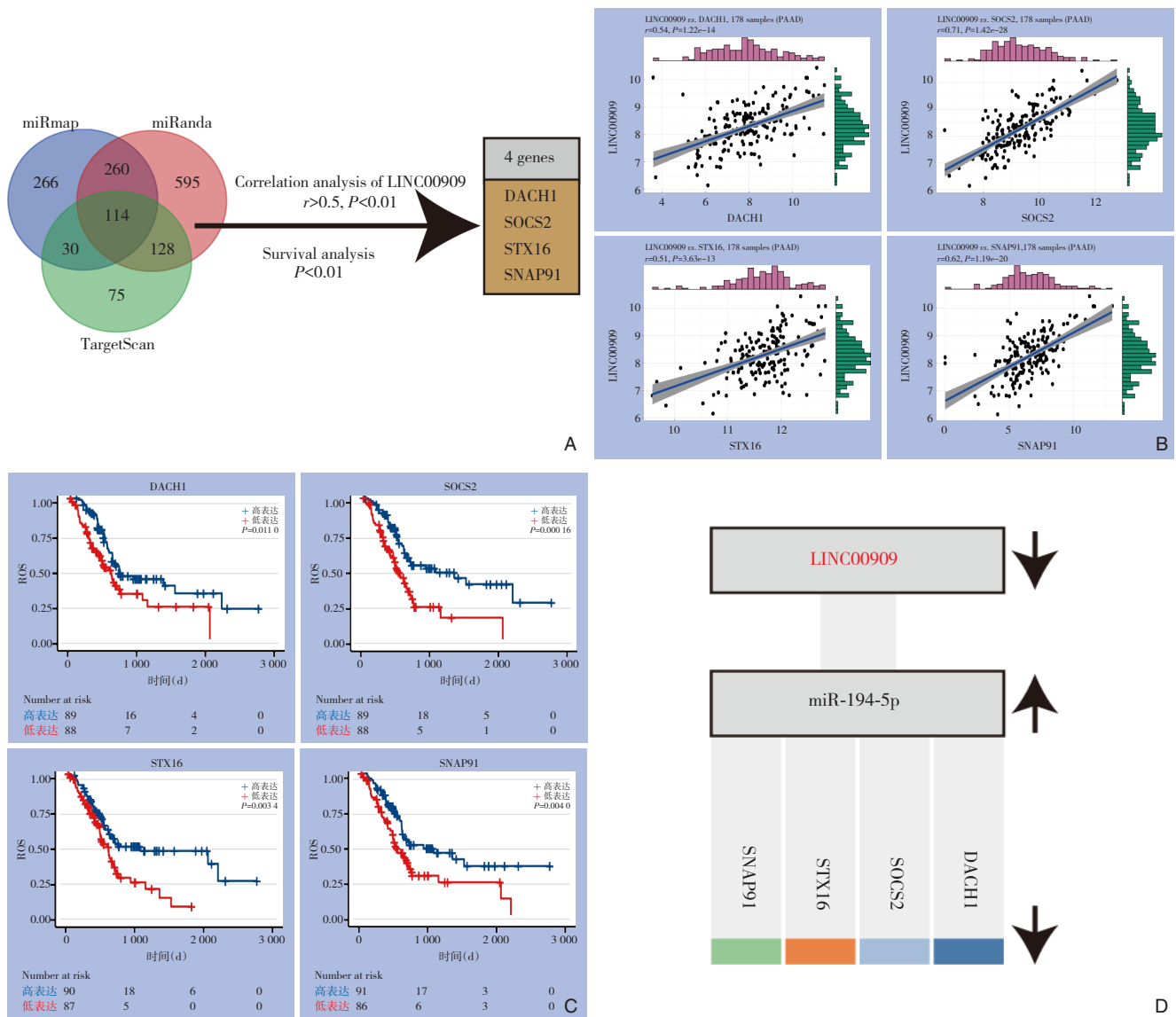


图3 LINC00909 ceRNA 网络的构建 A: 分析与 miR-194-5p 结合的潜在 mRNA; B: 基因表达相关性分析; C: 生存分析; D: ceRNA 网络示意图

Figure 3 Construction of LINC00909 ceRNA network A: Prediction of potential target genes of miR-194-5p; B: Gene expression correlation analysis; C: Survival analysis; D: Schematic diagram of ceRNA network

2.4 下调 LINC00909 对 PANC-1 细胞增殖、迁移及侵袭的影响

LINC00909 低表达组经 2 条 si-LINC00909 序列转染处理, qPCR 结果显示, PANC-1 细胞 LINC00909 低表达组 LINC00909 的表达水平降低 (P<0.05)

(图 4A)。MTT 结果显示, 与对照组相比, LINC00909 低表达组的细胞增殖活力增加 (P<0.05) (图 4B)。划痕实验结果显示, 与阴性对照组 (转染 si-NC) 比较, LINC00909 低表达组 (转染 si-LINC00909-1# 或 si-LINC00909-2#) 的划痕愈合率高

于对照组 ($P<0.05$) (图4C)。Transwell迁移和侵袭实验结果显示,与阴性对照组比较,LINC00909低

表达组的迁移与侵袭细胞数高于对照组 (均 $P<0.05$) (图4D)。

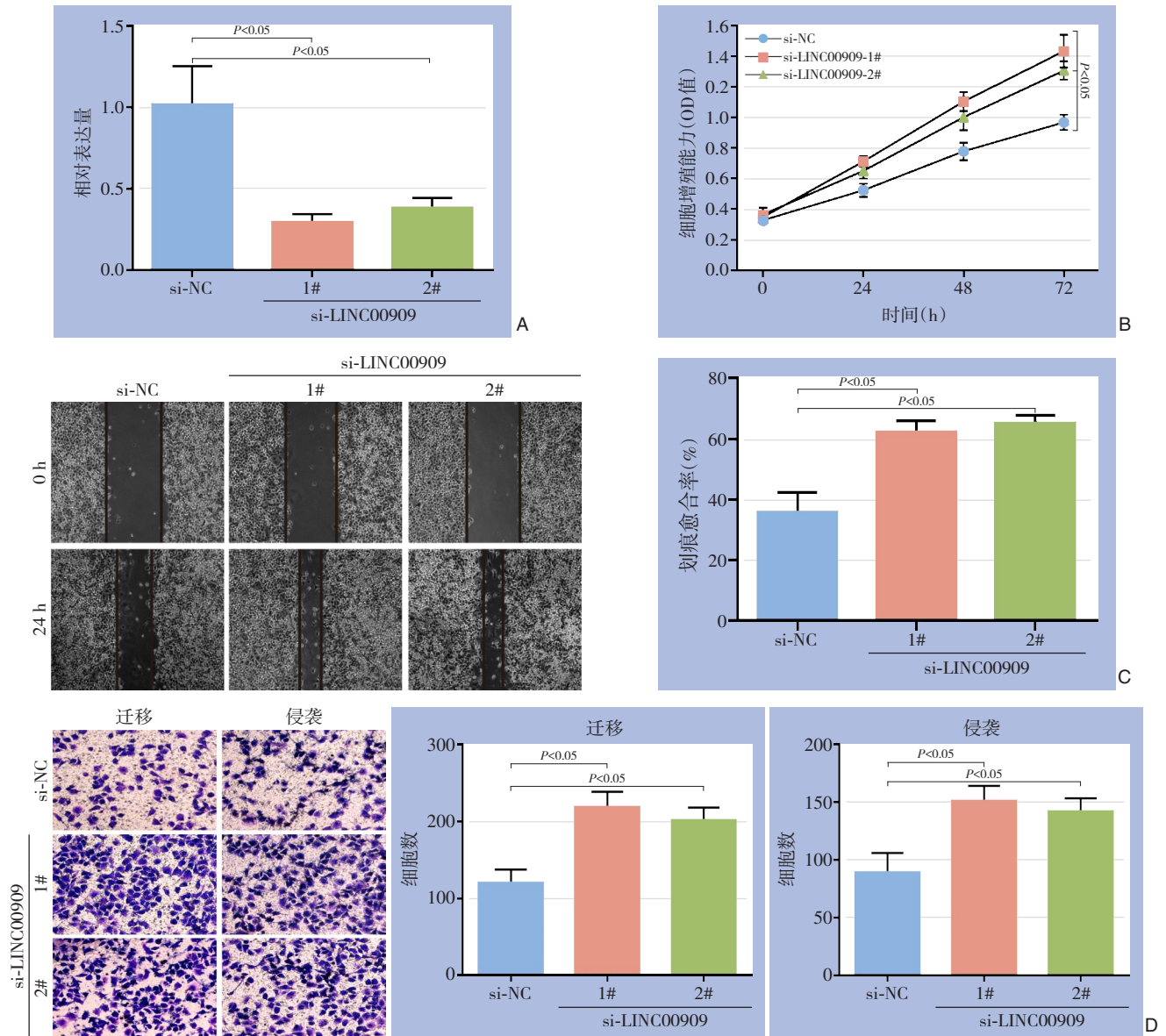


图4 下调LINC00909对PANC-1细胞增殖、迁移及侵袭的影响 A: 各组PANC-1细胞中LINC00909的表达水平; B: 各组PANC-1细胞的增殖曲线; C: 各组PANC-1细胞的划痕实验(×200); D: 各组PANC-1细胞的迁移及侵袭实验(×200)

Figure 4 The effects of down-regulation of LINC00909 on the proliferation, migration, and invasion of PANC-1 cells A: The expression level of LINC00909 in each group PANC-1 cells; B: The proliferation curve of PANC-1 cells in each group; C: Wound scratch assay of PANC-1 cells in each group (×200); D: Migration and invasion assays of PANC-1 cells in each group (×200)

2.5 LINC00909通过miR-194/DACH1轴抑制细胞增殖、迁移及侵袭

回复组经共转染 si-LINC00909-1#和 miR-194-5p 抑制物序列处理, qPCR结果显示, 下调LINC00909

后 DACH1 表达明显下降, 当同时下调LINC00909与 miR-194-5p 后 DACH1 表达明显升高 ($P<0.05$) (图5A)。MTT结果显示, 与LINC00909低表达组比较, 回复组细胞增殖活力降低 ($P<0.05$) (图

5B)。划痕实验结果显示,与LINC00909低表达组比较,回复组细胞划痕愈合能力降低 ($P<0.05$) (图5C)。Transwell 迁移和侵袭实验结果显示,与

LINC00909低表达组比较,回复组迁移与侵袭细胞数降低 (均 $P<0.05$) (图5D)。

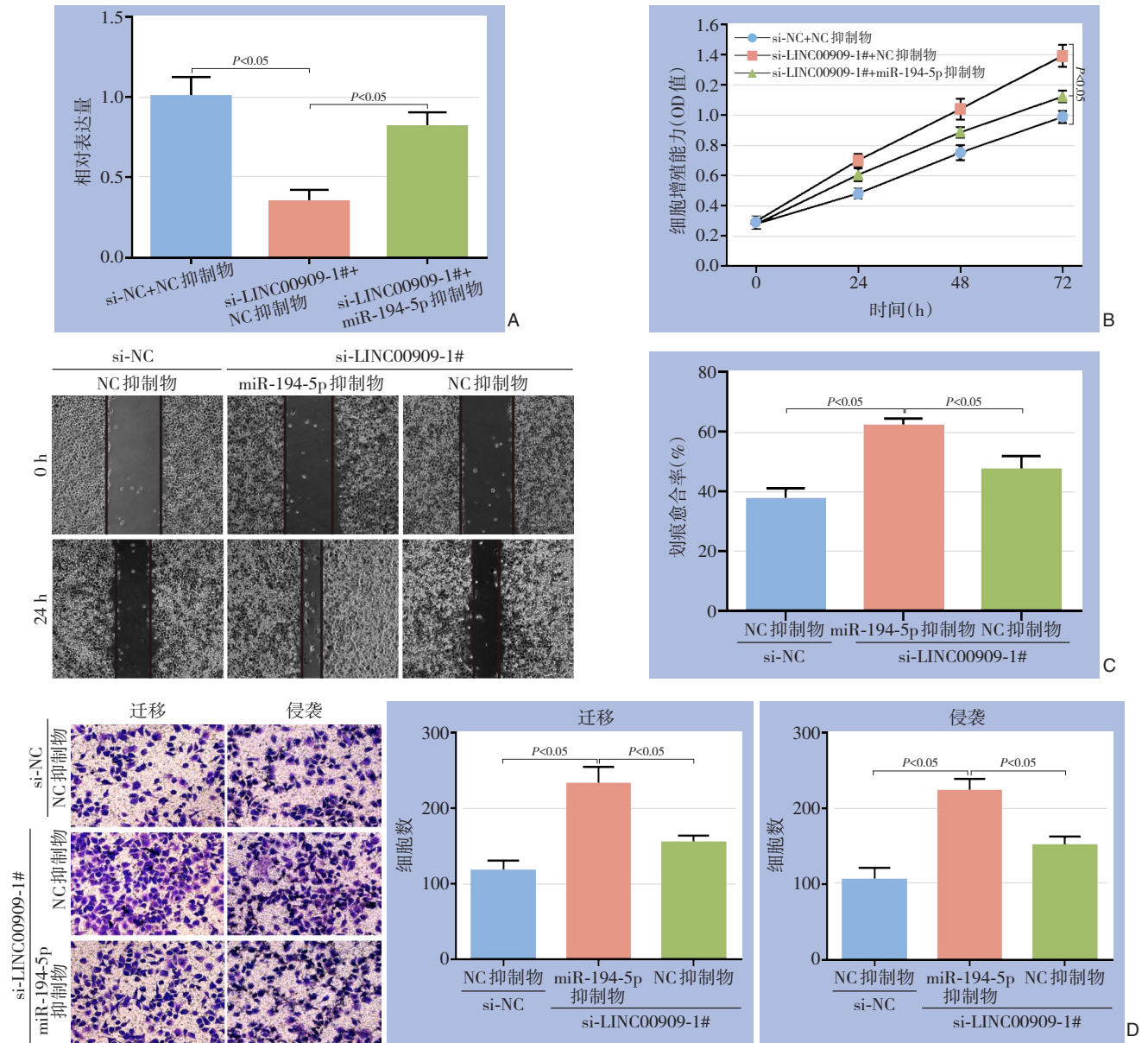


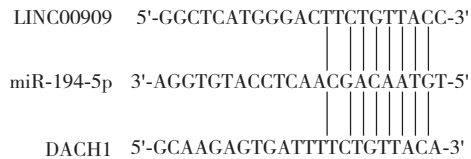
图5 LINC00909通过miR-194-5p/DACH1轴抑制PANC-1细胞增殖、迁移及侵袭 A: 各组PANC-1细胞DACH1水平; B: 各组PANC-1细胞的增殖曲线; C: 各组PANC-1细胞的划痕实验(×200); D: 各组PANC-1细胞的迁移及侵袭实验(×200)

Figure 5 LINC00909 inhibiting PANC-1 cell proliferation, migration, and invasion through miR-194-5p/DACH1 axis A: The expression level of DACH1 in each group PANC-1 cells; B: The proliferation curve of PANC-1 cells in each group; C: Wound scratch assay of PANC-1 cells in each group (×200); D: Migration and invasion assays of PANC-1 cells in each group (×200)

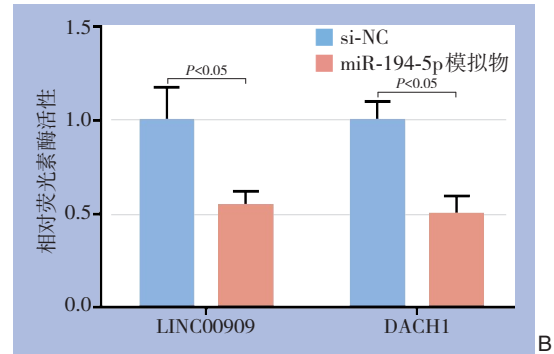
2.6 miR-194-5p 与 LINC00909 和 DACH1 的靶向关系验证

预测分析显示, LINC00909 和 DACH1 基因都含有 miR-194-5p 的结合位点 (图 6A)。双荧光素酶报

告基因实验表明, miR-194-5p 模拟物能够抑制含有其结合位点的 LINC00909 和 DACH1 质粒的荧光素酶活性, 差异有统计学意义 ($P < 0.05$) (图 6B)。



A



B

图6 miR-194-5p 与 LINC00909 和 DACH1 的靶向关系验证示意图; B: 相对荧光强度

A: LINC00909 和 DACH1 与预测的 miR-194-5p 结合位点对比及

Figure 6 Verification of the targeted relationship of miR-194-5p with LINC00909 and DACH1 A: Alignment and schematic diagram of the binding sites of predicted miR-194-5p to LINC00909 and DACH1; B: Relative fluorescence intensity

3 讨论

在最近的胰腺癌研究中发现, lncRNA 作为癌基因或抑癌基因参与胰腺癌的发生发展, 可作为 miRNA 的 ceRNA 来调节上皮间质转化等恶性转化过程^[15-17]。胰腺癌中一些 lncRNA, 如 THAP9-AS1、PLACT1 和 LINC00346 等已被证明发挥癌基因的功能^[18-21], 而一些 lncRNA 如 LINC01111、PXN-AS1 和 TUSC7 等作为肿瘤抑制因子发挥抑癌基因的功能^[22-24]。例如, THAP9-AS1 能够通过 miR-484/YAP 轴来促进胰腺癌生长^[18]。LINC01111 在胰腺癌患者中异常低表达, 且与患者总生存期呈正相关, 并通过 miR-3924/DUSP1 轴抑制胰腺癌的增殖与转移^[22]。LINC00909 已被报道可作为癌基因促进胶质瘤、结直肠癌及急性髓系白血病的发生发展, 但其在胰腺癌中的生物学功能尚不清楚。

本研究通过 TCGA 在线分析发现, LINC00909 在胰腺癌组织中低表达, 且与胰腺癌的预后呈正相关。体外干扰 LINC00909 表达, 检测其差异表达后 PANC-1 细胞增殖、迁移及侵袭能力的变化, 综合评估 LINC00909 在胰腺癌进展中的作用。CCK-8 实验结果显示, 下调 LINC00909 表达显著促进胰腺癌 PANC-1 细胞的增殖能力; 同时划痕愈合实验结果显示下调 LINC00909 表达显著促进 PANC-1 细胞

划痕愈合能力; Transwell 实验结果显示下调 LINC00909 表达显著促进 PANC-1 细胞迁移及侵袭能力, 以上结果表明 LINC00909 能够抑制胰腺癌细胞的增殖、迁移及侵袭过程。本研究证明在胰腺癌中 LINC00909 发挥抑癌基因的功能, 与已报道的在胶质瘤、结直肠癌及急性髓系白血病中的功能相反, 在胰腺癌中这种功能的差异可能是由于其调控下游靶标的不同导致的。

目前研究^[25-27]认为 lncRNA 通常可能作为 miRNA 的 ceRNA 来发挥生理作用。本研究首先通过生物信息学分析构建 LINC00909-miR-194-5p-DACH1/SOCS2/STX16/SNAP91 的 ceRNA 网络, 并在细胞学水平研究了 LINC00909-miR-194-5p-DACH1 轴的功能。荧光素酶报告实验证实 LINC00909 与 DACH1 都可靶向结合 miR-194-5p。进一步在下调 LINC00909 表达的细胞中抑制 miR-194-5p 表达, 发现由 LINC00909 下调引起的促生长、促迁移及促侵袭作用被明显抑制。有研究^[28-31]已证实, 细胞命运决定因子 DACH1 是在哺乳动物中发现的黑腹果蝇 Dachshund 基因同源的基因, 参与调控细胞增殖、迁移、分化、黏附等细胞活动, 是一种新发现的抑癌基因。DACH1 在大多数正常组织中均表达, 然而在很多恶性肿瘤如乳腺癌、子宫内膜癌、结直肠癌中表达降低或缺失, 在胰腺癌中 miR-194-5p

可靶向 DACH1 并下调其表达, 促进胰腺癌生长^[32]。近年来研究发现, miR-194-5p 可作为 LINC00909 调控 MUC1-C 的“海绵”分子, 参与 LINC00909 在胶质瘤中的作用^[11]。结合本研究 and 先前研究结果, 笔者推测 miR-194-5p 及其靶基因 DACH1 构成的信号轴可能是 LINC00909 在胰腺癌中发挥抑癌作用的机制。

综上所述, LINC00909 在胰腺癌细胞中低表达, 并通过 miR-194-5p/DACH1 信号轴来抑制胰腺癌细胞的增殖、迁移及侵袭, 发挥抑癌作用。该发现为 LINC00909 作为潜在的胰腺癌诊断和治疗靶点提供部分理论基础。

利益冲突: 所有作者均声明不存在利益冲突。

参考文献

- [1] Lin QJ, Yang F, Jin C, et al. Current status and progress of pancreatic cancer in China[J]. *World J Gastroenterol*, 2015, 21(26): 7988–8003. doi: 10.3748/wjg.v21.i26.7988.
- [2] 中华医学会外科学分会胰腺外科学组. 中国胰腺癌诊治指南(2021)[J]. *中华消化外科杂志*, 2021, 20(7):713–729. doi:10.3760/cma.j.cn115610-20210618-00289.
- [3] Study Group of Pancreatic Surgery in Chinese Society. Guidelines for the diagnosis and treatment of pancreatic cancer in China (2021)[J]. *Chinese Journal of Digestive Surgery*, 2021, 20(7):713–729. doi:10.3760/cma.j.cn115610-20210618-00289.
- [4] 杨永超, 李宜雄. 胰腺癌外科治疗的历史和现状[J]. *中国普通外科杂志*, 2018, 27(3): 269–283. doi: 10.3978/j.issn.1005-6947.2018.03.002.
- [5] Yang YC, Li YY. The surgical treatment of pancreatic cancer: history and present state[J]. *Chinese Journal of General Surgery*, 2018, 27(3):269–283. doi:10.3978/j.issn.1005-6947.2018.03.002.
- [6] Strobel O, Neoptolemos J, Jaeger D, et al. Optimizing the outcomes of pancreatic cancer surgery[J]. *Nat Rev Clin Oncol*, 2019, 16(1): 11–26. doi: 10.1038/s41571-018-0112-1.
- [7] Fernandes JCR, Acuña SM, Aoki JI, et al. Long non-coding RNAs in the regulation of gene expression: physiology and disease[J]. *Noncoding RNA*, 2019, 5(1):17. doi: 10.3390/ncrna5010017.
- [8] de Oliveira J C, Oliveira L C, Mathias C, et al. Long non-coding RNAs in cancer: Another layer of complexity[J]. *J Gene Med*, 2019, 21(1):e3065. doi: 10.1002/jgm.3065.
- [9] 周京涛, 刘佳, 努尔买买提·阿米都拉, 等. 上调 lncRNA SNHG12 与 miR-199a-5p/FZD6 轴对肝细胞癌细胞增殖、侵袭和上皮-间质转化的影响[J]. *中国普通外科杂志*, 2021, 30(2):173–186. doi: 10.7659/j.issn.1005-6947.2021.02.007.
- [10] Zhou JT, Liu J, Nuermaiti·AMD, et al. Influence of lncRNA SNHG12 up-regulation and miR-199a-5p/FZD6 axis on proliferation, invasion and epithelial-mesenchymal transition of hepatocellular carcinoma cells[J]. *Chinese Journal of General Surgery* 2021, 30(2): 173–186. doi: 10.7659/j.issn.1005-6947.2021.02.007.
- [11] Lin X, Zhuang S, Chen X, et al. LncRNA ITGB8-AS1 functions as a ceRNA to promote colorectal cancer growth and migration through integrin-mediated focal adhesion signaling[J]. *Mol Ther*, 2021, S1525-0016(21)00405-6. doi: 10.1016/j.ymthe.2021.08.011. [Online ahead of print]
- [12] Li K, Yao T, Zhang Y, et al. NEAT1 as a competing endogenous RNA in tumorigenesis of various cancers: Role, mechanism and therapeutic potential[J]. *Int J Biol Sci*, 2021, 17(13): 3428–3440. doi: 10.7150/ijbs.62728.
- [13] Wang Y, Ma M, Li C, et al. GAS6-AS1 overexpression increases GIMAP6 expression and inhibits lung adenocarcinoma progression by sponging miR-24-3p[J]. *Front Oncol*, 2021, 11: 645771. doi: 10.3389/fonc.2021.645771.
- [14] Liu Z, Lu C, Hu H, et al. LINC00909 promotes tumor progression in human glioma through regulation of miR-194/MUC1-C axis[J]. *Biomed Pharmacother*, 2019, 116: 108965. doi: 10.1016/j.biopha.2019.108965.
- [15] Ma L, Wang YY, Jiang P. LncRNA LINC00909 promotes cell proliferation and metastasis in pediatric acute myeloid leukemia via miR-625-mediated modulation of Wnt/ β -catenin signaling[J]. *Biochem Biophys Res Commun*, 2020, 527(3): 654–661. doi: 10.1016/j.bbrc.2020.05.001.
- [16] Cheng L, Han T, Zhang Z, et al. Identification and Validation of Six Autophagy-related Long Non-coding RNAs as Prognostic Signature in Colorectal Cancer[J]. *Int J Med Sci*, 2021, 18(1):88–98. doi: 10.7150/ijms.49449.
- [17] 倪志强, 王永恒, 彭书旺, 等. LINC00909 靶向 miR-365a-5p/FGFBP1 分子轴调控结肠癌细胞增殖、迁移及侵袭的实验研究[J]. *中国肿瘤外科杂志*, 2021, 13(2):152–159. doi:10.3969/j.issn.1674-4136.2021.02.010.
- [18] Ni ZQ, Wang YH, Peng SW, et al. LINC00909 regulates the proliferation, migration and invasion of colon cancer cells by targeting miR-365a-5p/FGFBP1 molecular axis[J]. *Chinese Journal of Surgical Oncology*, 2021, 13(2): 152–159. doi: 10.3969/j.issn.1674-4136.2021.02.010.
- [19] Shen J, Hong L, Yu D, et al. LncRNA XIST promotes pancreatic cancer migration, invasion and EMT by sponging miR-429 to modulate ZEB1 expression[J]. *Int J Biochem Cell Biol*, 2019, 113: 17–26. doi: 10.1016/j.biocel.2019.05.021.

- [16] Luo Y, Wang Q, Teng L, et al. LncRNA DANCR promotes proliferation and metastasis in pancreatic cancer by regulating miRNA-33b[J]. FEBS Open Bio, 2020, 10(1):18-27. doi: [10.1002/2211-5463.12732](https://doi.org/10.1002/2211-5463.12732).
- [17] Cao W, Zhou G. LncRNA SNHG12 contributes proliferation, invasion and epithelial-mesenchymal transition of pancreatic cancer cells by absorbing miRNA-320b[J]. Biosci Rep, 2020, 40(6): BSR20200805. doi: [10.1042/BSR20200805](https://doi.org/10.1042/BSR20200805).
- [18] Li N, Yang G, Luo L, et al. LncRNA THAP9-AS1 promotes pancreatic ductal adenocarcinoma growth and leads to a poor clinical outcome via sponging miR-484 and interacting with YAP[J]. Clin Cancer Res, 2020, 26(7):1736-1748. doi: [10.1158/1078-0432.CCR-19-0674](https://doi.org/10.1158/1078-0432.CCR-19-0674).
- [19] Ren X, Chen C, Luo Y, et al. lncRNA-PLACT1 sustains activation of NF- κ B pathway through a positive feedback loop with I κ B α /E2F1 axis in pancreatic cancer[J]. Mol Cancer, 2020, 19(1):35. doi: [10.1186/s12943-020-01153-1](https://doi.org/10.1186/s12943-020-01153-1).
- [20] Shi W, Zhang C, Ning Z, et al. Long non-coding RNA LINC00346 promotes pancreatic cancer growth and gemcitabine resistance by sponging miR-188-3p to derepress BRD4 expression[J]. J Exp Clin Cancer Res, 2019, 38(1):60. doi: [10.1186/s13046-019-1055-9](https://doi.org/10.1186/s13046-019-1055-9).
- [21] Peng WX, He RZ, Zhang Z, et al. LINC00346 promotes pancreatic cancer progression through the CTCF-mediated Myc transcription[J]. Oncogene, 2019, 38(41): 6770-6780. doi: [10.1038/s41388-019-0918-z](https://doi.org/10.1038/s41388-019-0918-z).
- [22] Pan S, Shen M, Zhou M, et al. Long noncoding RNA LINC01111 suppresses pancreatic cancer aggressiveness by regulating DUSP1 expression via microRNA-3924[J]. Cell Death Dis, 2019, 10(12): 883. doi: [10.1038/s41419-019-2123-y](https://doi.org/10.1038/s41419-019-2123-y).
- [23] Yan J, Jia Y, Chen H, et al. Long non-coding RNA PXN-AS1 suppresses pancreatic cancer progression by acting as a competing endogenous RNA of miR-3064 to upregulate PIP4K2B expression[J]. J Exp Clin Cancer Res, 2019, 38(1):390. doi: [10.1186/s13046-019-1379-5](https://doi.org/10.1186/s13046-019-1379-5).
- [24] Yue L, Guo J. LncRNA TUSC7 suppresses pancreatic carcinoma progression by modulating miR-371a-5p expression[J]. J Cell Physiol, 2019, 234(9):15911-15921. doi: [10.1002/jcp.28248](https://doi.org/10.1002/jcp.28248).
- [25] Tam C, Wong JH, Tsui SKW, et al. LncRNAs with miRNAs in regulation of gastric, liver, and colorectal cancers: updates in recent years[J]. Appl Microbiol Biotechnol, 2019, 103(12): 4649-4677. doi: [10.1007/s00253-019-09837-5](https://doi.org/10.1007/s00253-019-09837-5).
- [26] Tang XJ, Wang W, Hann SS. Interactions among lncRNAs, miRNAs and mRNA in colorectal cancer[J]. Biochimie, 2019, 163: 58-72. doi: [10.1016/j.biochi.2019.05.010](https://doi.org/10.1016/j.biochi.2019.05.010).
- [27] Wang J, Yang Y, Ma Y, et al. Potential regulatory role of lncRNA-miRNA-mRNA axis in osteosarcoma[J]. Biomed Pharmacother, 2020, 121:109627. doi: [10.1016/j.biopha.2019.109627](https://doi.org/10.1016/j.biopha.2019.109627).
- [28] Hu X, Zhang L, Li Y, et al. Organoid modelling identifies that DACH1 functions as a tumour promoter in colorectal cancer by modulating BMP signalling[J]. EBioMedicine, 2020, 56: 102800. doi: [10.1016/j.ebiom.2020.102800](https://doi.org/10.1016/j.ebiom.2020.102800).
- [29] Yu J, Jiang P, Zhao K, et al. Role of DACH1 on Proliferation, Invasion, and Apoptosis in Human Lung Adenocarcinoma Cells[J]. Curr Mol Med, 2021, doi: [10.2174/1566524021666210119094633](https://doi.org/10.2174/1566524021666210119094633). [Online ahead of print]
- [30] Zhou Q, Li W, Kong D, et al. DACH1 suppresses epithelial to mesenchymal transition (EMT) through Notch1 pathway and reverses progesterin resistance in endometrial carcinoma[J]. Cancer Med, 2019, 8(9):4380-4388. doi: [10.1002/cam4.2317](https://doi.org/10.1002/cam4.2317).
- [31] Yu S, Yi M, Xu L, et al. CXCL1 as an Unfavorable prognosis factor negatively regulated by DACH1 in non-small cell lung cancer[J]. Front Oncol, 2020, 9:1515. doi: [10.3389/fonc.2019.01515](https://doi.org/10.3389/fonc.2019.01515).
- [32] Zhang J, Zhao C Y, Zhang S H, et al. Upregulation of miR-194 contributes to tumor growth and progression in pancreatic ductal adenocarcinoma[J]. Oncol Rep, 2014, 31(3): 1157-1164. doi: [10.3892/or.2013.2960](https://doi.org/10.3892/or.2013.2960).

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