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CircACAP2调节miR-139-5p/HOXA9信号轴对脑胶质瘤细胞增殖、迁移和侵袭的影响

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摘要　该文探究CircACAP2调节微小RNA-139-5p(miR-139-5p)/同源盒基因A9(HOXA9)信号轴对脑胶质瘤细胞增殖、迁移和侵袭的影响。收集2020年12月~2024年12月在青岛市胶州中心医院进行治疗的150例脑胶质瘤组织以及150例因为外伤等原因进行颅内减压术患者的正常脑组织, 并以脑胶质瘤细胞系SW1088为研究对象, 分为CK组、沉默-NC组、沉默-CircACAP2组、miR-NC组、miR-139-5p mimic组、沉默-CircACAP2+inhibitor NC组、沉默-CircACAP2+miR-139-5p inhibitor组。qRT-PCR检测组织及SW1088细胞中CircACAP2、miR-139-5p和*HOXA9* mRNA表达情况; CCK-8法、划痕实验、Transwell法、Western blot法分别检测细胞增殖、迁移、侵袭相关蛋白表达情况; 双荧光素酶实验验证miR-139-5p与CircACAP2、HOXA9靶向关系。结果显示, 脑胶质瘤组织相比于正常脑组织CircACAP2、*HOXA9* mRNA、HOXA9蛋白表达水平升高, miR-139-5p表达水平降低(P<0.05)。沉默-CircACAP2组相比于CK组、沉默-NC组的CircACAP2表达水平、HOXA9表达水平、细胞存活率、划痕愈合率、侵袭细胞数, 以及Ki-67和Vimentin蛋白表达水平降低, miR-139-5p表达水平升高(P<0.05); miR-139-5p mimic组相比于CK组、miR-NC组HOXA9表达水平、细胞存活率、划痕愈合率、侵袭细胞数, 以及Ki-67和Vimentin蛋白表达水平降低, miR-139-5p表达水平升高(P<0.05); 沉默-CircACAP2+miR-139-5p inhibitor组相比于沉默-CircACAP2组、沉默-CircACAP2+inhibitor NC组miR-139-5p表达水平降低, *HOXA9* mRNA表达水平、细胞存活率、划痕愈合率、侵袭细胞数, 以及Ki-67、Vimentin、HOXA9蛋白表达水平升高(P<0.05); CircACAP2和HOXA9分别与miR-139-5p存在靶向关系。结果表明, 沉默CircACAP2可上调miR-139-5p的表达, 进而抑制HOXA9的表达, 从而抑制脑胶质瘤细胞增殖、迁移和侵袭。

关键词　CircACAP2; miR-139-5p/HOXA9信号轴; 脑胶质瘤; 增殖; 迁移; 侵袭

Impacts of CircACAP2 on the Proliferation, Migration, and Invasion of Glioma Cells by Regulating miR-139-5p/HOXA9 Signaling Axis

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Abstract　This study aims to investigate the effects of CircACAP2 on the proliferation, migration, and invasion of glioma cells by regulating the miR-139-5p (microRNA-139-5p)/HOXA9 (homeobox gene A9) signaling axis. From December 2020 to December 2024, 150 cases of glioma tissue of patients and 150 cases of normal brain tissue from patients who underwent intracranial decompression surgery due to trauma and other reasons were collected in the hospital. The glioma cell line SW1088 was used as the research object and classified into CK

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group, silencing-NC group, silencing-CircACAP2 group, miR-NC group, miR-139-5p mimic group, silencing-CircACAP2+inhibitor NC group, and silencing-CircACAP2+miR-139-5p inhibitor group. qRT-PCR was used to detect the CircACAP2, miR-139-5p, and *HOXA9* mRNA in tissues and SW1088 cells. CCK-8 method, scratch assay, Transwell method, and Western blot method were used to detect cell proliferation, migration, invasion, and related proteins, respectively. The dual luciferase assay was used to verify the targeting relationship between miR-139-5p and CircACAP2, HOXA9. The results showed that the expression levels of CircACAP2, *HOXA9* mRNA, and HOXA9 protein were increased, while the expression level of miR-139-5p was decreased in glioma tissues compared with normal brain tissues ($P<0.05$). Compared with the CK group and silencing-NC group, the CircACAP2 group showed decreased CircACAP2, HOXA9, cell survival rate, scratch healing rate, number of invasive cells, the expression of Ki-67, and Vimentin proteins, and increased miR-139-5p ($P<0.05$). Compared with the CK group and miR-NC group, the miR-139-5p mimic group showed decreased HOXA9, cell survival rate, scratch healing rate, number of invasive cells, the expression of Ki-67, and Vimentin proteins, and increased miR-139-5p ($P<0.05$). Compared with the silencing-CircACAP2+miR-139-5p inhibitor group and silencing-CircACAP2+inhibitor NC group, the silencing-CircACAP2+miR-139-5p inhibitor group showed reduced miR-139-5p, increased *HOXA9* mRNA, cell survival rate, scratch healing rate, number of invasive cells, the expression of Ki-67, Vimentin, and HOXA9 proteins ($P<0.05$). CircACAP2 and *HOXA9* had targeted relationships with miR-139-5p, respectively. The results indicated that silencing CircACAP2 could up-regulate the expression of miR-139-5p, which in turn inhibited the expression of HOXA9, thereby suppressing the proliferation, migration, and invasion of glioma cells.

Keywords CircACAP2; miR-139-5p/HOXA9 signaling axis; glioma; proliferation; migration; invasion

脑胶质瘤是一类起源于神经上皮组织的肿瘤, 在中枢神经系统及原发性脑肿瘤中极为常见, 其发病率居高不下, 对人类健康造成严重威胁[1]。目前, 脑胶质瘤的临床治疗方法主要依赖于手术切除, 但是该疾病具有极强的侵袭性, 极易向周围组织浸润, 若手术过程中肿瘤组织未被彻底切除, 会进一步增大复发风险; 化疗也是常用的治疗手段, 但是会产生一定的副作用[2]。尽管近年来关于脑胶质瘤的诊断及治疗都取得了一定的突破, 但其患者5年生存率仍处于较低水平[3]。因此, 对脑胶质瘤发生、发展进程的分子机制进行深入研究, 积极寻找有效治疗靶点至关重要。环状RNA(circular RNA, circRNA)拥有共价闭环结构, 属于非编码RNA, 在基因调控、细胞分化等多种生物过程中发挥作用[4]。CircACAP2属于circRNA的一种, 已有相关研究报道, CircACAP2影响胃癌细胞的增殖及凋亡[5]。但CircACAP2是否影响脑胶质瘤并不十分清楚。微小RNA(microRNA, miRNA)具有关键生物功能, 其表达变化会影响癌症进展, 既可作为促癌因素, 也可作为抑癌因素。已有报道称, 微小RNA-139-5p(microRNA-139-5p, miR-139-5p)参与神经胶质瘤的进展过程[6]。同源盒基因A9(homeobox A9, HOXA9)在乳腺癌组织中表达水平呈现升高的趋势[7]。网站预测CircACAP2、*HOXA9*和miR-139-5p之间存在结合位点, 但CircACAP2是否通过调控miR-139-5p/HOXA9信号轴对脑胶质瘤细胞增殖、迁移和侵袭产生影响尚未明确。因此本研究旨在探究CircACAP2调节miR-139-5p/HOXA9信号轴对脑胶质瘤细胞增殖、迁移和侵袭的影响。

1 材料与方法

1.1 组织与细胞

收集2020年12月~2024年12月在青岛市胶州中心医院进行治疗的150例脑胶质瘤患者组织以及150例因为外伤等原因进行颅内减压术的患者正常脑组织, 本研究经青岛市胶州中心医院伦理委员会审核批准, 批准号: 胶中伦审论文第(20240816-009)号。脑胶质瘤细胞系SW1088购自上海盈湾生物科技有限公司, 货号: C1332。

1.2 主要试剂与仪器

RPMI-1640培养基(货号: XB003)购自广东环凯生物科技有限公司; qRT-PCR试剂盒(货号: YLK-PCR0126)购自优利科(东莞)生物科技有限公司; CCK-8试剂盒(货号: CA1210-5000T)购自上海吉至生化科技有限公司; Ki-67(货号: ab16667)、Vimentin(货

号: ab8978)、HOXA9(货号: ab152453)、GAPDH(货号: ab181602)抗体及IgG二抗(货号: ab205718)购自英国Abcam公司; qRT-PCR仪(型号: qTOWER3G touch)购自德国Analytik Jena AG公司; 酶标仪(型号: CLARIOstar Plus)购自北京融京科技发展有限公司; 凝胶成像系统(型号: OI1000)购自山西光仪科技有限公司; 显微镜(型号: IX53)购自日本Olympus公司。

1.3 方法

1.3.1 细胞培养 将SW1088细胞采用添加10%胎牛血清(fetal bovine serum, FBS)的RPMI-1640培养基进行培养, 待细胞生长至对数期, 将其接种到6孔板(每孔5×10^5个细胞), 之后继续培养24 h, 收集细胞用于后续实验。

1.3.2 细胞转染与分组 收集处于对数生长期的SW1088细胞, 进行不同的转染和分组, 共分为以下几组, (1) CK组: 不进行转染处理, 作空白对照; (2) 沉默-NC组: 向SW1088细胞中转染si-NC, 作转染操作及非特异性干扰的对照; (3) 沉默-CircACAP2组: 向SW1088细胞中转染si-CircACAP2, 研究下调CircACAP2对SW1088细胞的影响; (4) miR-NC组: 向SW1088细胞中转染miR-NC, 作miR-139-5p mimic转染的对照; (5) miR-139-5p mimic组: 向SW1088细胞中转染miR-139-5p mimic, 研究过表达miR-139-5p对SW1088细胞的影响; (6) 沉默-CircACAP2+inhibitor NC组: 向SW1088细胞中共转染si-CircACAP2和inhibitor NC, 排除共转染过程及非特异性抑制剂对细胞的影响; (7) 沉默-CircACAP2+miR-139-5p inhibitor组: 向SW1088细胞中共转染si-CircACAP2和miR-139-5p inhibitor, 分析CircACAP2和miR-139-5p之间可能存在的调控机制。进行后续实验。

1.3.3 qRT-PCR检测组织及SW1088细胞中CircACAP2、miR-139-5p和*HOXA9* mRNA表达情况 收集组织及培养的SW1088细胞, 借助Trizol试剂提取总RNA, 在反转录试剂盒作用下得到cDNA, 以此为模板开展qRT-PCR扩增实验。分别以*U6*和*GAPDH*作为内参, 运用$2^{-\Delta\Delta Ct}$法计算相对表达量。引物如表1。

1.3.4 CCK-8法检测细胞增殖 将经分组处理后的细胞以每孔1×10^4个细胞接种于96孔板, 培养48 h后, 每孔加入10 μL CCK-8溶液, 反应120 min后, 使用酶标仪检测$D_{450\,nm}$, 并对细胞存活率进行计算。

1.3.5 划痕实验检测细胞迁移 收集各组细胞接种至6孔板(每孔1×10^6个细胞), 待细胞贴壁后, 使用无菌枪头垂直于孔板底部表面, 划出宽度均匀的划痕。在显微镜下对划痕区域进行拍照, 作为0 h的初始数据。随后, 吸去原有培养基, 加入不含血清培养基, 继续培养24 h。在相同显微镜参数下观察划痕区域, 拍照并记录, 即24 h结果。对0 h和24 h的划痕宽度进行对比, 评估细胞的迁移能力。

1.3.6 Transwell法检测细胞侵袭 先在Transwell上室加入基质胶, 取不同组别的细胞分别接种到Transwell上室(5×10^4/mL), Transwell下室添加含有血清的培养基(600 μL), 培养24 h后, 25 ℃条件下多聚甲醛固定20 min、0.1%结晶紫染色15 min。随机选取不同的视野拍照并统计侵袭细胞数量。

1.3.7 Western blot检测相关蛋白表达 收集各组细胞, 使用RIPA试剂提取细胞内总蛋白, BCA试剂盒检测蛋白浓度, 之后进行电泳、转膜、封闭。分别添加一抗Ki-67(1:1 000)、Vimentin(1:1 000)、HOXA9(1:1 000)、GAPDH(1:5 000), 4 ℃摇床孵育过夜, 之后洗膜, 加二抗(1:5 000), 37 ℃孵育2 h。运用Image Lab™软件定量蛋白条带。

1.3.8 双荧光素酶报告基因检测 借助生物信息学手段, 对CircACAP2、*HOXA9*和miR-139-5p之间的结合位点展开分析, 随后构建CircACAP2及HOXA9野生型(WT)、突变型(MUT)载体(WT型载

表1 qRT-PCR引物序列

Table 1 qRT-PCR primer sequences

基因 Gene	上游引物(5′→3′) Forward primer (5′→3′)	下游引物(5′→3′) Reverse primer (5′→3′)
miR-139-5p	ACA CTC CAG CTG GGT CTA CAG TGC ACG TGT C	TGG TGT CGT GGA GTC G
U6	GTG CAG GGT CCG AGG T	CTC GCT TCG GCA GCA CA
CircACAP2	GAA TGG GAT TCG AGA CCT G	TTC TTC CAA AGC TGC CTG T
HOXA9	GTG GTT CTC CTC CAG TTG ATA G	AGT TGG CTG CTG GGT TAT T
GAPDH	ACA GCA ACA GGG TGG TGG AC	AAC GCT TCA CGA ATT TGC GT

体包含预测的结合位点序列，MUT型载体结合位点序列被破坏），将构建好的载体分别与miR-NC和miR-139-5p mimic共转染至SW1088细胞(与miR-NC共转染组作为阴性对照，排除转染操作及非特异性干扰，与miR-139-5p mimic共转染组验证靶向结合效应)，转染操作完成48 h后，对细胞内的相对荧光素酶活性进行检测。

1.4 统计学分析

SPSS 25.0分析数据。数据表示为$\bar{x}\pm s$，两组数据比较用t检验，多组数据比较用单因素方差分析，两两比较用SNK-q检验。P<0.05表示差异有统计学意义。

2 结果

2.1 CircACAP2、miR-139-5p、HOXA9在组织中的表达

脑胶质瘤组织相比于正常脑组织，CircACAP2、*HOXA9* mRNA、HOXA9蛋白表达水平升高，miR-139-5p表达水平降低(P<0.05)。见图1和表2。

2.2 CircACAP2、miR-139-5p、*HOXA9* mRNA在SW1088细胞中的表达

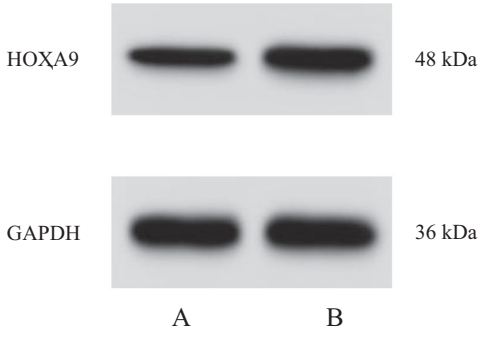
沉默-CircACAP2组相比于CK组、沉默-NC组CircACAP2、*HOXA9* mRNA表达水平降低，miR-139-5p表达水平升高(P<0.05)；miR-139-5p mimic组相比于CK组、miR-NC组*HOXA9* mRNA表达水平降低，miR-139-5p表达水平升高(P<0.05)；沉默-CircACAP2+miR-139-5p inhibitor组相比于沉默-CircACAP2组、沉默-CircACAP2+inhibitor NC组miR-139-5p表达水平降低，*HOXA9* mRNA表达水平升高(P<0.05)。见表3。

2.3 各组SW1088细胞增殖能力比较

沉默-CircACAP2组相比于CK组、沉默-NC组细胞存活率降低(P<0.05)；miR-139-5p mimic组相比于CK组、miR-NC组细胞存活率降低(P<0.05)；沉默-CircACAP2+miR-139-5p inhibitor组相比于沉默-CircACAP2组、沉默-CircACAP2+inhibitor NC组细胞存活率升高(P<0.05)。见表4。

2.4 各组SW1088细胞迁移能力比较

沉默-CircACAP2组相比于CK组、沉默-NC组划痕愈合率降低(P<0.05)；miR-139-5p mimic组相比于CK组、miR-NC组划痕愈合率降低(P<0.05)；沉默-CircACAP2+miR-139-5p inhibitor组相比于沉默-CircACAP2组、沉默-CircACAP2+inhibitor NC组划痕愈合率升高(P<0.05)。见图2和表5。



A: 正常脑组织; B: 脑胶质瘤组织。

A: normal brain tissue; B: glioma tissue.

图1 Western blot检测HOXA9蛋白表达

Fig.1 Western blot was used to detect the expression of HOXA9 proteins

表2 CircACAP2、miR-139-5p、HOXA9在组织中的表达

Table 2 The expression of CircACAP2, miR-139-5p, and HOXA9 in tissues

类别 Category	CircACAP2	miR-139-5p	*HOXA9* mRNA	HOXA9蛋白 HOXA9 protein
Normal brain tissue	1.03±0.13	0.98±0.10	1.01±0.11	0.56±0.07
Glioma tissue	1.65±0.18[a]	0.59±0.07[a]	1.46±0.16[a]	0.93±0.11[a]

$\bar{x}\pm s$; n=150; [a]P<0.05, 与正常脑组织相比。

$\bar{x}\pm s$; n=150; [a]P<0.05 compared with normal brain tissue.

2.5 各组SW1088细胞侵袭能力比较

沉默-CircACAP2组相比于CK组、沉默-NC组侵袭细胞数减少($P<0.05$); miR-139-5p mimic组相比于CK组、miR-NC组侵袭细胞数减少($P<0.05$); 沉默-CircACAP2+miR-139-5p inhibitor组相比于沉默-CircACAP2组、沉

表3 CircACAP2、miR-139-5p、*HOXA9* mRNA在SW1088细胞中的表达

Table 3 The expression of CircACAP2, miR-139-5p, and *HOXA9* mRNA in SW1088 cells

组别 Groups	CircACAP2	miR-139-5p	*HOXA9* mRNA
CK group	0.97±0.10	1.02±0.10	1.01±0.10
sh-NC group	1.01±0.12	1.05±0.11	1.04±0.12
sh-CircACAP2 group	0.53±0.06^{ab}	1.67±0.18^{ab}	0.67±0.08^{ab}
miR-NC group	0.98±0.11	1.03±0.11	1.03±0.11
miR-139-5p mimic group	0.99±0.11	1.36±0.15^{ac}	0.82±0.09^{ac}
sh-CircACAP2+inhibitor NC group	0.56±0.07	1.69±0.19	0.65±0.07
sh-CircACAP2+miR-139-5p inhibitor group	0.55±0.07	1.32±0.17^{de}	0.81±0.09^{de}

$\bar{x}\pm s$; n=6; $^{a}P<0.05$, 与CK组相比; $^{b}P<0.05$, 与沉默-NC组相比; $^{c}P<0.05$, 与miR-NC组相比; $^{d}P<0.05$, 与沉默-CircACAP2组相比; $^{e}P<0.05$, 与沉默-CircACAP2+inhibitor NC组相比。

$\bar{x}\pm s$; n=6; $^{a}P<0.05$ compared with CK group; $^{b}P<0.05$ compared with sh-NC group; $^{c}P<0.05$ compared with miR-NC group; $^{d}P<0.05$ compared with sh-CircACAP2 group; $^{e}P<0.05$ compared with sh-CircACAP2+inhibitor NC group.

表4 各组细胞存活率比较

Table 4 Comparison of cell viability among groups

组别 Groups	存活率/% Survival rate /%
CK group	100.00±0.00
sh-NC group	93.63±5.17
sh-CircACAP2 group	49.83±5.03^{ab}
miR-NC group	93.11±5.09
miR-139-5p mimic group	40.25±4.32^{ac}
sh-CircACAP2+inhibitor NC group	50.37±5.08
sh-CircACAP2+miR-139-5p inhibitor group	72.64±7.65^{de}

$\bar{x}\pm s$; n=6; $^{a}P<0.05$, 与CK组相比; $^{b}P<0.05$, 与沉默-NC组相比; $^{c}P<0.05$, 与miR-NC组相比; $^{d}P<0.05$, 与沉默-CircACAP2组相比; $^{e}P<0.05$, 与沉默-CircACAP2+inhibitor NC组相比。

$\bar{x}\pm s$; n=6; $^{a}P<0.05$ compared with CK group; $^{b}P<0.05$ compared with sh-NC group; $^{c}P<0.05$ compared with miR-NC group; $^{d}P<0.05$ compared with sh-CircACAP2 group; $^{e}P<0.05$ compared with sh-CircACAP2+inhibitor NC group.

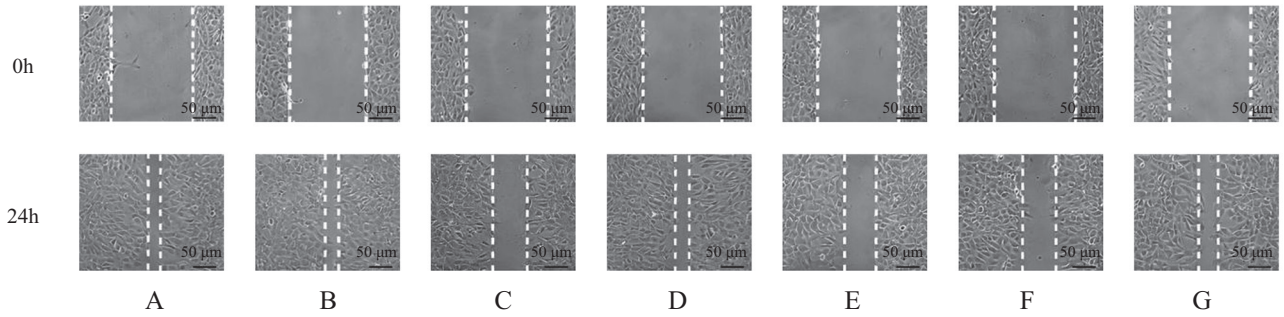
表5 各组细胞划痕愈合率比较

Table 5 Comparison of cell scratch wound healing rate among groups

组别 Groups	划痕愈合率/% Scratch wound healing rate /%
CK group	90.23±9.27
sh-NC group	91.05±9.35
sh-CircACAP2 group	62.37±6.41^{ab}
miR-NC group	92.01±9.46
miR-139-5p mimic group	61.27±6.32^{ac}
sh-CircACAP2+inhibitor NC group	66.78±6.87
sh-CircACAP2+miR-139-5p inhibitor group	79.56±8.11^{de}

$\bar{x}\pm s$; n=6; $^{a}P<0.05$, 与CK组相比; $^{b}P<0.05$, 与沉默-NC组相比; $^{c}P<0.05$, 与miR-NC组相比; $^{d}P<0.05$, 与沉默-CircACAP2组相比; $^{e}P<0.05$, 与沉默-CircACAP2+inhibitor NC组相比。

$\bar{x}\pm s$; n=6; $^{a}P<0.05$ compared with CK group; $^{b}P<0.05$ compared with sh-NC group; $^{c}P<0.05$ compared with miR-NC group; $^{d}P<0.05$ compared with sh-CircACAP2 group; $^{e}P<0.05$ compared with sh-CircACAP2+inhibitor NC group.



A: CK组; B: 沉默-NC组; C: 沉默-CircACAP2组; D: miR-NC组; E: miR-139-5p mimic组; F: 沉默-CircACAP2+inhibitor NC组; G: 沉默-CircACAP2+miR-139-5p inhibitor组。

A: CK group; B: sh-NC group; C: sh-CircACAP2 group; D: miR-NC group; E: miR-139-5p mimic group; F: sh-CircACAP2+inhibitor NC group; G: sh-CircACAP2+miR-139-5p inhibitor group.

图2 各组SW1088细胞迁移能力

Fig.2 Migration ability of SW1088 cells in each group

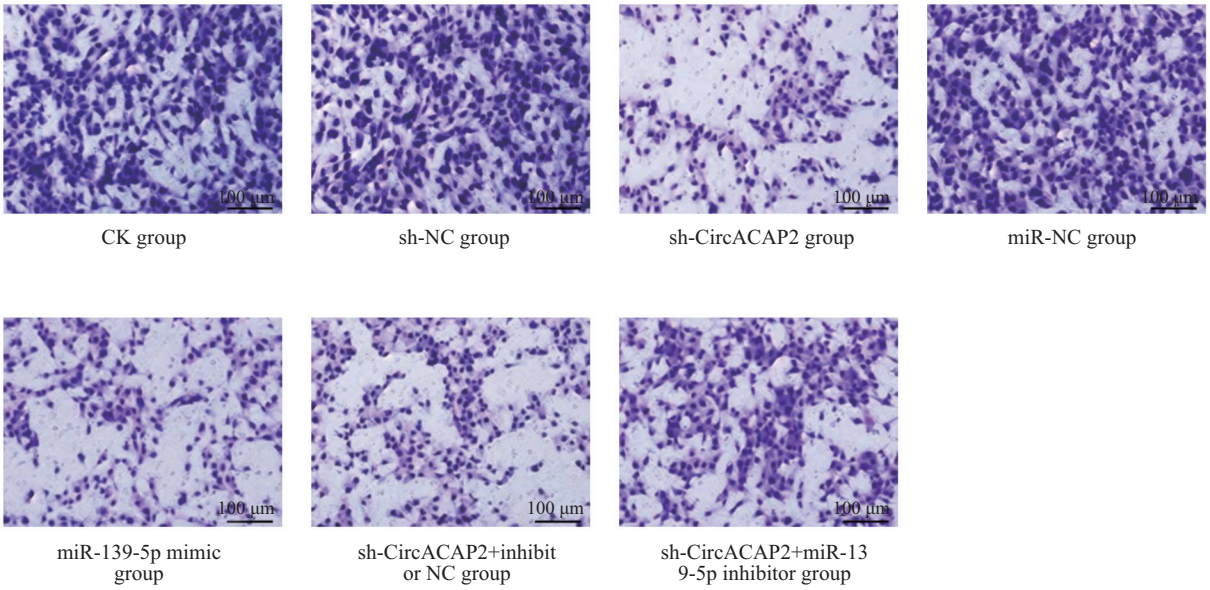


图3 各组SW1088细胞侵袭能力

Fig.3 Invasion ability of SW1088 cells in each group

默-CircACAP2+inhibitor NC组侵袭细胞数增加(P<0.05)。见图3和表6。

2.6 各组SW1088细胞相关蛋白表达

沉默-CircACAP2组相比于CK组、沉默-NC组Ki-67、Vimentin、HOXA9蛋白表达水平降低(P<0.05); miR-139-5p mimic组相比于CK组、miR-NC组Ki-67、Vimentin、HOXA9蛋白表达水平降低(P<0.05); 沉默-CircACAP2+miR-139-5p inhibitor组相比于沉默-CircACAP2组、沉默-CircACAP2+inhibitor NC组Ki-67、Vimentin、HOXA9蛋白表达水平升高(P<0.05)。见图4和表7。

2.7 miR-139-5p与CircACAP2和*HOXA9*的靶向关系验证

TargetScanHuman网站预测miR-139-5p分别与CircACAP2、*HOXA9*存在结合位点。见图5和图6。miR-139-5p mimic+CircACAP2-WT组较miR-NC+CircACAP2-WT组、miR-139-5p mimic+HOXA9-WT组较miR-NC+HOXA9-WT组相对荧光素酶活性降低(P<0.05); miR-139-5p mimic+CircACAP2-MUT组较miR-NC+CircACAP2-MUT组、miR-139-5p mimic+HOXA9-MUT组较miR-NC+HOXA9-MUT组相对荧光素酶活性无差异(P>0.05)。见表8和表9。

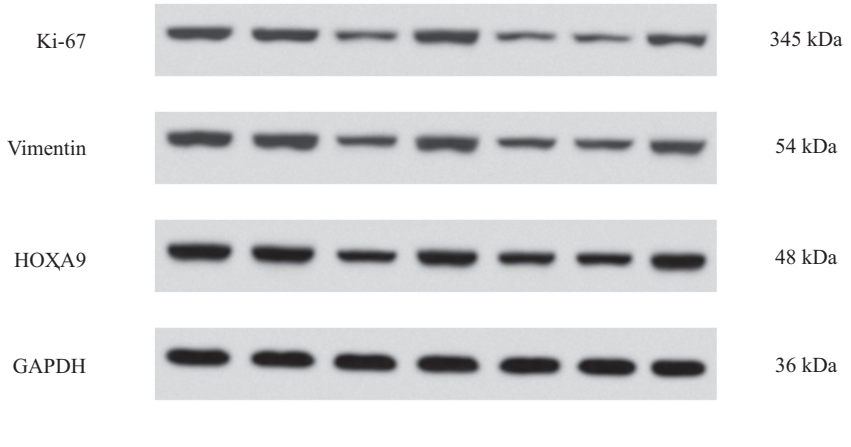
表6 各组细胞侵袭能力比较

Table 6 Comparison of cell invasion ability among groups

组别 Groups	侵袭细胞数 Number of invasive cells
CK group	162.35±17.25
sh-NC group	161.78±17.14
sh-CircACAP2 group	82.57±8.39[ab]
miR-NC group	156.37±16.77
miR-139-5p mimic group	81.06±8.34[ac]
sh-CircACAP2+inhibitor NC group	80.35±8.12
sh-CircACAP2+miR-139-5p inhibitor group	128.59±13.26[de]

$\bar{x}\pm s$; n=6; [a]P<0.05, 与CK组相比; [b]P<0.05, 与沉默-NC组相比; [c]P<0.05, 与miR-NC组相比; [d]P<0.05, 与沉默-CircACAP2组相比; [e]P<0.05, 与沉默-CircACAP2+inhibitor NC组相比。

$\bar{x}\pm s$; n=6; [a]P<0.05 compared with CK group; [b]P<0.05 compared with sh-NC group; [c]P<0.05 compared with miR-NC group; [d]P<0.05 compared with sh-CircACAP2 group; [e]P<0.05 compared with sh-CircACAP2+inhibitor NC group.



A: CK组; B: 沉默-NC组; C: 沉默-CircACAP2组; D: miR-NC组; E: miR-139-5p mimic组; F: 沉默-CircACAP2+inhibitor NC组; G: 沉默-CircACAP2+miR-139-5p inhibitor组。

A: CK group; B: sh-NC group; C: sh-CircACAP2 group; D: miR-NC group; E: miR-139-5p mimic group; F: sh-CircACAP2+inhibitor NC group; G: sh-CircACAP2+miR-139-5p inhibitor group.

图4 Western blot检测Ki-67、Vimentin、HOXA9蛋白表达情况

Fig.4 Western blot was used to detect the expression of Ki-67, Vimentin, and HOXA9 proteins

表7 各组细胞相关蛋白水平比较

Table 7 Comparison of related protein levels in cells among groups

组别 Groups	Ki-67/GAPDH	Vimentin/GAPDH	HOXA9/GAPDH
CK group	0.82±0.11	0.91±0.11	1.11±0.12
sh-NC group	0.79±0.08	0.92±0.10	1.13±0.13
sh-CircACAP2 group	0.37±0.04[ab]	0.45±0.06[ab]	0.57±0.06[ab]
miR-NC group	0.80±0.10	0.90±0.09	1.10±0.11
miR-139-5p mimic group	0.27±0.03[ac]	0.40±0.05[ac]	0.59±0.06[ac]
sh-CircACAP2+inhibitor NC group	0.31±0.04	0.43±0.05	0.58±0.07
sh-CircACAP2+miR-139-5p inhibitor group	0.60±0.07[de]	0.71±0.08[de]	0.94±0.10[de]

$\bar{x}\pm s$; n=6; [a]P<0.05, 与CK组相比; [b]P<0.05, 与沉默-NC组相比; [c]P<0.05, 与miR-NC组相比; [d]P<0.05, 与沉默-CircACAP2组相比; [e]P<0.05, 与沉默-CircACAP2+inhibitor NC组相比。

$\bar{x}\pm s$; n=6; [a]P<0.05 compared with CK group; [b]P<0.05 compared with sh-NC group; [c]P<0.05 compared with miR-NC group; [d]P<0.05 compared with sh-CircACAP2 group; [e]P<0.05 compared with sh-CircACAP2+inhibitor NC group.

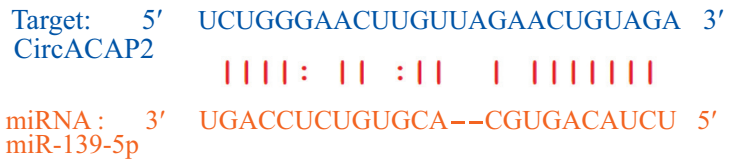


图5 预测miR-139-5p、CircACAP2结合位点

Fig.5 Prediction of binding sites between miR-139-5p and CircACAP2

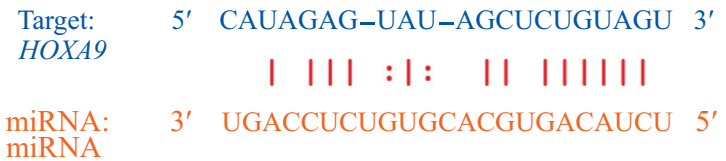


图6 预测miR-139-5p、*HOXA9*结合位点

Fig.6 Prediction of binding sites between miR-139-5p and *HOXA9*

表8 细胞中相对荧光素酶活性比较

Table 8 Comparison of relative luciferase activity in cells

组别 Groups	相对荧光素酶活性 Relative luciferase activity
miR-NC+CircACAP2-WT group	1.04±0.13
miR-139-5p mimic+CircACAP2-WT group	0.53±0.06[a]
miR-NC+CircACAP2-MUT group	1.01±0.12
miR-139-5p mimic+CircACAP2-MUT group	0.98±0.10

$\bar{x}\pm s$; n=6; [a]P<0.05, 与miR-NC+CircACAP2-WT组相比。

$\bar{x}\pm s$; n=6; [a]P<0.05 compared with miR-NC+CircACAP2-WT group.

表9 细胞中相对荧光素酶活性比较

Table 9 Comparison of relative luciferase activity in cells

组别 Groups	相对荧光素酶活性 Relative luciferase activity
miR-NC+HOXA9-WT group	1.02±0.11
miR-139-5p mimic+HOXA9-WT group	0.51±0.06[a]
miR-NC+HOXA9-MUT group	0.99±0.10
miR-139-5p mimic+HOXA9-MUT group	1.03±0.13

$\bar{x}\pm s$; n=6; [a]P<0.05, 与miR-NC+HOXA9-WT组相比。

$\bar{x}\pm s$; n=6; [a]P<0.05 compared with miR-NC+HOXA9-WT group.

3 讨论

脑胶质瘤作为一种侵袭性肿瘤，虽已开发有新的治疗方案，但其患者生存率仍处于较低水平[8]。目前，脑胶质瘤具有侵袭性较强、预后情况差以及患者生存期偏短等特点，因此，深入探究脑胶质瘤发生的潜在机制，对探寻有效的治疗靶点、提升其治疗效果意义重大[9]。

circRNA呈闭合环状，具有较高的稳定性与保守性，广泛存在于真核生物系统。大量研究表明，circRNA在肿瘤组织中存在异常表达，能够参与调控肿瘤细胞表型，进而对肿瘤的发生、发展过程产生影响[10-11]。CircACAP2参与多种肿瘤的进展过程，如在头颈部鳞状细胞癌中，CircACAP2可通过靶向miR-21-5p/STAT3信号轴，影响其上皮–间质转化，进而影响细胞生物学进展[12]。庄玉芬等[13]研究显示，CircACAP2表达水平在乳腺癌组织及患者血清中升高。CircACAP2可通过对miR-193a-5p/GPX4信号轴进行调控，影响宫颈癌恶性进展[14]。Ki-67的表达水平可直接反映出细胞的增殖活跃程度，其水平越高，说明细胞分裂越活跃[15]。Vimentin是发生上皮–间质转化的关键标志物，在癌症的转移中通过多种机制促进细胞的迁移与侵袭[16]。本研究结果显示，

脑胶质瘤组织较正常脑组织中CircACAP2表达水平升高。对CircACAP2进行沉默后发现, SW1088细胞存活率、划痕愈合率、侵袭细胞数, 以及Ki-67和Vimentin蛋白表达水平降低, 表明沉默CircACAP2会抑制SW1088细胞增殖、迁移和侵袭。

过往多项研究表明, 在多种肿瘤细胞(如前列腺癌[17]、非小细胞肺癌[18])中, miR-139-5p呈现异常表达状态。WANG等[19]研究发现, miR-139-5p在肝细胞癌中的表达相比于正常组织降低, 且与肝癌患者的预后密切相关。WANG等[20]研究发现, miR-139-5p卵巢癌中表达水平降低, 与肿瘤的分化以及临床分期有关。研究显示, HOXA9与宫颈癌的病变程度密切相关[21]。李妍等[22]研究发现, 下调circ-SFMBT2的表达, 可对miR-491-5p/HOXA9轴进行调控, 抑制急性髓系白血病的细胞增殖、迁移和侵袭。本研究结果显示, miR-139-5p表达在脑胶质瘤组织下调, HOXA9上调, 表明miR-139-5p、HOXA9参与脑胶质瘤发生。沉默CircACAP2后, miR-139-5p表达水平升高, HOXA9表达水平降低。过表达miR-139-5p后细胞存活率、划痕愈合率、侵袭细胞数, 以及Ki-67和Vimentin、HOXA9蛋白表达水平降低, 与沉默CircACAP2结果一致。同时沉默CircACAP2、miR-139-5p表达发现, 沉默miR-139-5p能够抵消部分沉默CircACAP2对SW1088细胞增殖、迁移、侵袭的影响。网站预测及实验结果显示, miR-139-5p与CircACAP2、*HOXA9*存在结合位点, 提示CircACAP2可能通过调节miR-139-5p/HOXA9信号轴, 进而影响脑胶质瘤细胞增殖、迁移和侵袭。

综上所述, 沉默CircACAP2可上调miR-139-5p的表达, 抑制HOXA9的表达, 进而抑制脑胶质瘤细胞增殖、迁移和侵袭。该研究为脑胶质瘤的治疗提供新的思路与方法, 不过目前仅停留在细胞层面, 后续将继续开展多层次、多维度的深入研究, 以实现更大突破。

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