

LncRNA DANCER 调节 miR-656/BMPRI1A 轴对脑胶质瘤细胞恶性行为的影响

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[摘 要] **目的:**探讨长链非编码 RNA (long non-coding RNA, LncRNA) 分化拮抗非蛋白编码 RNA (differentiation antagonizing non-protein coding RNA, DANCER) 调节微小 RNA-656 (miR-656)/骨形态发生蛋白受体 1A (bone morphogenetic protein receptor type 1A, BMPRI1A) 轴对脑胶质瘤细胞免疫微环境的影响。**方法:**实时荧光定量聚合酶链式反应 (quantitative real-time polymerase chain reaction, qRT-PCR) 检测脑胶质瘤细胞中 DANCER、miR-656、BMPRI1A 的表达水平。通过转染或共转染至 U87 细胞, 分为沉默 (short hairpin, sh)-DANCER 组、过表达 (plasmid cytome galoirus, pc)DNA 3.1 DANCER 组、阴性对照 (negative control, NC) sh 组、pcDNA 3.1 组、sh-DANCER + miR-656 抑制剂组、sh-DANCER + NC 抑制剂组、sh-DANCER + pcDNA 3.1 BMPRI1A 组、sh-DANCER + pcDNA 3.1 组, 并以未处理的 U87 细胞为空白组。CCK8 检测 U87 细胞增殖; Transwell 实验检测侵袭与迁移; 流式细胞仪检测细胞凋亡; qRT-PCR 检测细胞中 DANCER、miR-656、BMPRI1A mRNA 表达; DANCER 与 miR-656 靶向关系通过双荧光素酶验证; BMPRI1A 及免疫逃逸因子 [程序性死亡受体 1 (programmed death-1, PD-1) 和程序性死亡配体 1 (programmed death-ligand 1, PD-L1)] 通过 Western blot 检测。**结果:**U87、A172、LN229 和 U251 细胞较 NHA 细胞中 DANCER、BMPRI1A mRNA 表达显著增加, miR-656 表达显著降低 ($P < 0.05$); 与空白组、sh-DANCER 组相比, sh-DANCER 组 U87 细胞增殖率, 侵袭、迁移数, DANCER、BMPRI1A mRNA、BMPRI1A、PD-1、PD-L1 表达显著降低, 凋亡率、miR-656 表达显著增加 ($P < 0.05$); 与 pcDNA 3.1 组相比, pcDNA 3.1 DANCER 组 U87 细胞增殖率, 侵袭、迁移数, DANCER、BMPRI1A mRNA、BMPRI1A、PD-1、PD-L1 表达显著增加, 凋亡率、miR-656 表达显著降低 ($P < 0.05$); 与 sh-DANCER + NC 抑制剂组相比, sh-DANCER + miR-656 抑制剂组 U87 细胞增殖率, 侵袭、迁移数, BMPRI1A mRNA、BMPRI1A、PD-1、PD-L1 表达显著增加, 凋亡率、miR-656 表达显著降低 ($P < 0.05$), DANCER 表达的差异无统计学意义 ($P > 0.05$); 与 sh-DANCER + pcDNA 3.1 组相比, sh-DANCER + pcDNA 3.1 BMPRI1A 组 U87 细胞增殖率, 侵袭、迁移数, BMPRI1A mRNA、BMPRI1A、PD-1、PD-L1 表达显著增加, 凋亡率显著降低 ($P < 0.05$), miR-656 表达、DANCER 表达差异无统计学意义 ($P > 0.05$)。DANCER、miR-656 具有靶向负调节关系。**结论:**LncRNA DANCER 调节 miR-656/BMPRI1A 轴改善脑胶质瘤细胞免疫微环境, 抑制癌细胞恶性行为发展。

[关键词] LncRNA DANCER; miR-656/BMPRI1A 轴; 脑胶质瘤细胞; 免疫微环境; 恶性行为发展

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Effect of LncRNA DANCER on the immune microenvironment of glioma cells by regulating the miR-656/BMPRI1A axis

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ABSTRACT Objective: To investigate the effect of long non-coding RNA (LncRNA) differentiation antagonizing non-protein coding RNA (DANCER) on the immune microenvironment of glioma cells by regulating the miR-656/bone morphogenetic protein receptor type 1A (BMPRI1A) axis. **Methods:** The expression levels of DANCER, miR-656 and BMPRI1A in glioma cells were detected by quantitative real-time polymerase chain reaction (qRT-PCR). The U87 cells were transfected or co-transfected to form the following groups: sh-DANCER (transfected with sh-DANCER), overexpression (pcDNA 3.1) DANCER (transfected with pcDNA 3.1 DANCER), NC sh (transfected with negative control sh), pcDNA 3.1 (transfected with pcDNA 3.1 vector), sh-DANCER + miR-656 inhibitor (co-transfected with sh-DANCER and miR-656 inhibitor), sh-DANCER + NC inhibitor (co-transfected with sh-DANCER and NC inhibitor), sh-DANCER + pcDNA 3.1 BMPRI1A (co-transfected with sh-DANCER and pcDNA 3.1 BMPRI1A), and sh-DANCER + pcDNA 3.1 (co-transfected with sh-DANCER and pcDNA 3.1 BMPRI1A). The un-

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treated U87 cells were used as the blank group. The proliferation of U87 cells was detected by CCK-8; invasion and migration were detected by Transwell assay; apoptosis was detected by flow cytometry; the expression of DANCER, miR-656, and BMPR1A mRNA in cells was detected by qRT-PCR; the targeting relationship between DANCER and miR-656 was verified by dual-luciferase; and BMPR1A and immune escape factors [programmed death receptor 1 (PD-1) and programmed death-ligand 1 (PD-L1)] were detected by Western blot. **Results:** The mRNA expressions of DANCER and BMPR1A in U87, A172, LN229 and U251 cells were significantly increased, while the expression of miR-656 was significantly decreased compared with those in NHA cells ($P < 0.05$). Compared with the blank group and sh-DANCER group, the proliferation rate, invasion, migration number, DANCER, BMPR1A mRNA, BMPR1A, PD-1, PD-L1 expression of U87 cells in the sh-DANCER group were obviously reduced, while the apoptosis rate and miR-656 expression were obviously increased ($P < 0.05$). Compared with the pcDNA 3.1 group, the proliferation rate, invasion, migration number, DANCER, BMPR1A mRNA, BMPR1A, PD-1, and PD-L1 expression of U87 cells in the pcDNA 3.1 DANCER group were obviously increased, while the apoptosis rate and miR-656 expression were obviously reduced ($P < 0.05$). Compared with the sh-DANCER + NC inhibitor group, the proliferation rate, invasion, migration number, BMPR1A mRNA, BMPR1A, PD-1, and PD-L1 expression of U87 cells in the sh-DANCER + miR-656 inhibitor group were obviously increased, and the apoptosis rate and miR-656 expression were obviously reduced ($P < 0.05$), while the expression of DANCER was not obvious ($P > 0.05$). Compared with the sh-DANCER + pcDNA 3.1 group, the proliferation rate, invasion, migration number, BMPR1A mRNA, BMPR1A, PD-1, and PD-L1 expression of U87 cells in the sh-DANCER + pcDNA 3.1 BMPR1A group obviously increased, and the apoptosis rate obviously decreased ($P < 0.05$), while here was no statistically obvious difference in miR-656 expression and DANCER expression ($P > 0.05$). DANCER and miR-656 had a targeted negative regulatory relationship. **Conclusion:** LncRNA DANCER improves the immune microenvironment of glioma cells and inhibits the malignant behavior development of cancer cells by regulating the miR-656/BMPR1A axis. **KEY WORDS** LncRNA DANCER; MiR-656/BMPR1A axis; Glioma cells; Immune microenvironment; Malignant behavior development

神经胶质瘤源于中枢神经系统,是全世界最常诊断的颅内恶性肿瘤,占原发性颅内肿瘤的75%以上,发病率、恶性程度较高且预后不良^[1-2]。手术切除、放射治疗和化学治疗是神经胶质瘤治疗方法,然而其临床结果仍不令人满意^[3]。因此了解胶质瘤进展的分子机制对该肿瘤的治疗意义重大。长链非编码RNA(long non-coding RNA,LncRNA)可调节某些肿瘤的进展和转移,包括神经胶质瘤^[4]。LncRNA分化拮抗非蛋白编码RNA(differentiation antagonizing non-protein coding RNA,DANCER)作为LncRNA家族成员,在多种癌症中高度表达,并与肿瘤进展呈正相关^[5]。Yu等^[6]研究表明,DANCER在胶质瘤细胞中高表达,且增殖水平与DANCER表达呈正相关,凋亡水平则呈负相关。微小RNA(miRNA)是一类约21~22 nt的非编码小RNA,可以靶向mRNA、识别mRNA 3'-非翻译区(untranslated region,UTR)的位点并调节其稳定性,与许多人类癌症有关,包括胶质瘤细胞^[7]。有学者发现,miR-656表达在神经胶质瘤细胞系和组织中显著下调,且与靶点骨形态发生蛋白受体1A(bone morphogenetic protein receptor type 1A,BMPR1A)表达呈负相关^[8]。但其miR-656/BMPR1A轴是否受DANCER调控进而影响神经胶质瘤发展尚未见报道,生物信息学预测LncRNA DANCER与miR-656存在靶向结合位点,故本研究旨在探究LncRNA DANCER调节miR-656/BMPR1A轴

在脑胶质瘤细胞中的作用机制。

1 材料与方法

1.1 主要材料与仪器

人神经胶质瘤细胞系(U87、A172、LN229和U251)和正常人星形胶质细胞(normal human astrocyte,NHA)购自美国ATCC(American Typer Culture Collection)细胞库,采用Roswell Park Memorial研究所-1640培养基[10%(体积分数)胎牛血清,双抗生素]在37℃和5%(体积分数)CO₂的培养箱中培养细胞。

cDNA合成试剂盒(批号:RR037A)购自日本Takara公司,沉默DANCER重组载体(sh-DANCER)、过表达DANCER重组载体(pcDNA 3.1-DANCER)、过表达BMPR1A重组载体(pcDNA 3.1-BMPR1A)、miR-656模拟物、miR-656抑制剂及其相应阴性对照[negative control(NC)sh、空载体pcDNA 3.1、NC抑制剂、NC模拟物,均购自中国上海基因制药有限公司,CCK-8试剂(批号:C0038)购自北京索莱宝科技有限公司,基质胶(批号:354234)购自美国BD Biosciences公司,结晶紫染色液(批号:C3886)及Annexin V-FITC/PI凋亡检测试剂盒(批号:K2350)购自美国Sigma-Aldrich公司,BMPR1A抗体(批号:ab219403)、程序性死亡受体1(programmed death-1,PD-1)抗体(批号:ab137116)及程序性死亡配体1

(programmed death-ligand 1, PD-L1) 抗体 (批号: ab237726) 均购自英国 Abcam 公司。

Synergy H1 全功能酶标仪购自美国伯腾仪器有限公司, 倒置显微镜购自日本 Olympus 公司, 流式细胞仪购自美国 Thermo Fisher Scientific 公司。

本研究开始前已经温州市人民医院伦理委员会审查批准 (伦理 KY-2025 01-022 号)。

1.2 实时荧光定量聚合酶链式反应 (quantitative real-time polymerase chain reaction, qRT-PCR) 检测细

胞中 DANCER、miR-656、BMPR1A 表达水平

使用 TRIzol 试剂从神经胶质瘤细胞系中提取总 RNA, 对 BMPR1A mRNA 和 DANCER 进行检测, 以 cDNA 合成 SuperMix 试剂盒将 mRNA 转录为 cDNA, 通过使用 qPCR Master 试剂盒进行实时 PCR 反应; 以 miRNA qPCR 试剂盒检测 miR-656 表达, 其中 U6 用作 miRNA 检测的内参照, β -actin 用作 mRNA 和 LncRNA 检测的内部对照, 采用 $2^{-\Delta\Delta Ct}$ 分析 DANCER、miR-656、BMPR1A 表达水平 (表 1)。

表 1 qRT-PCR 引物序列
Table 1 Sequence of qRT-PCR primers

Primer name	Orientation	Sequence(5' to 3')
DANCER	Forward	GCGCCACTATGTAGCGGGTT
	Reverse	TCAATGGCTTGTGCCTGTAGTT
miR-656	Forward	GTCAGAAAAATGGAGTAACCTTA
	Reverse	GTCAGAAAAATGGAGTAACCTTA
BMPR1A	Forward	TAGTTCGCTGAACCAATAAAGG
	Reverse	GTCAGAAAAATGGAGTAACCTTA
β -actin	Forward	GCTAATATCTATAATC
	Reverse	GAGGCTATCTTCATAGAT
U6	Forward	CTCGCTTCGGCAGCAC
	Reverse	AACGCTTCACGAATTTCGCT

qRT-PCR, quantitative real-time polymerase chain reaction; LncRNA, long non-coding RNA; DANCER, differentiation antagonizing non-protein coding RNA; BMPR1A, bone morphogenetic protein receptor type 1A.

1.3 细胞分组及处理

以 U87 细胞进行转染或共转染分组: 利用 Lipofectamine 3000 试剂将 sh-DANCER、pcDNA 3.1 DANCER、NC sh、pcDNA 3.1 质粒分别转染细胞, 并依次标记为 sh-DANCER 组、pcDNA 3.1 DANCER 组、NC sh 组、pcDNA 3.1 组; Lipofectamine 3000 试剂将 sh-DANCER 分别与 miR-656 抑制剂、NC 抑制剂、pcDNA 3.1、pcDNA 3.1 BMPR1A 共转染至细胞, 并依次标记为 sh-DANCER + miR-656 抑制剂组、sh-DANCER + NC 抑制剂组、sh-DANCER + pcDNA 3.1 BMPR1A 组、sh-DANCER + pcDNA 3.1 组, 以未处理的 U87 细胞为空白组, 48 h 后分析各组指标变化。

1.4 CCK-8 法检测细胞增殖

以每孔 2 000 个细胞接种在 96 孔板中并培养细胞, 在处理后的各组细胞中加入 CCK-8 试剂, Synergy H1 酶标仪检测 450 nm 波长下的光密度值并用于计算细胞增殖。

1.5 Transwell 实验检测细胞迁移、侵袭

使用涂有基质胶的 Transwell 系统进行侵入试验, 上室预先涂有基质胶并加入无血清培养基中 (5×10^5 个细胞), 含有 10% 胎牛血清 (fetal bovine

serum, FBS) 的培养基加入底部, 以诱导细胞侵袭, 24 h 后弃去培养基, 用于固定侵袭的细胞, 并用 0.5% (质量分数) 结晶紫染色。选择 5 个视野, 将侵袭的细胞在倒置显微镜下拍照计数。迁移实验除不涂基质胶, 其余同上。

1.6 流式细胞术检测细胞凋亡

转染后 48 h 收集细胞, 并用磷酸盐缓冲液重悬细胞, 避光条件下, 将约 10 μ L 染色溶液 (包括膜联蛋白 V-异硫氰酸荧光素、碘化丙锭) 滴入样品中并孵育, 流式细胞仪分析细胞凋亡率。

1.7 qRT-PCR 检测 U87 细胞中 DANCER、miR-656、BMPR1A 表达水平

检测方法同 1.3 小节。

1.8 验证 DANCER、miR-656 的靶向关系

将 DANCER 的片段 (包含与 miR-656 的相互作用位点) 克隆到 PmirGLO 载体中, 命名为 DANCER-野生型 (wild type, WT)。随后利用 Mut Express II 快速诱变试剂盒使结合位点发生突变, 并被插入到质粒中, 命名为 DANCER-突变型 (mutant type, MUT)。用 DANCER-MUT、DANCER-WT 以及等量的 NC mimics 和 miR-656 mimics 共转染细胞, 随后检测相对荧光

素酶活性。

1.9 Western blot 检测 BMPR1A、PD-1、PD-L1 蛋白表达水平

使用放射免疫沉淀法 (radio-immunoprecipitation assay,RIPA) 裂解缓冲液分离总蛋白,在 4 ℃ 下以 12 000 r/min 离心 20 min,收集上清液,使用二喹啉甲酸 (bicinchoninic acid,BCA) 方法估算总蛋白。蛋白质通过电泳分离后,转移到特定的膜上,膜中的蛋白质与 BMPR1A、PD-1、PD-L1 一抗在 4 ℃ 孵育过夜,将膜洗涤三次后,与辣根过氧化物酶 (horse radish peroxidase,HRP) 标记的二抗在室温下孵育。使用增强型化学发光 (enhanced chemiluminescence,ECL)显示蛋白质条带,并使用 ImageJ 软件定量分析条带强度。

表 2 比较细胞中 DANCER、miR-656、BMPR1A mRNA 的表达
Table 2 Comparison of mRNA expression of DANCER, miR-656 and BMPR1A in cells

Group	DANCER	miR-656	BMPR1A mRNA
NHA	0.94 ± 0.10	0.92 ± 0.10	1.02 ± 0.11
LN229	1.51 ± 0.16 *	0.66 ± 0.07 *	1.41 ± 0.15 *
U251	1.55 ± 0.16 *	0.62 ± 0.07 *	1.47 ± 0.15 *
A172	1.62 ± 0.17 *	0.58 ± 0.06 *	1.57 ± 0.16 *
U87	2.03 ± 0.21 *	0.42 ± 0.05 *	2.03 ± 0.21 *

Data are $\bar{x} \pm s$, $n = 6$. * $P < 0.05$, compared with NHA cells. DANCER, differentiation antagonizing non-protein coding RNA; BMPR1A, bone morphogenetic protein receptor type 1A; NHA, normal human astrocytes.

2.2 各组 U87 细胞中的细胞增殖率变化

与空白组、sh-DANCER 组相比,sh-DANCER 组 U87 细胞增殖率显著降低 ($P < 0.05$);与 pcDNA 3.1 组相比,pcDNA 3.1 DANCER 组增殖率显著增加 ($P < 0.05$);与 sh-DANCER + NC 抑制剂组相比,sh-DANCER + miR-656 抑制剂组增殖率显著增加 ($P < 0.05$);与 sh-DANCER + pcDNA 3.1 组相比,sh-DANCER + pcDNA 3.1 BMPR1A 组增殖率显著增加 ($P < 0.05$,表 3)。

2.3 各组 U87 细胞中侵袭、迁移变化

与空白组、sh-DANCER 组相比,sh-DANCER 组 U87 细胞侵袭、迁移数显著降低 ($P < 0.05$);与 pcDNA 3.1 组相比,pcDNA 3.1 DANCER 组侵袭、迁移数显著增加 ($P < 0.05$);与 sh-DANCER + NC 抑制剂组相比,sh-DANCER + miR-656 抑制剂组侵袭、迁移数显著增加 ($P < 0.05$);与 sh-DANCER + pcDNA 3.1 组相比,sh-DANCER + pcDNA 3.1 BMPR1A 组侵袭、迁移数显著增加 ($P < 0.05$,图 1、2,表 4)。

2.4 各组 U87 细胞中凋亡率变化

与空白组、sh-DANCER 组相比,sh-DANCER 组 U87 细胞凋亡率显著增加 ($P < 0.05$);与 pcDNA 3.1 组相比,pcDNA 3.1 DANCER 组凋亡率显著降低

1.10 统计学分析

采用 SPSS 26.0 统计学软件,符合正态分布且方差齐的计量资料以 $\bar{x} \pm s$ 表示,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 SNK- q 检验, $P < 0.05$ 认为差异有统计学意义。

2 结果

2.1 细胞 DANCER、miR-656、BMPR1A mRNA 表达水平

U87、A172、LN229 和 U251 细胞较 NHA 细胞中 DANCER、BMPR1A mRNA 表达显著增加,miR-656 表达显著降低 ($P < 0.05$,表 2)。鉴于 U87 细胞中以上三个基因的表达差异大,故作为本研究对象。

($P < 0.05$);与 sh-DANCER + NC 抑制剂组相比,sh-DANCER + miR-656 抑制剂组凋亡率显著降低 ($P < 0.05$);与 sh-DANCER + pcDNA 3.1 组相比,sh-DANCER + pcDNA 3.1 BMPR1A 组凋亡率显著降低 ($P < 0.05$,图 3、表 5)。

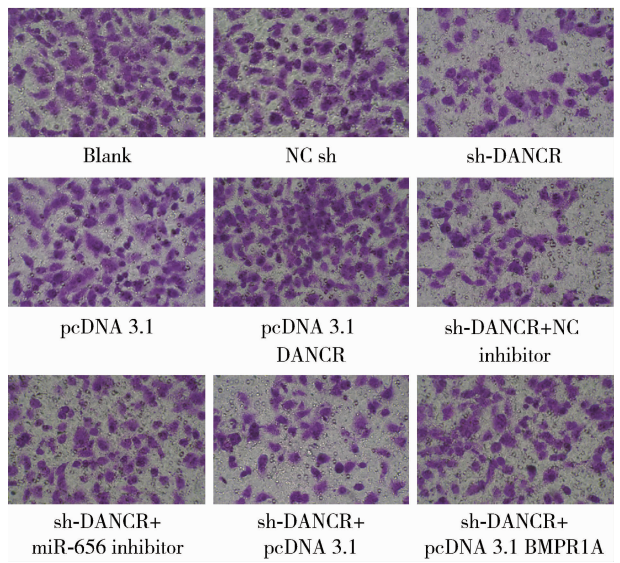
表 3 各组 U87 细胞增殖率的变化
Table 3 Changes in proliferation rate of U87 cells in each group

Group	Proliferation rate/%
Blank	94.22 ± 4.51
NC sh	94.11 ± 4.49
sh-DANCER	47.52 ± 4.82 ^{a,b}
pcDNA 3.1	94.24 ± 4.49
pcDNA 3.1 DANCER	125.34 ± 6.85 ^c
sh-DANCER + NC inhibitor	47.67 ± 4.79
sh-DANCER + miR-656 inhibitor	72.14 ± 7.31 ^d
sh-DANCER + pcDNA 3.1	47.72 ± 4.81
sh-DANCER + pcDNA 3.1 BMPR1A	72.54 ± 7.38 ^e

Data are $\bar{x} \pm s$, $n = 6$. a, $P < 0.05$, compared with the blank group; b, $P < 0.05$, compared with the NC sh group; c, $P < 0.05$, compared with pcDNA 3.1 group; d, $P < 0.05$, compared with the sh-DANCER + NC inhibitor group; e, $P < 0.05$, compared with the sh-DANCER + pcDNA 3.1 group. NC, negative control; sh, short hairpin; DANCER, differentiation antagonizing non-protein coding RNA; BMPR1A, bone morphogenetic protein receptor type 1A.

2.5 各组 U87 细胞中 DANCER、miR-656、BMPR1A mRNA 表达

与空白组、sh-DANCER 组相比, sh-DANCER 组 U87 细胞 DANCER、BMPR1A mRNA 表达显著降低, miR-656 表达显著增加 ($P < 0.05$); 与 pcDNA 3.1 组相比, pcDNA 3.1 DANCER 组 DANCER、BMPR1A mRNA 表达显著增加, miR-656 表达显著降低 ($P < 0.05$); 与 sh-DANCER + NC 抑制剂组相比, sh-DANCER + miR-656 抑制剂组 BMPR1A mRNA 表达显著增加, miR-656 表达显著降低 ($P < 0.05$), DANCER 表达差异无统计学意义 ($P > 0.05$); 与 sh-DANCER + pcDNA 3.1 组相比, sh-DANCER + pcDNA 3.1 BMPR1A 组 BMPR1A mRNA 表达显著增加, miR-656 表达、DANCER 表达差异无统计学意义 ($P > 0.05$, 表 6)。



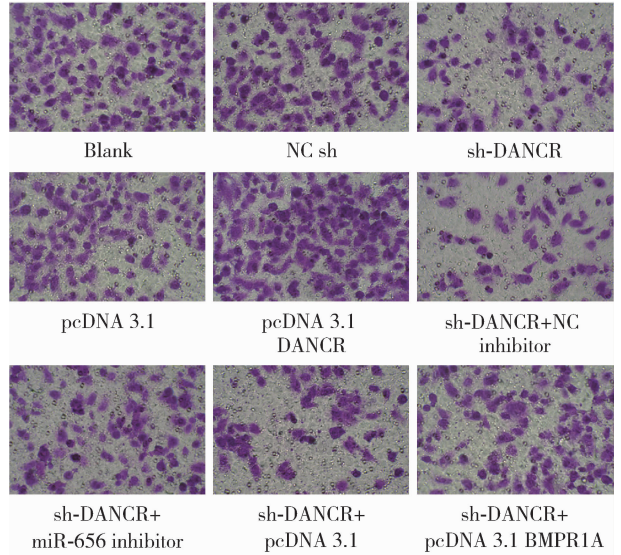
NC, negative control; sh, short hairpin; DANCER, differentiation antagonizing non-protein coding RNA; BMPR1A, bone morphogenetic protein receptor type 1A.

图 1 观察 U87 细胞侵袭变化

Figure 1 Observation of invasion changes of U87 cells

2.6 验证 DANCER、miR-656 的靶向关系

DANCER、miR-656 预测结合位点见图 4。共转染 miR-656 模拟物与 DANCER-WT 的荧光素酶活性较 NC 模拟物与 DANCER-WT 降低 ($P < 0.05$, 表 7)。



NC, negative control; sh, short hairpin; DANCER, differentiation antagonizing non-protein coding RNA; BMPR1A, bone morphogenetic protein receptor type 1A.

图 2 观察 U87 细胞迁移变化

Figure 2 Observation of migration changes of U87 cells

2.7 各组 U87 细胞中 BMPR1A、PD-1、PD-L1 蛋白表达水平

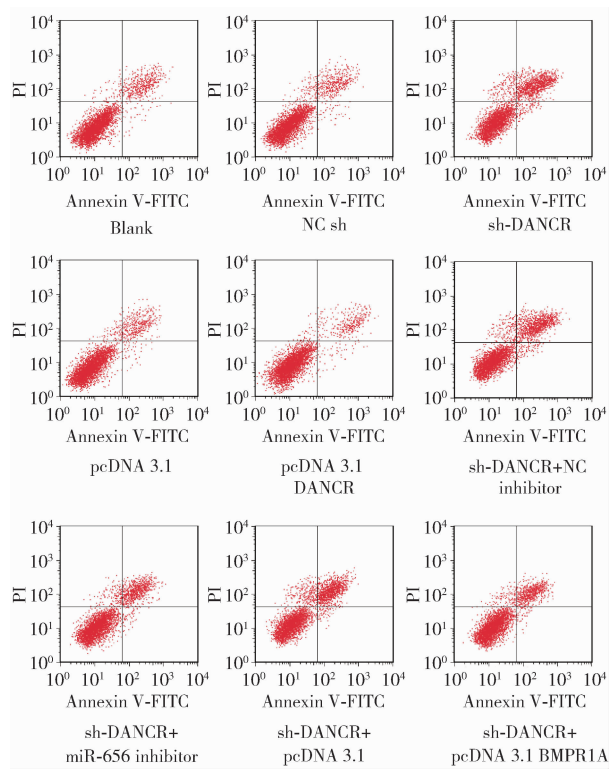
与空白组、sh-DANCER 组相比, sh-DANCER 组 U87 细胞 BMPR1A、PD-1、PD-L1 蛋白表达显著降低 ($P < 0.05$); 与 pcDNA 3.1 组相比, pcDNA 3.1 DANCER 组 BMPR1A、PD-1、PD-L1 蛋白表达显著增加 ($P < 0.05$); 与 sh-DANCER + NC 抑制剂组相比, sh-DANCER + miR-656 抑制剂组 BMPR1A、PD-1、PD-L1 蛋白表达显著增加 ($P < 0.05$); 与 sh-DANCER + pcDNA 3.1 组相比, sh-DANCER + pcDNA 3.1 BMPR1A 组 BMPR1A、PD-1、PD-L1 蛋白表达显著增加 ($P < 0.05$, 图 5, 表 8)。

表 4 U87 细胞侵袭、迁移数变化

Table 4 Changes in invasion and migration numbers of U87 cells

Groups	Invasion count	Migration count
Blank	112.46 ± 11.34	102.61 ± 10.47
NC sh	108.94 ± 11.27	105.81 ± 10.62
sh-DANCER	70.88 ± 7.18 ^{a,b}	51.46 ± 5.24 ^{a,b}
pcDNA 3.1	111.74 ± 11.20	104.55 ± 10.53
pcDNA 3.1 DANCER	150.54 ± 15.21 ^c	145.84 ± 14.75 ^c
sh-DANCER + NC inhibitor	71.44 ± 7.21	52.37 ± 5.31
sh-DANCER + miR-656 inhibitor	108.72 ± 11.04 ^d	91.48 ± 9.25 ^d
sh-DANCER + pcDNA 3.1	71.67 ± 7.20	52.53 ± 5.29
sh-DANCER + pcDNA 3.1 BMPR1A	109.34 ± 11.07 ^e	91.66 ± 9.28 ^e

Data are $\bar{x} \pm s$, $n=6$. a, $P < 0.05$, compared with the blank group; b, $P < 0.05$, compared with the NC sh group; c, $P < 0.05$, compared with pcDNA 3.1 group; d, $P < 0.05$, compared with the sh-DANCER + NC inhibitor group; e, $P < 0.05$, compared with the sh-DANCER + pcDNA 3.1 group. NC, negative control; sh, short hairpin; DANCER, differentiation antagonizing non-protein coding RNA; BMPR1A, bone morphogenetic protein receptor type 1A.



NC, negative control; sh, short hairpin; DANCER, differentiation antagonizing non-protein coding RNA; BMPRI1A, bone morphogenetic protein receptor type 1A.

图3 观察 U87 细胞凋亡变化

Figure 3 Observation of apoptosis changes of U87 cells

3 讨论

神经胶质瘤是最常见的中枢神经系统原发性恶

性肿瘤,侵袭性较强,导致患者死亡率增加^[9],神经胶质瘤的发生受遗传和环境因素以及不当的生活方式等因素的影响^[10],识别新的治疗靶点在神经胶质瘤的发展、侵袭和转移过程中,有助于治疗效果和总生存期的改善^[11],因此,迫切需要寻找新的治疗靶点。

表5 U87 细胞各组细胞的凋亡率

Table 5 The apoptosis rates of U87 cells in each group

Group	Apoptosis rate/%
Blank	12.51 ± 1.28
NC sh	12.63 ± 1.29
sh-DANCER	37.82 ± 3.86 ^{a,b}
pcDNA 3.1	12.58 ± 1.28
pcDNA 3.1 DANCER	7.67 ± 0.79 ^c
sh-DANCER + NC inhibitor	37.64 ± 3.79
sh-DANCER + miR-656 inhibitor	21.04 ± 2.18 ^d
sh-DANCER + pcDNA 3.1	37.74 ± 3.82
sh-DANCER + pcDNA 3.1 BMPRI1A	21.17 ± 2.24 ^e

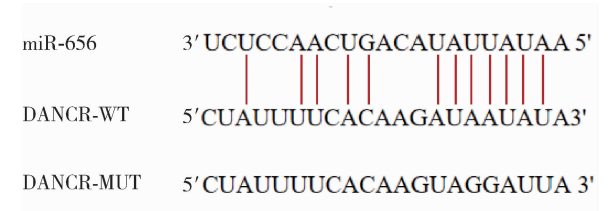
Data are $\bar{x} \pm s$, $n = 6$. a, $P < 0.05$, compared with the blank group; b, $P < 0.05$, compared with the NC sh group; c, $P < 0.05$, compared with pcDNA 3.1 group; d, $P < 0.05$, compared with the sh-DANCER + NC inhibitor group; e, $P < 0.05$, compared with the sh-DANCER + pcDNA 3.1 group. NC, negative control; sh, short hairpin; DANCER, differentiation antagonizing non-protein coding RNA; BMPRI1A, bone morphogenetic protein receptor type 1A.

表6 U87 细胞 DANCER、miR-656、BMPRI1A mRNA 表达变化

Table 6 Changes in mRNA expression of DANCER, miR-656, and BMPRI1A in U87 cells

Group	DANCER	miR-656	BMPRI1A mRNA
Blank	1.05 ± 0.11	0.98 ± 0.10	0.94 ± 0.10
NC sh	1.06 ± 0.11	1.02 ± 0.11	0.98 ± 0.10
sh-DANCER	0.45 ± 0.05 ^{a,b}	1.67 ± 0.18 ^{a,b}	0.55 ± 0.06 ^{a,b}
pcDNA 3.1	0.97 ± 0.10	0.92 ± 0.10	1.05 ± 0.11
pcDNA 3.1 DANCER	1.55 ± 0.16 ^c	0.57 ± 0.06 ^c	1.72 ± 0.18 ^c
sh-DANCER + NC inhibitor	0.48 ± 0.05	1.64 ± 0.18	0.58 ± 0.06
sh-DANCER + miR-656 inhibitor	0.46 ± 0.05	1.08 ± 0.11 ^d	0.88 ± 0.09 ^d
sh-DANCER + pcDNA 3.1	0.47 ± 0.05	1.62 ± 0.18	0.56 ± 0.06
sh-DANCER + pcDNA 3.1 BMPRI1A	0.42 ± 0.05	1.59 ± 0.11	0.83 ± 0.09 ^e

Data are $\bar{x} \pm s$, $n = 6$. a, $P < 0.05$, compared with the blank group; b, $P < 0.05$, compared with the NC sh group; c, $P < 0.05$, compared with pcDNA 3.1 group; d, $P < 0.05$, compared with the sh-DANCER + NC inhibitor group; e, $P < 0.05$, compared with the sh-DANCER + pcDNA 3.1 group. NC, negative control; sh, short hairpin; DANCER, differentiation antagonizing non-protein coding RNA; BMPRI1A, bone morphogenetic protein receptor type 1A.



DANCER, differentiation antagonizing non-protein coding RNA; WT, wild-type; MUT, mutant.

图4 DANCER、miR-656 结合位点

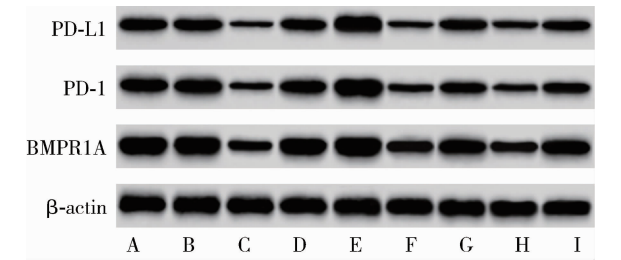
表7 DANCER、miR-656 靶向关系

Table 7 Verification of targeting relationship between DANCER and miR-656

Group	relative luciferase activity
NC mimics + DANCER-WT	1.07 ± 0.11
miR-656 mimics + DANCER-WT	0.62 ± 0.07 ^a
NC mimics + DANCER-MUT	1.03 ± 0.11
miR-656 mimics + DANCER-MUT	1.06 ± 0.11

Data are $\bar{x} \pm s$. $n = 6$. a, $P < 0.05$, compared with the NC mimics + DANCER-WT. NC, negative control; DANCER, differentiation antagonizing non-protein coding RNA; WT, wild-type; MUT, mutant.

Figure 4 Binding sites of DANCER and miR-656



A, blank group; B, NC sh group; C, sh-DANCR group; D, pcDNA 3.1 group; E, pcDNA 3.1 DANCR group; F, sh-DANCR + NC inhibitor group; G, sh-DANCR + miR-656 inhibitor group; H, sh-DANCR + pcDNA 3.1 group; I, sh-DANCR + pcDNA 3.1 BMPR1A group. NC, negative control; sh, short hairpin; DANCR, differentiation antagonizing non-protein coding RNA; BMPR1A, bone morphogenetic protein receptor type 1A.

图5 细胞中 BMPR1A、PD-1、PD-L1 蛋白表达

Figure 5 Expression of BMPR1A, PD-1, and PD-L1 proteins in cells

LncRNA 虽不能编码蛋白质,但长度超过 200

表8 U87 细胞 BMPR1A、PD-1、PD-L1 表达变化

Table 8 Changes in the expression of BMPR1A, PD-1, and PD-L1 in U87 cells

Groups	BMPR1A/β-actin	PD-1/β-actin	PD-L1/β-actin
Blank	1.24 ± 0.13	0.83 ± 0.09	0.77 ± 0.08
NC sh	1.27 ± 0.13	0.87 ± 0.09	0.71 ± 0.08
sh-DANCR	0.65 ± 0.07 ^{a,b}	0.41 ± 0.05 ^{a,b}	0.34 ± 0.04 ^{a,b}
pcDNA 3.1	1.25 ± 0.13	0.85 ± 0.09	0.75 ± 0.08
pcDNA 3.1 DANCR	1.68 ± 0.17 ^c	1.37 ± 0.14 ^c	1.14 ± 0.12 ^c
sh-DANCR + NC inhibitor	0.68 ± 0.07	0.48 ± 0.05	0.36 ± 0.04
sh-DANCR + miR-656 inhibitor	0.92 ± 0.10 ^d	0.78 ± 0.08 ^d	0.64 ± 0.07 ^d
sh-DANCR + pcDNA 3.1	0.65 ± 0.07	0.46 ± 0.05	0.37 ± 0.04
sh-DANCR + pcDNA 3.1 BMPR1A	0.97 ± 0.10 ^e	0.72 ± 0.08 ^e	0.66 ± 0.07 ^e

Data are $\bar{x} \pm s$, $n=6$. a, $P<0.05$, compared with the blank group; b, $P<0.05$, compared with the NC sh group; c, $P<0.05$, compared with pcDNA 3.1 group; d, $P<0.05$, compared with the sh-DANCR + NC inhibitor group; e, $P<0.05$, compared with the sh-DANCR + pcDNA 3.1 group. NC, negative control; sh, short hairpin; DANCR, differentiation antagonizing non-protein coding RNA; BMPR1A, bone morphogenetic protein receptor type 1A.

LncRNA 发挥作用的调控机制很复杂,研究者尚未完全了解,但其可通过表观遗传重塑、转录激活和转录后修饰等机制参与影响疾病发展^[18]。据报道,LncRNAs 通过直接结合 miRNA 中的互补序列并竞争性阻断 miRNA,与其靶 mRNA 的相互作用而发挥其竞争性内源性 RNA 的作用^[19]。如 Jia 等^[20]研究表明,DANCR 可通过靶向 miR-5194/VEGFB 信号治疗黑色素瘤。为了确定 DANCR 在神经胶质瘤中的调控模式,本研究通过数据库和荧光素酶报告分析确定 miR-656 是 DANCR 的潜在靶点,qRT-PCR 结果显示 miR-656 在胶质瘤细胞系表达显著降低,且与 Guo 等^[8]提出的 miR-656 通过抑制 BMPR1A 从而抑制胶质瘤发生的结论相一致。本研究进行相关实验验证,结果同样证实了抑制 miR-656 表达或

个核苷酸,参与转录和转录后调控,与各种癌症类型的发展有关,因此可作为癌症诊断和预后的生物标志物^[12]。越来越多的研究表明,作为一种新发现的 LncRNA,DANCR 在多种人类癌症中呈现异常表达,并在癌症进展中发挥重要作用^[13],如 DANCR 通过靶向 miR-185-5p 促进前列腺癌的恶性行为发展^[14]。PD-1/PD-L1 信号通路是免疫治疗的重要手段,阻断其发展有助于肿瘤微环境的改善,抑制肿瘤免疫逃逸^[15]。本研究发现 DANCR 在神经胶质瘤细胞中高表达,与先前研究相吻合^[16-17],沉默 DANCR 表达可明显抑制 PD-1、PD-L1 表达,阻止 U87 细胞的恶性行为发展,但过表达 DANCR 则提高 PD-1、PD-L1 表达,促进 U87 细胞恶性行为发展,提示 DANCR 可能通过改善肿瘤微环境来参与神经胶质瘤的发展,但其机制尚不明确。

过表达 BMPR1A 逆转了沉默 DANCR 对 U87 细胞恶性行为的抑制作用,表明 DANCR 参与神经胶质瘤的发展,可能与调节 miR-656/BMPR1A 轴有关。

综上所述,沉默 LncRNA DANCR 可改善肿瘤微环境,抑制 U87 细胞恶性生物学行为,可能与 miR-656/BMPR1A 轴有关,但由于涉及靶点较多,机制复杂,本课题组后续会继续开展相关机制研究。

利益冲突 所有作者均声明不存在利益冲突。

作者贡献声明 王欧洋:提出研究思路,设计研究方案,收集、整理、分析数据,撰写论文;朱鹏磊:协助细胞试验,收集、整理数据;林杰:协助收集、整理数据;吴昊:总体把关和审定论文。所有作者均参与论文修改,并对最终文稿进行阅读和确认。

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