·脑胶质瘤·

长链非编码RNA SNORD3A在脑胶质瘤中的表达变化及功能研究

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【摘要】 **目的** 探讨长链非编码(lncRNA)SNORD3A在胶质瘤组织和胶质瘤细胞系中的表达变化,以及对胶质瘤细胞增殖和侵袭能力的影响。**方法** 采用生物信息学方法分析美国国家生物技术信息中心(NCBI)GEO数据库收录的GSE58276中差异表达的lncRNA,采集2017年6月至2019年8月手术切除的胶质瘤组织标本30例,实时荧光定量聚合酶链反应(PCR)检测lncRNA SNORD3A表达水平;小干扰RNA转染胶质瘤细胞系T98G和U251,CCK-8细胞增殖实验检测胶质瘤细胞增殖能力、Transwell细胞侵袭实验检测胶质瘤细胞侵袭能力、Western blotting法检测胶质瘤细胞c-Myc mRNA和蛋白表达变化。**结果** 与对照组相比,胶质瘤组织lncRNA SNORD3A表达水平升高($P=0.000$);与HEB细胞相比,胶质瘤细胞系T98G、U87、U251和U373 lncRNA SNORD3A表达升高(均$P<0.05$)。si-SNORD3A-1组和si-SNORD3A-2组T98G细胞($P=0.001$,0.007)和U251细胞($P=0.002$,0.009)lncRNA SNORD3A表达水平低于对照组;转染后24、48和72 h,si-SNORD3A-1组和si-SNORD3A-2组T98G细胞(均$P=0.000$)和U251细胞(均$P=0.000$)增殖能力低于对照组;转染后48 h,si-SNORD3A-1组和si-SNORD3A-2组穿过小室的T98G和U251细胞数目少于对照组(均$P=0.000$)、c-Myc mRNA和蛋白表达水平低于对照组(均$P<0.01$)。**结论** lncRNA SNORD3A可能通过靶向c-Myc蛋白促进T98G和U251细胞的增殖和侵袭。

【关键词】 神经胶质瘤; RNA,长链非编码; 细胞增殖; 肿瘤侵润; 肿瘤细胞,培养的

Expression and function of lncRNA SNORD3A in glioma

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【Abstract】 **Objective** To explore the expression of long non-coding RNA (lncRNA) SNORD3A in glioma tissues and cell lines and its effect on proliferation and invasion of glioma cells. **Methods** We analyzed the differentially expressed lncRNAs in the National Center for Biotechnology Information (NCBI) GEO (GSE58276) by bioinformatics. A total of 30 glioma tissue specimen were collected from June 2017 to August 2019, lncRNA SNORD3A expression was detected by real-time fluorescence quantitative polymerase chain reaction (PCR). The si-SNORD3A was transfected into T98G and U251 cells. CCK-8 was used to analyze the proliferative capacity of cells. Transwell assay was used to detect cell invasion changes. The expression of c-Myc protein was detected by Western blotting. **Results** Compared with the control group, the expression level of lncRNA SNORD3A in glioma tissue was increased ($P=0.000$). Compared with HEB cells, the expression levels of T98G, U87, U251 and U373 lncRNA SNORD3A in glioma cell lines were elevated ($P<0.05$, for all). The expression levels of lncRNA SNORD3A in T98G cells ($P=0.001$, 0.007) and U251 cells ($P=0.002$, 0.009) in si-SNORD3A-1 group and si-SNORD3A-2 group were lower than those in control group. At 24, 48 and 72 h after transfection, the proliferation capacity of T98G cells ($P=0.000$, for all) and U251 cells ($P=0.000$, for all) in si-SNORD3A-1 and si-SNORD3A-2 groups were lower than those in control group. At 48 h after transfection, the number of T98G and U251 cells passing through the

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chamber in si-SNORD3A-1 and si-SNORD3A-2 groups were less than those in control group ($P = 0.000$, for all), and the levels of c-Myc mRNA and protein expression were lower than control group ($P < 0.01$, for all). **Conclusions** lncRNA SNORD3A promotes proliferation and invasion of T98G and U251 cells by partly targeting c-Myc.

【Key words】 Glioma; RNA, long noncoding; Cell proliferation; Neoplasm invasiveness; Tumor cells, cultured

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Conflicts of interest: none declared

脑胶质瘤是好发于成人的最具侵袭性的原发性恶性脑肿瘤，呈浸润性生长，手术难以全切除，对放射治疗和药物化疗抵抗[1-2]。手术后通常需采取辅助放射治疗和药物化疗的联合治疗方案，尽管如此，患者中位生存期也仅有14.6～20.9个月[3-4]。近年来，随着对胶质瘤分子生物学特征研究的进展，探寻表达异常的长链非编码RNA（lncRNA）及其对胶质瘤发生发展的影响成为新的研究热点。lncRNA系指一类长度＞200个核苷酸且不具编码功能的RNA，大部分由RNA聚合酶Ⅱ转录而成，包含5'-Cap和3'-Poly（A）结构域，绝大部分来自基因间区长链非编码RNA（lincRNA）或者其他转录本的正义或反义转录产物[5-6]。lncRNA不仅参与调控人类许多重要生理学过程，如染色质修饰、基因印迹、转录调控和转录后调控等，而且与肿瘤的发生发展相关[7-9]。晚近研究显示，lncRNA表达异常可能伴随DNA损伤、免疫逃逸和肿瘤细胞代谢异常，还可能与多种肿瘤上皮间质转化（EMT）和肿瘤干细胞（TSCs）调节密切相关，提示靶向lncRNA可能成为治疗肿瘤的新方法[10]。lncRNA SNORD3A是一种核仁小RNA（snoRNA），参与核糖体RNA前体的加工，其在病毒性疾病的发生与发展机制中起重要作用[11]，但其在胶质瘤中的研究鲜有文献报道。本研究拟对lncRNA SNORD3A在胶质瘤组织和胶质瘤细胞系中的表达变化，以及其对T98G和U251细胞增殖、侵袭的影响和可能的分子机制进行探讨，旨在推进胶质瘤相关lncRNA研究进展，丰富胶质瘤治疗方法，为改善患者预后提供重要依据。

材料与方法

一、实验材料

1. 胶质瘤组织标本来源　采集2017年6月至2019年8月在安徽医科大学附属省立医院神经外科手术切除的胶质瘤标本共计30例，男性18例，女性12例；年龄14～68岁，平均为46.30岁；术后经病理证实为胶质瘤，其中WHO Ⅰ～Ⅱ级8例、WHO Ⅲ～Ⅳ级22例。阴性对照标本（对照组）为我院神经外科同期收治的30例颅脑创伤（TBI）患者的手术切除相对正常脑组织标本，男性25例，女性5例；年龄为37～74岁，平均54岁。所有组织标本均置于-80 ℃低温环境中保存备用。本研究经安徽医科大学附属省立医院人类研究道德伦理委员会批准同意，患者或其家属对所有检查项目均知情同意，并签署知情同意书。

2. 胶质瘤细胞系来源　人脑胶质瘤细胞系T98G、U87、U251和U373，以及人脑正常胶质细胞株HEB均购自中国科学院上海生命科学研究院细胞资源中心，传代至第3～4代。

3. 试剂与仪器　（1）药品与试剂：体积分数为10%的胎牛血清购自美国CLARK Bioscience公司，DMEM培养基为美国HyClone公司产品，Lipofectamine 3000转染试剂由美国Thermo Fisher Scientific公司提供，体积分数为0.25%的细胞胰酶消化液、RIPA裂解液和蛋白上样缓冲液均购自上海碧云天生物技术有限公司，CCK-8细胞增殖或细胞毒性检测试剂盒为合肥睿捷生物科技有限公司产品，Transwell小室购自美国Corning公司，Matrigel基质胶由美国BD公司提供，Trizol裂解液为美国Invitrogen公司产品，体积分数为80%的乙醇溶液［焦碳酸二乙酯（DEPC）处理水配置］由北京克尔慧科技有限公司提供，GoScript™ Reverse Transcription System试剂盒由美国Promega公司提供，Ⅰ抗为抗c-Myc抗体（290 μg/ml）和辣根过氧化物酶（HRP）标记的山羊抗兔IgG Ⅱ抗（0.20 mg/ml），

均购自生工生物工程(上海)股份有限公司。(2)仪器与设备:Infinite M200多功能酶标仪为瑞士Tecan公司产品,倒置相差荧光显微镜由日本Olympus公司提供,NanoDrop One微量核酸蛋白浓度测定仪购自美国Thermo Fisher Scientific公司,Biorad Powerpac Basic电泳仪购自美国Bio-Rad公司。

二、实验方法

1. 生物信息学分析 对美国国家生物技术信息中心(NCBI)GEO数据库收录的RNA高通量测序结果(ID号:GSE58276)进行比较分析,以人类*hg19*基因作为参考基因组,选择实验组(胶质瘤组织)RNA表达量/对照组(相对正常脑组织)RNA表达量>2或<0.50且$P<0.05$的lncRNA进行后续实验验证。

2. 细胞培养与分组 将人脑胶质瘤细胞系T98G、U87、U251和U373,以及人脑正常胶质细胞株HEB均置于含10%胎牛血清的DMEM培养基中、于37℃、5%二氧化碳、饱和湿度环境中进行培养,并于每48~72小时更换培养基1次,稳定传代至第3~4代。根据细胞转染的小干扰RNA(siRNA)种类不同分为空白对照组、si-SNORD3A-1组(正向引物序列为:5'-AGAAGAACGAUCAUCAAUG-3',反向引物序列为:5'-CAUUGAUGAUCGUUCUUCU-3')以及si-SNORD3A-2组(正向引物序列:5'-UAGUAACACACUAUAGAAAUG-3',反向引物序列:5'-CAUUUCUAUAGUGUGUUACUA-3')。

3. 小干扰RNA转染 将处于对数生长期的T98G和U251细胞接种于6孔板,调整其细胞数为600×10^3个/孔,继续于含10%胎牛血清的DMEM培养基中培养12~16 h,严格按照Lipofectamine 3000转染试剂盒说明书进行siRNA转染,6~8 h后重新置于含10%胎牛血清的DMEM培养基中培养48 h,以0.25%细胞胰酶消化液收集细胞,实时荧光定量聚合酶链反应(PCR)检测细胞转染率,PCR反应条件为95 ℃变性15 s、60 ℃收集荧光34 s,循环40次,再60 ℃退火15 s,获得细胞转染siRNA后的拷贝数(CT值),以甘油醛-3-磷酸脱氢酶(GAPDH)为内参照物,根据公式$\triangle Ct=Ct(siRNA)-Ct(GAPDH)$计算$2^{-\triangle\triangle Ct}$值,每组设置3个复孔,取平均值,如果siRNA转染组$2^{-\triangle\triangle Ct}$值/对照组$2^{-\triangle\triangle Ct}$值<1,证实lncRNA SNORD3A被沉默。

4. CCK-8细胞增殖实验 将处于对数生长期的T98G和U251细胞接种于96孔板,调整其细胞数至4×10^3个/孔,于含10%胎牛血清的DMEM培养基中培养16~24 h后进行siRNA转染,继续培养6~8 h后更换新鲜培养基,每孔加入CCK-8 10 μl,并以培养基补充至终体积100 μl,培养4 h。分别于转染siRNA后即刻、24 h、48 h和72 h,采用多功能酶标仪在450 nm波长处测定光密度值(OD值),为保证实验的准确性,每组设置5个复孔,取平均值。

5. Transwell细胞侵袭实验 将处于对数生长期的T98G和U251细胞接种于6孔板,调整细胞数为600×10^3个/孔,于含10%胎牛血清的DMEM培养基中培养16~24 h后进行siRNA转染,继续培养36 h后收集细胞,以不含血清的DMEM培养基重悬,并置于含体积分数为4%~7% Matrigel基质胶的Transwell小室上室中,Transwell小室下室加入含10%胎牛血清的DMEM培养基,继续培养48 h,以体积分数为4%的多聚甲醛溶液固定,结晶紫染色,倒置相差荧光显微镜(×100)下观察并计数侵入下室且结晶紫染色阳性(呈紫红色)的细胞,每组均设置3个复孔,取平均值。

6. 实时荧光定量PCR法检测lncRNA SNORD3A相对表达量 将转染48 h的T98G和U251细胞以磷酸盐缓冲液清洗1次,加入Trizol裂解液1 ml,充分裂解细胞后转移至1.50 ml离心管,滴加三氯甲烷溶液200 μl振荡,于4 ℃、离心半径8.60 cm、12 000 r/min离心15 min,收集上清液,转移至新的离心管,加入等体积异丙醇混匀,室温静置10 min后重复离心15 min,弃上清液,加入80%乙醇溶液(DEPC处理水配置)1 ml重悬,于4 ℃、离心半径8.60 cm、7500 r/min离心5 min,倒置风干,再滴加不含RNA酶的蒸馏水30 μl溶解,-80 ℃保存备用。提取总RNA 300~500 ng,严格按照GoScript™ Reverse Transcription System试剂盒说明书反转录为cDNA,以GAPDH(上游引物序列:5'-CTTCATTGACCTCAACTACATGG-3',下游引物序列:5'-CTCGCTCCTGGAAGATGGTGAT-3')为内参照物,微量核酸蛋白浓度测定仪检测lncRNA SNORD3A(上游引物序列:5'-TTCTCTGAACGTGTAGAGCA-3',下游引物序列为:5'-TACGGAGAGAAGAACGATCA-3')相对表达量。

7. Western blotting法检测c-Myc蛋白表达水平 在转染48 h的T98G和U251细胞中加入RIPA裂解液,冰上裂解10 min,用细胞铲刮下细胞后振

荡，于25 ℃、离心半径为8.60 cm、13 000 r/min离心15 min，收集上清液。微量核酸蛋白浓度测定仪测定总蛋白，加入蛋白上样缓冲液（1∶5），100 ℃水浴变性15 min，行十二烷基磺酸钠-聚丙烯酰胺凝胶电泳（SDS-PAGE），电压100 V、80 min，再将蛋白转至聚偏二氟乙烯（PVDF）膜，电流300 mA、90 min，PVDF膜以体积分数为5%脱脂牛奶封闭2 h，采用TBST缓冲液冲洗3次，再滴加Ⅰ抗（抗c-Myc抗体），于4 ℃孵育过夜后回收Ⅰ抗，PVDF膜采用TBST缓冲液冲洗3次（×10 min），再加入辣根过氧化物酶标记的山羊抗兔IgGⅡ抗，于4 ℃孵育2 h，TBST缓冲液连续冲洗PVDF膜3次，加入显影液孵育1 min后显影。

三、统计分析方法

采用SPSS 16.0统计软件进行数据处理与分析。呈正态分布的计量资料以均数±标准差（$\bar{x}\pm s$）表示，采用两独立样本的t检验或单因素方差分析，两两比较行LSD-t检验。以$P\leqslant 0.05$为差异具有统计学意义。

结　果

一、胶质瘤组织和胶质瘤细胞中lncRNA SNORD3A表达变化

对照组lncRNA SNORD3A表达量为0.20±0.18，胶质瘤组lncRNA SNORD3A表达量为1.02±0.21，与对照组相比，胶质瘤组织标本中的lncRNA SNORD3A表达升高（$t=-13.182$，$P=0.000$）；与HEB细胞相比，胶质瘤细胞系T98G、U87、U251和U373 lncRNA SNORD3A表达水平均升高（$P<0.05$），尤以T98G细胞（$P=0.013$，0.003，0.037，0.031）和U251细胞（$P=0.000$，0.037，0.000，0.002）lncRNA SNORD3A表达升高最显著（表1，2）。进一步检索GEPIA数据库（http://gepia.cancer-pku.cn），选取163例胶质瘤标本和207例正常脑组织标本进行比较，结果发现胶质瘤组织lncRNA SNORD3A表达水平亦高于正常脑组织（$P<0.05$，图1），与本研究结果相一致。上述结果提示沉默lncRNA SNORD3A可能抑制胶质瘤的进展。

二、lncRNA SNORD3A的siRNA转染率

实时荧光定量PCR法显示，si-SNORD3A-1组和si-SNORD3A-2组T98G和U251细胞lncRNA SNORD3A表达水平均低于对照组（T98G细胞：$P=0.001$，0.007；U251细胞：$P=0.002$，0.009；表3，4）。

表1 胶质瘤细胞系与HEB细胞lncRNA SNORD3A表达水平的比较（$\bar{x}\pm s$，$2^{-\Delta\Delta Ct}$）

Table 1. Comparison of lncRNA SNORD3A expression levels in glioma cell lines and HEB cells ($\bar{x}\pm s$, $2^{-\Delta\Delta Ct}$)

级别	例数	lncRNA SNORD3A	F值	P值
HEB细胞（1）	3	1.00±0.33		
T98G细胞（2）	3	12.15±2.30		
U87细胞（3）	3	2.65±0.94	59.388	0.000
U251细胞（4）	3	17.44±1.90		
U373细胞（5）	3	6.83±1.65		

表2 胶质瘤细胞系与HEB细胞lncRNA SNORD3A表达水平的两两比较

Table 2. Pairwise comparison of lncRNA SNORD3A expression levels in glioma cell lines and HEB cells

组间两两比	t值	P值	组间两两比	t值	P值
（1）∶（2）	-8.315	0.013	（2）∶（4）	-3.080	0.037
（1）∶（3）	-2.872	0.045	（2）∶（5）	3.258	0.031
（1）∶（4）	-14.792	0.000	（3）∶（4）	-13.493	0.000
（1）∶（5）	-6.013	0.004	（3）∶（5）	-3.822	0.019
（2）∶（3）	6.628	0.003	（4）∶（5）	-7.321	0.002

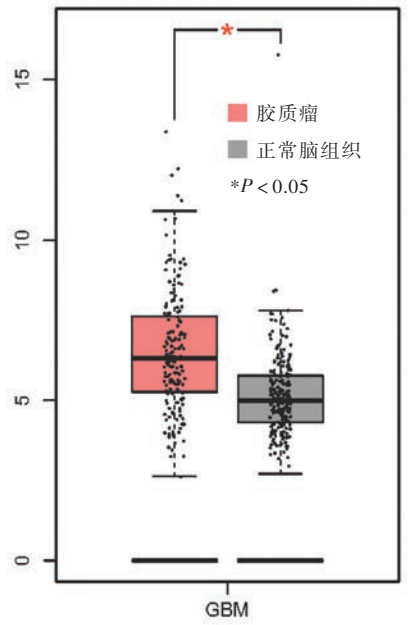


图1 检索GEPIA数据库显示，胶质瘤组织标本（163例）lncRNA SNORD3A表达水平明显高于正常脑组织标本（207例）

Figure 1 Searching the GEPIA database showed expression level of lncRNA SNORD3A in glioma tissue samples (163 cases) was significantly higher than that in normal brain tissue samples (207 cases).

三、lncRNA SNORD3A促进胶质瘤细胞增殖

CCK-8细胞增殖实验结果显示，转染后24、48和72 h si-SNORD3A-1组和si-SNORD3A-2组T98G

表3 siRNA组与对照组T98G和U251细胞lncRNA SNORD3A表达水平的比较($\bar{x} \pm s$, $2^{-\Delta\Delta Ct}$)

Table 3. Comparison of lncRNA SNORD3A expression levels in T98G and U251 cells between siRNA group and control group ($\bar{x} \pm s$, $2^{-\Delta\Delta Ct}$)

组别	例数	T98G	U251
对照组(1)	3	1.00±0.18	1.00±0.14
si-SNORD3A-1组(2)	3	0.03±0.01	0.28±0.10
si-SNORD3A-2组(3)	3	0.36±0.11	0.41±0.16
*F*值		47.037	23.364
*P*值		0.000	0.001

表4 siRNA组与对照组T98G和U251细胞lncRNA SNORD3A表达水平的两两比较

Table 4. Pairwise comparison of lncRNA SNORD3A expression levels in T98G and U251 cells between siRNA group and control group

组间两两比	T98G		U251	
	*t*值	*P*值	*t*值	*P*值
(1)与(2)	9.174	0.001	7.097	0.002
(1)与(3)	5.108	0.007	4.751	0.009
(2)与(3)	-5.090	0.035	-1.206	0.294

表5 siRNA组与对照组不同观察时间点T98G和U251细胞增殖活性的比较($\bar{x} \pm s$, $OD_{450\,nm}$)

Table 5. Comparison of proliferation activity in T98G and U251 cells between siRNA group and control group at different observation time points ($\bar{x} \pm s$, $OD_{450\,nm}$)

组别	例数	转染即刻	转染后24 h	转染后48 h	转染后72 h
T98G					
对照组(1)	5	0.16±0.01	0.42±0.02	0.77±0.03	2.27±0.07
si-SNORD3A-1组(2)	5	0.16±0.01	0.24±0.01	0.49±0.03	1.68±0.11
si-SNORD3A-2组(3)	5	0.16±0.01	0.31±0.02	0.50±0.03	1.66±0.10
*F*值		0.000	165.667	120.697	65.017
*P*值		1.000	0.000	0.000	0.000
U251					
对照组(1)	5	0.56±0.17	1.07±0.03	1.69±0.02	2.66±0.10
si-SNORD3A-1组(2)	5	0.56±0.17	0.74±0.02	1.12±0.08	1.83±0.15
si-SNORD3A-2组(3)	5	0.56±0.17	0.81±0.04	1.18±0.06	1.96±0.18
*F*值		0.000	165.315	150.662	46.297
*P*值		1.000	0.000	0.000	0.000

表6 siRNA组与对照组同一观察时间点T98G和U251细胞增殖活性的两两比较

Table 6. Pairwise comparison of proliferation activity in T98G and U251 cells between siRNA group and control group at different observation time points

组间两两比	转染后24 h		转染后48 h		转染后72 h	
	*t*值	*P*值	*t*值	*P*值	*t*值	*P*值
T98G						
(1)与(2)	19.642	0.000	13.490	0.000	9.887	0.000
(1)与(3)	9.893	0.000	13.508	0.000	11.188	0.000
(2)与(3)	-7.551	0.000	-0.569	0.585	0.204	0.844
U251						
(1)与(2)	15.573	0.000	16.304	0.000	10.260	0.000
(1)与(3)	9.915	0.000	17.708	0.000	7.705	0.000
(2)与(3)	-3.362	0.015	-1.613	0.146	-1.261	0.243

和U251细胞增殖能力减弱，且低于对照组(均$P=0.000$)，提示沉默lncRNA SNORD3A可以显著抑制胶质瘤细胞的增殖能力(表5,6)。

四、lncRNA SNORD3A促进胶质瘤细胞侵袭

Transwell细胞侵袭实验显示，转染后48 h于倒置相差荧光显微镜下观察可见，细胞均匀固定于下室，贴壁生长，细胞形态完整，T98G细胞呈圆形、紫红色，U251细胞呈梭形、紫蓝色；si-SNORD3A-1组和si-SNORD3A-2组穿过小室的细胞数目明显少于对照组，表明沉默lncRNA SNORD3A可以显著抑制

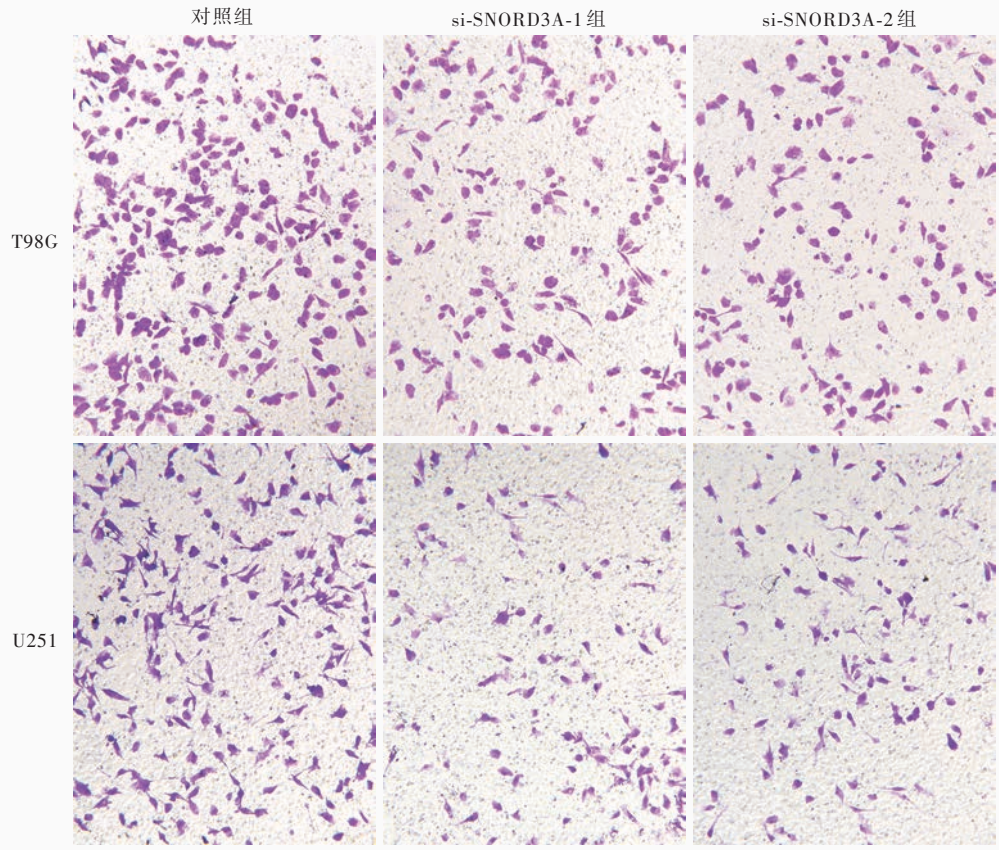


图2 倒置相差荧光显微镜观察显示，T98G细胞形态完整，贴壁生长，胞核呈圆形、紫色，胞质呈多角形、紫红色；U251细胞形态完整，贴壁生长，呈梭形，突触较长且相互连接，胞核呈紫蓝色、胞质呈紫色；si-SNORD3A-1组和si-SNORD3A-2组穿过小室的细胞数目明显少于对照组 结晶紫染色 低倍放大

Figure 2 Inverted phase contrast fluorescence microscopy findings T98G cells were morphologically intact, adherent growth, the nucleus was round, stained purple; cytoplasm was polygonal, and stained purple-red. U251 cells have complete morphology, adherent growth, fusiform shape, long synapses and interconnections, the nucleus was stained purple blue, and the cytoplasm was purple. The number of cells invaded into the lower chamber of control group was more than that of si-SNORN3A-1 group and si-SNORN3A-2 group. Crystal violet staining low power magnified

表7 siRNA组与对照组T98G和U251细胞侵袭能力的比较($\bar{x}\pm s$，%)

Table 7. Comparison of invasive ability of T98G and U251 cells in siRNA group and control group ($\bar{x}\pm s$, %)

组别	例数	T98G	U251
对照组(1)	3	1.00±0.03	1.00±0.03
si-SNORD3A-1组(2)	3	0.71±0.03	0.51±0.02
si-SNORD3A-2组(3)	3	0.66±0.01	0.50±0.01
*F*值		147.870	544.377
*P*值		0.000	0.001

表8 siRNA组与对照组T98G和U251细胞侵袭能力的两两比较

Table 8. Pairwise comparison of invasive ability of T98G and U251 cells in siRNA group and control group

组间两两比	T98G		U251	
	*t*值	*P*值	*t*值	*P*值
(1)与(2)	11.707	0.000	24.751	0.000
(1)与(3)	18.755	0.000	26.306	0.000
(2)与(3)	2.137	0.099	1.167	0.308

胶质瘤细胞侵袭能力(均$P=0.000$；图2；表7，8)。

五、lncRNA SNORD3A通过靶向c-Myc促进胶质瘤进展

实时荧光定量PCR法结果显示，转染后48 h可见si-SNORD3A-1组和si-SNORD3A-2组T98G和U251细胞c-Myc mRNA表达降低，且低于对照组(均$P<0.01$；表9，10)；Western blotting法结果显示，si-SNORD3A-1组和si-SNORD3A-2组T98G和U251细胞c-Myc蛋白表达低于对照组(均$P<0.01$；表9，10；图3)。提示沉默lncRNA SNORD3A抑制胶质瘤进展是通过抑制c-Myc的表达而发挥作用的。

讨 论

根据组织病理学对胶质瘤进行诊断和WHO分

表9 siRNA组与对照组T98G和U251细胞c-Myc mRNA和蛋白表达水平的比较($\bar{x}\pm s$,%)

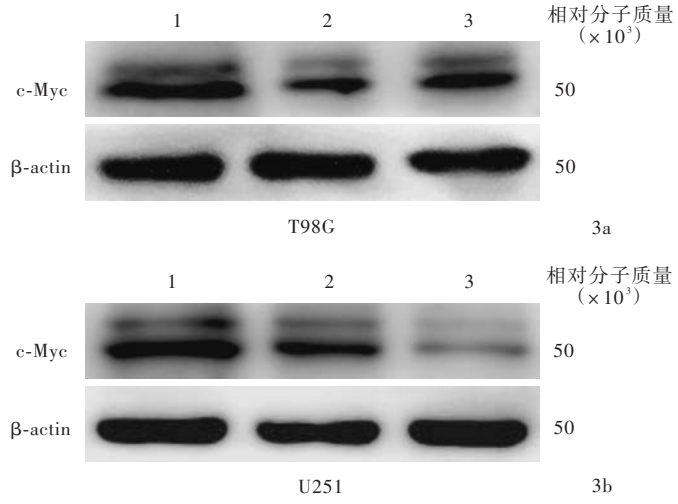
Table 9. Comparison of c-Myc mRNA and protein expression levels of T98G and U251 cells between siRNA group and control group ($\bar{x}\pm s$, %)

组别	例数	T98G		U251	
		c-Myc mRNA	c-Myc	c-Myc mRNA	c-Myc
对照组(1)	3	1.00±0.13	1.00±0.06	1.00±0.17	1.00±0.02
si-SNORD3A-1组(2)	3	0.52±0.05	0.53±0.02	0.31±0.10	0.62±0.01
si-SNORD3A-2组(3)	3	0.50±0.04	0.71±0.02	0.45±0.11	0.26±0.01
*F*值		34.236	115.793	23.360	1898.519
*P*值		0.001	0.000	0.001	0.000

表10 siRNA组与对照组T98G和U251细胞c-Myc mRNA和蛋白表达水平的两两比较

Table 10. Pairwise comparison of c-Myc mRNA and the protein expression levels of T98G and U251 cells between siRNA group and control group

组间两两比	c-Myc mRNA		c-Myc		组间两两比	c-Myc mRNA		c-Myc	
	*t*值	*P*值	*t*值	*P*值		*t*值	*P*值	*t*值	*P*值
T98G					U251				
(1)与(2)	6.047	0.004	12.977	0.000	(1)与(2)	6.072	0.004	26.696	0.000
(1)与(3)	6.317	0.003	7.956	0.001	(1)与(3)	4.722	0.009	59.462	0.000
(2)与(3)	0.302	0.778	-10.841	0.000	(2)与(3)	-1.521	0.203	42.097	0.000



1,对照组;2,si-SNORD3A-1组;3,si-SNORD3A-2组

图3 Western blotting法检测c-Myc蛋白表达可见 3a si-SNORD3A-1组和si-SNORD3A-2组T98G细胞c-Myc蛋白表达水平低于对照组 3b si-SNORD3A-1组和si-SNORD3A-2组中U251细胞c-Myc蛋白表达水平低于对照组

Figure 3 Western blotting findings The expression levels of c-Myc protein in T98G cells of si-SNORD3A-1 and si-SNORD3A-2 groups were lower than those in control group (Panel 3a). The expression levels of c-Myc protein of U251 cells in si-SNORD3A-1 group and si-SNORD3A-2 group were lower than those in control group (Panel 3b).

类,主要包括星形细胞瘤、胶质母细胞瘤、少突胶质细胞瘤、室管膜瘤和髓母细胞瘤等[12],除WHO Ⅰ级胶质瘤[如毛细胞型星形细胞瘤、多形性黄色瘤型星形细胞瘤(PXA)和室管膜下巨细胞型星形细胞瘤(SEGA)]边界较为清晰,WHO Ⅱ级(如少突胶质细胞瘤和星形细胞瘤)、Ⅲ级(如间变性星形细胞瘤和间变性室管膜瘤)、Ⅳ级(如胶质母细胞瘤)胶质瘤均呈浸润性生长且临床较为常见。胶质瘤呈侵袭性生长的特性导致手术难以完全切除,加之肿瘤对放射治疗和药物化疗抵抗,患者即使接受积极的治疗,中位生存期仍较短。此外,胶质瘤细胞的增殖和侵袭能力对维持胶质瘤恶性生物学特征亦发挥重要作用[13-14]。

越来越多的研究表明,lncRNA在肿瘤的发生发展过程中发挥重要作用,可影响肿瘤细胞的增殖、凋亡、侵袭、迁移、血管生成、干细胞转化等。lncRNA PVT1通过调节骨形态发生蛋白(BMP)信号转导通路促进胶质瘤的发生与发展[15];lncRNA NEAT1竞争性结合微小RNA-132(miRNA-132),通过靶向SOX2调控胶质瘤细胞的迁移和侵袭[16]。lncRNA SNORD3A在胶质瘤中呈异常高表达,但目前尚无关于lncRNA SNORD3A的任何文献报道。

本研究率先在胶质瘤组织和胶质瘤细胞系中验证lncRNA SNORD3A的高表达，且与数据库分析结果相一致；进一步采用siRNA沉默lncRNA SNORD3A，探究其在胶质瘤发生发展过程中的作用，结果显示，lncRNA SNORD3A沉默后可以显著抑制T98G和U251细胞的增殖和侵袭能力，表明lncRNA SNORD3A在胶质瘤中作为促癌因子，可能成为胶质瘤治疗的新靶点。

为探究lncRNA SNORD3A影响胶质瘤发生发展的可能机制，我们检索StarBase v2.0数据库，发现lncRNA SNORD3A与miRNA-185-5P的种子区紧密结合（http://starbase.sysu.edu.cn）。研究显示，miRNA-185-5P在胶质瘤中呈低表达，且通过靶向高迁移率族蛋白A2（HMGA2）抑制胶质瘤U87和U251细胞增殖和侵袭[17]，提示lncRNA SNORD3A可能通过竞争性结合miRNA-185-5P，抑制miRNA-185-5P的抑癌作用，以促进胶质瘤的发生发展。

*c-myc*是一种人类癌基因，参与肿瘤的多种生物学特征。早期研究表明，*c-myc*转录靶点参与多种生物学过程，如代谢、细胞生长、细胞周期调节和细胞凋亡等[18]。已知c-Myc蛋白作为磷脂酰肌醇3-激酶（PI3K）/丝氨酸/苏氨酸激酶（AKT）信号转导通路的下游蛋白，在胶质瘤中呈异常高表达且能够促进胶质瘤细胞的增殖和侵袭[19-20]。本研究提示，lncRNA SNORD3A可以影响c-Myc mRNA和蛋白表达水平，沉默lncRNA SNORD3A后，c-Myc mRNA和蛋白表达降低。miRNA-185-5P通过靶向HMGA2蛋白抑制PI3K/AKT信号转导通路的激活，进而抑制胶质瘤细胞的增殖、侵袭[17]。上述研究结果提示，lncRNA SNORD3A可能通过竞争性结合miRNA-185-5P而抑制其表达、激活PI3K/AKT信号转导通路和上调下游c-Myc蛋白表达，从而在胶质瘤中发挥促癌作用。

然而，lncRNA SNORD3A在胶质瘤中的作用和具体机制尚待进一步研究。本研究缺乏lncRNA SNORD3A过表达质粒的构建和研究、lncRNA SNORD3A与miRNA-185-5P结合位点的探究，以及lncRNA SNORD3A对体外成瘤的影响，尚待在今后的研究中进一步完善。

利益冲突 无

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34th International Conference of Alzheimer´s Disease International

Time: March 18–21, 2020

Venue: Singapore

Website: https://adi2020.org/

The 34th International Conference of Alzheimer´s Disease International (ADI) will take place in Singapore on March 18–21, 2020. This unique, multi-disciplinary event brings together all those with an interest in dementia, including researchers, scientists, clinicians, allied healthcare professionals, people living with dementia, family members, care professionals, and staff and volunteers of Alzheimer associations.

ADI is proud that its international conference is the longest running and one of the largest international conferences on dementia, attracting over 1,000 delegates from over 100 countries.

Featuring a range of international keynote speakers and a high standard of scientific and non-scientific content, the conference programme enables participants to learn about the latest advances in the prevention, diagnosis, treatment, care and management of dementia.

ADI is the international federation of Alzheimer associations around the world, in official relations with the World Health Organization (WHO). ADI currently has 100 members, each member being the Alzheimer association in their country who support people living with dementia and their families. ADI´s vision is prevention, care and inclusion today, and cure tomorrow.

Alzheimer´s Disease Association (ADA) was formed in 1990 because of a growing concern for the needs of persons living with dementia and their caregivers. Through our work, the Association hopes to reduce stigma by increasing awareness and understanding of dementia; enabling and involving persons living with dementia to be integrated and accepted in the community; and leading in the quality of dementia care services for persons living with dementia and their families.

27th Congress of the European Society for Pediatric Neurosurgery

Time: May 3–6, 2020

Venue: Athens, Greece

Website: https://www.erasmus.gr/microsites/1179

The 27th Congress of the European Society for Pediatric Neurosurgery (ESPN) will take place in Athens, Greece on May 3–6, 2020. ESPN aims to organize an outstanding scientific and educational congress to facilitate the spread and exchange of knowledge, skills and attitudes, between experts, researchers, clinicians and trainees, and to continue the development of pediatric neurosurgery in Europe.

The ESPN Congress is organized on a biennial basis (traditionally in late April-early May) by the acting ESPN President in his country of origin. It is ESPN´s main educational activity since its first organization in 1967 in Vienna, Austria, which constituted the Society´s inaugural occasion. The Scientific Program is drafted by the ESPN Scientific Committee, in collaboration with the ESPN President. Plenary sessions host a series of invited lectures delivered by renowned experts in the field of Pediatric Neurosurgery and sister disciplines. Abstract submission on topics covering all major aspects of concurrent Pediatric Neurosurgery research and clinical practice lavishly provides for oral and poster presentations. Vibrant discussion is an integral part of all sessions, with the strategic positioning of Panel Tables on major issues addressed.