

研究论文

MiR-218-5p抑制A2B5⁺/CD133⁻亚群胶质瘤干细胞的干性维持及侵袭性

吴至武^{1#} 王杭州^{2#} 李炎炎¹ 李学涛¹ 孙 婷¹ 陈桂林¹ 谢学顺¹ 周幽心^{1*} 杜子威¹(¹苏州大学附属第一医院神经外科暨脑神经研究室, 苏州 215006; ²苏州大学附属儿童医院, 苏州 215003)

摘要 该文目的为探讨miR-218-5p对表达A2B5⁺/CD133⁻的胶质瘤干细胞(glioma stem cells, GSCs)亚群干性维持及侵袭性的影响。实时荧光定量PCR(qRT-PCR)检测人脑胶质瘤组织及细胞株中miR-218-5p的表达; 上、下调miR-218-5p慢病毒转染表达A2B5⁺/CD133⁻的胶质瘤干细胞SHG-139s, 二次球体形成实验、免疫荧光及qRT-PCR检测SHG-139s的标志物A2B5、CD133、Nestin、PLAGL2、ALDH1及SOX2的表达变化; Transwell实验、qRT-PCR及免疫荧光检测SHG-139s基质金属蛋白酶9(matrix metalloproteinase 9, MMP9)的表达变化。结果显示, miR-218-5p在胶质瘤中低表达, 上调miR-218-5p可显著降低SHG-139s成球率, 同时明显降低SHG-139s中上述干细胞标志物的表达; Transwell实验显示, 上调miR-218-5p能显著抑制SHG-139s的侵袭能力, 使其MMP9表达受到明显抑制。因此, miR-218-5p可能作为抑癌基因在胶质瘤中发挥作用, 高表达的miR-218-5p可抑制SHG-139s的干性维持及其侵袭能力。

关键词 miR-218-5p; A2B5⁺/CD133⁻胶质瘤干细胞; 干性维持; 侵袭

MiR-218-5p Inhibited the Stemness Maintaining and Invasion of A2B5⁺/CD133⁻ Subgroup Human Glioma Stem Cells

Wu Zhiwu^{1#}, Wang Hangzhou^{2#}, Li Yanyan¹, Li Xuetao¹, Sun Ting¹, Chen Guilin¹, Xie Xueshun¹, Zhou Youxin^{1*}, Du Ziwei¹(¹Neurosurgery and Brain & Nerve Research Laboratory, the First Affiliated Hospital of Soochow University, Suzhou 215006, China;²The Children's Hospital of Soochow University, Suzhou 215003, China)

Abstract The article was aimed at detecting the expression of miR-218-5p in human gliomas, and exploring its influence in stemness maintaining and invasion of A2B5⁺/CD133⁻ human glioma stem cells (SHG-139s). qRT-PCR was used to detect miR-218-5p expression in noncancerous brain tissue, human glioma tissue, human glioma cell lines and human glioma stem cell lines. Lentivirus vectors encoding miR-218-5p and anti-miR-218-5p were constructed and stably transfected into A2B5⁺/CD133⁻ SHG-139s cells. Neurosphere formation assay, Transwell assay, immunofluorescence and qRT-PCR analysis were used to explore the role of miR-218-

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#共同第一作者

*通讯作者。Tel: 0512-67781170, E-mail: zhouyouxin@suda.edu.cn

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#These authors contributed equally to this work

*Corresponding author. Tel: +86-512-67781170, E-mail: zhouyouxin@suda.edu.cn

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5p in SHG-139s. qRT-PCR analysis showed that *miR-218-5p* expression was lower in human glioma tissues and cells than in noncancerous brain tissue and normal human astrocyte cells, and lower in A2B5⁺/CD133⁻ (SHG-139s) cells than in CD133⁺ cells (SU2, U87s) cells. Immunofluorescence staining and qRT-PCR showed that *miR-218-5p* reduced the expressions of stem cell markers (A2B5, nestin, PLAGL2, ALDH1, Sox2), compared to controls; However, qRT-PCR analysis showed that upregulated *miR-218-5p* expression led to a decline in CD133 expression, although this result was not confirmed by immunofluorescence staining. *MiR-218-5p* reduced SHG-139s cell invasiveness in Transwell assay and reduced MMP9 expression detected by qRT-PCR and immunofluorescence analysis. *MiR-218-5p* is implicated as a tumor suppressor gene in glioma that functions by upregulating *miR-218-5p* expression, which inhibits the stem cell and invasive properties of SHG-139s.

Keywords *miR-218-5p*; A2B5⁺/CD133⁻ glioma stem cells; stemness maintaining; invasion

胶质瘤是颅内常见的恶性肿瘤, 具有很强的侵袭性、高死亡率和复发率, 尤其是胶质母细胞瘤 (glioblastoma, GBM), 患者平均生存时间大约为15个月^[1]。胶质瘤干细胞(glioma stem cells, GSCs)是胶质瘤发生和复发的根源, 因此治疗胶质瘤的关键是彻底切除肿瘤的同时全面消除胶质瘤干细胞。目前有关研究证实, 胶质瘤中存在CD133⁺的细胞, 而这群不表达CD133的细胞同样具有GSCs的生物学特性^[2-3], 由此推测, GSCs的特性并不是由一种标志物决定的, 而可能由多种肿瘤干细胞标志物共同决定。相关研究已证实, *MiR-218*在胶质瘤中低表达, 上调*miR-218*的表达后能显著促进胶质瘤干细胞的分化, 抑制胶质瘤细胞的增殖及侵袭性。然而, 作为*miR-218*的前体, *miR-218-5p*在胶质瘤中的作用是否一致, 目前国内外仍未见相关报道。本室近年培养出了一株表达A2B5⁺/CD133⁻的胶质瘤干细胞株, 将其命名为SHG-139s^[4]。通过基因芯片技术筛选发现, *miR-218-5p*在SHG-139s中表达显著降低, 因此推测, *miR-218-5p*对这株A2B5⁺/CD133⁻人脑胶质瘤干细胞(SHG-139s)的干细胞特性及侵袭性可能起着重要的调控作用。

1 材料与方法

1.1 组织标本及来源

收集2008年5月至2013年1月苏州大学附属第一医院神经外科手术切除并经病理证实的人脑胶质瘤组织标本53例、脑外伤减压术切除的非瘤脑组织6例, 均储存于液氮中。按照WHO 2007年的分类标准, 对53例人脑胶质瘤组织标本进行良恶性分级, 其中I级6例、II级16例、III级17例、IV级14例; 男35例, 平均年龄44.6岁; 女18例, 平均年龄47.5岁。

1.2 细胞来源及细胞培养

人胶质瘤细胞株SHG-44、U87、U373、A172和正常人脑星形细胞株1800购于中国科学院上海细胞库; SHG-139s为本室近年新培养出的一株人脑胶质瘤干细胞株, 应用神经干细胞培养基(neural stem cells medium, NSCM)成功获得其干细胞, 表达标志物A2B5⁺/CD133⁻; U87s为U87在NSCM中培养获取的肿瘤干细胞; SU2由苏州大学附属第二医院神经外科黄强教授惠赠。胶质瘤细胞株培养于含10%胎牛血清的DMEM和RPMI 1640培养基中, GSCs培养于含EGF (20 ng/mL)、bFGF (20 ng/mL)、1/100 N2的DMEM/F12无血清培养基中; 在含5% CO₂的37 °C恒温培养箱中培养, 每2~3 d换液一次, 胰酶消化传代培养。

1.3 主要试剂和抗体

细胞培养液DMEM和RPMI 1640购于美国Thermo公司, DMEM/F12、N2购于美国Gibco公司, bFGF(碱性成纤维细胞生长因子)、EGF(表皮生长因子)购于美国Invitrogen公司。*miR-218-5p*引物和All-in-One™ *miRNA* qRT-PCR Detection Kit购于GeneCopoeia公司。普通逆转录及荧光定量PCR反应试剂盒购于美国Thermo公司。*miR-218-5p*的慢病毒由上海吉凯基因化学技术有限公司构建和包装。一抗: 兔抗人Nestin、SOX2、CD133、PLAGL2、ALDH1、MMP9(matrix metalloproteinase 9)单克隆抗体及鼠抗人A2B5 IgM购于Abcam公司, 红色荧光二抗山羊抗兔IgG、山羊抗鼠IgM购于Thermo公司。

1.4 构建稳定表达*miR-218-5p*的SHG-139s

将SHG-139s细胞分成空白组、阴性对照组、*miR-218-5p*过表达组和*miR-218-5p*干扰组。将SHG-139s单细胞悬液接种到6孔板中, 细胞融合至30%~50%时, 用已构建好的病毒滴度在1×10⁸ TU的

表1 胶质瘤干细胞标志物qRT-PCR引物序列
Table 1 The primer sequences of biomarks of glioma stem cells for qRT-PCR

基因 Gene	引物序列(5'→3') Primer sequence (5'→3')
<i>CD133</i>	Upstream: ATT GAC TTC TTG GTG CTG TTG A Downstream: GAT GGA GTT ACG CAG GTT TCT C
<i>SOX2</i>	Upstream: TGT CAA GGC AGA GAA GAG AGT G Downstream: GCC GCC GAT GAT TGT TAT TAT
<i>PLAGL2</i>	Upstream: GGG CTT GCT GAC TTC TCT TCA T Downstream: AAT CCT CTT GCC TGT CTT CTG T
<i>ALDH1</i>	Upstream: CGC CAG ACT TAC CTG TCC TAC T Downstream: TCA ACA TCC TCC TTA TCT CCT TCT
<i>Nestin</i>	Upstream: CTT GCC TGC TAC CCT TGA GAC Downstream: GTT TCC TCC CAC CCT GTG T
<i>GAPDH</i>	Upstream: ACC ACA GTC CAT GCC ATC AC Downstream: TCC ACC ACC CTG TTG CTG TA

相应慢病毒, 按MOI=1分别加入各孔, 空白组中不加病毒, polybrene(4~10 μg/mL)用于提高病毒的转染效率, 然后将6孔板置于含5% CO₂的37 °C恒温培养箱中培养, 8 h后换液培养, 3~4 d后细胞涂片于荧光显微镜下观察GFP表达率。

1.5 组织及细胞中总RNA的提取及定量分析

分别取液氮中保存的人脑胶质瘤组织、非瘤脑组织及培养细胞, 加入Trizol, 匀浆后严格按照Trizol法抽提总RNA并测定RNA浓度。 *MiR-218-5p*的定量检测根据All-in-One™ miRNA qRT-PCR Detection Kit试剂盒说明书要求, PCR反应条件为: 95 °C预变性10 min; 95 °C变性10 s, 60 °C退火20 s, 72 °C延伸10 s, 在Roche480定量PCR仪进行40个循环反应, 结果进行2^{-ΔΔCt}值分析。肿瘤干细胞标志物的定量检测采用Thermo公司的逆转录和PCR反应试剂盒, 反应条件为: 95 °C预变性10 min; 95 °C变性10 s, 60 °C退火20 s, 72 °C延伸10 s, 在Roche480定量PCR仪进行45个循环反应, 结果进行2^{-ΔΔCt}值分析。肿瘤干细胞标志物及内参*GAPDH*的引物序列见表1。

1.6 SHG-139s二次球体形成实验及肿瘤干细胞标志物免疫荧光染色

将各对照组的SHG139s单细胞无限稀释后, 按每孔100个细胞接种于24孔板中, 每组设8个复孔, 培养14 d后将超过20个细胞的细胞球计数, 结果以8个复孔的平均值表示^[5]。将稳定转染的SHG-139s细胞悬液接种于灭菌的经多聚赖氨酸处理的载玻片上, 室温下自然风干后用PBS漂洗, 4%多聚甲醛室温固定15 min, PBS漂洗, 0.25% Triton X-100室温通透5~15 min, PBS漂洗, 5%胎牛血清(BSA)封闭30 min, 加一抗湿盒中孵育, 4 °C过夜。第二天弃去一抗, 加带红色荧光的二抗,

湿盒中室温避光孵育1 h。弃去二抗液体, PBS冲洗细胞后吸干, 用含DAPI(DNA染色剂)的封片剂封片, 激光共聚焦显微镜(Leica公司)下观察及分析结果。表达量以荧光强度表示, 采用Image J软件分析。

1.7 SHG-139s细胞侵袭实验

将Matrigel胶按照1:5稀释后, 按每孔50 μL均匀铺于Transwell小室, 37 °C孵育3 h。将转染96 h后的SHG-139s细胞用0.05%胰酶+0.02% EDTA吹散, 离心5 min, 干细胞培养液重悬细胞后以每孔2×10⁵个接种于Transwell小室, 上室200 μL, 下室加入500 μL含20%胎牛血清的DMEM培养基。在含5% CO₂的37 °C恒温培养箱中培养48 h后, 用棉签擦净上层小室内细胞, 室温下无水甲醇固定30 min, 再用1%结晶紫染色5 min, 随即在显微镜下随机取6个视野计数, 重复3次取其平均值。基质金属蛋白酶MMP9的qRT-PCR检测及细胞免疫荧光染色方法同上, *MMP9*基因引物序列: 上游5'-CGG ACC AAG GAT ACA GTT TGT T-3', 下游5'-GTT CAG GGC GAG GAC CAT AG-3'; *GAPDH*引物序列: 上游5'-ACC ACA GTC CAT GCC ATC AC-3', 下游5'-TCC ACC ACC CTG TTG CTG TA-3'。

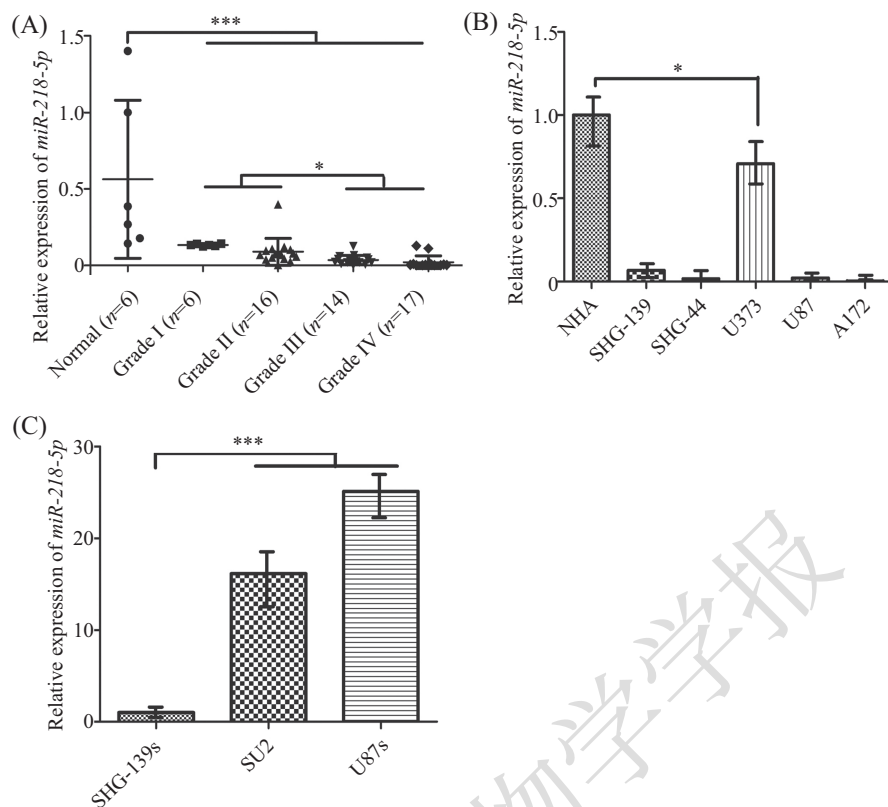
1.8 统计方法

应用SPSS 19.0软件进行数据分析, 两组间均数比较采用*t*检验, 多组间数据分析采用单因素方差分析, *P*<0.05为差异显著。

2 结果

2.1 *miR-218-5p*在人脑胶质瘤组织、非瘤脑组织及胶质瘤细胞株中的表达

通过qRT-PCR检测了53例人脑胶质瘤组织、6

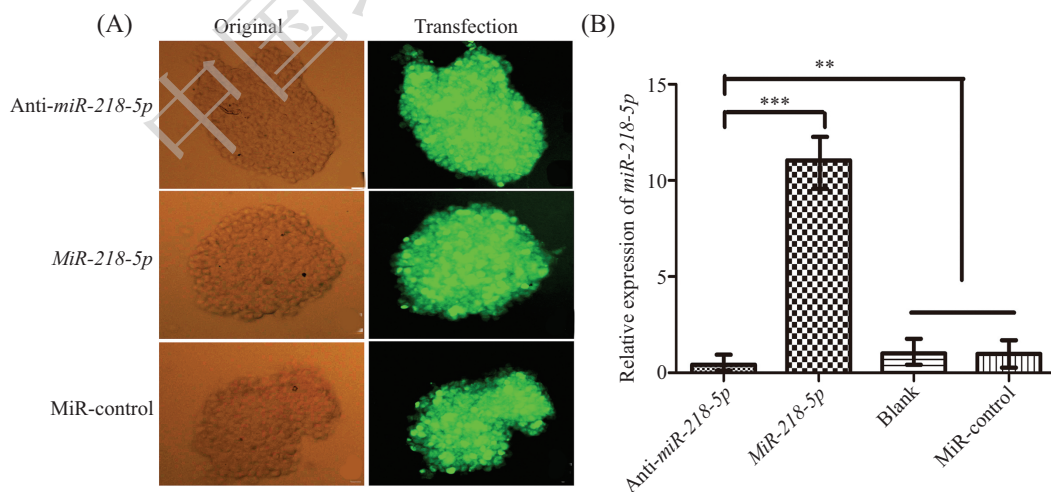


A: *miR-218-5p*在胶质瘤组织中的表达显著低于非瘤脑组织,在低级别组中(Grade I、II)的表达高于高级别组(Grade III、IV); B: *miR-218-5p*在胶质瘤细胞株中的表达显著低于正常星形细胞株(NHA); C: *miR-218-5p*在表达A2B5⁺/CD133⁻的SHG-139s中的表达显著低于表达CD133⁺的SU2和U87s。* $P<0.05$, *** $P<0.001$ 。

A: *miR-218-5p* expression in human glioma tissues was lower than that in noncancerous brain tissue, with higher expression in low grade groups (Grade I, II) than that in high grade groups (Grade III, IV); B: *miR-218-5p* expression in human glioma cell lines was lower than that in normal human astrocyte cells; C: *miR-218-5p* expression in A2B5⁺/CD133⁻ cells (SHG-139s) was lower than that in CD133⁺ glioma cell lines (SU2, U87s). * $P<0.05$, *** $P<0.001$.

图1 qRT-PCR检测*miR-218-5p*在胶质瘤组织及胶质瘤细胞中的表达水平

Fig.1 Relative expression of *miR-218-5p* in glioma tissues and cell lines determined by qRT-PCR



A: 带有绿色荧光(GFP)的慢病毒转染SHG-139s后具有很强的荧光表达,证实已获得稳定转染的SHG-139s干细胞株(200×); B: 转染后各组中*miR-218-5p*的表达水平,图示干扰组中*miR-218-5p*的表达显著低于过表达组。** $P<0.01$, *** $P<0.001$ 。

A: strong fluorescence detected in transfected SHG-139s cells stably transfected the lentivirus GV159-GFP vector expressing anti-*miR-218-5p*, *miR-218-5p* and miR-control (200×); B: the expression of *miR-218-5p* in different groups of SHG-139s, and it was lower in anti-*miR-218-5p* group than that in *miR-218-5p* group. ** $P<0.01$, *** $P<0.001$.

图2 稳定转染绿色荧光蛋白慢病毒的SHG-139s细胞

Fig.2 Successfully stable transfection of obtained SHG-139s cells with recombinant lentiviruses expressing green fluorescent protein

例非瘤脑组织及5例胶质瘤细胞株中*miR-218-5p*的表达水平。结果显示, *miR-218-5p*在正常脑组织中表达显著高于胶质瘤组织(图1A); 其在体外培养的正常人脑星形细胞株1800(NHA)中的表达显著高于在胶质瘤细胞株U87、U373、A172、SHG-139s及SHG-44中的表达(图1B)。此外, *miR-218-5p*在A2B5⁺/CD133⁺的SHG-139s中的表达水平要比在表达CD133⁺的U87s和SU2细胞中更低(图1C), 结果具有显著的统计学差异。

2.2 转染效率的鉴定

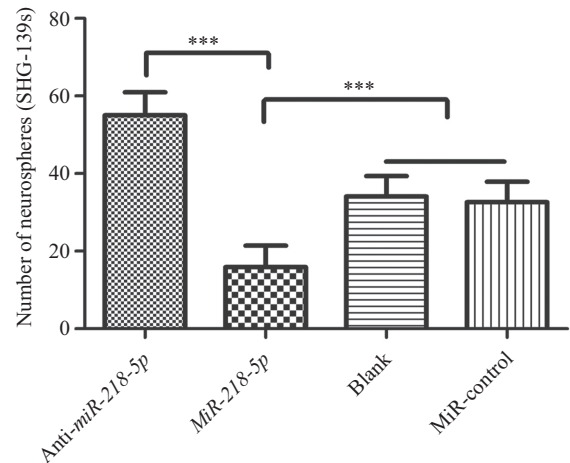
为了检测和观察转染效率, 将转染后72~96 h的细胞于荧光显微镜下观察绿色荧光的表达情况。经证实, 阳性表达率均在80%以上(图2A)。进一步用qRT-PCR检测各转染组细胞中*miR-218-5p*的表达水平, 发现*miR-218-5p*在各组之间的表达有明显差异, 干扰组中*miR-218-5p*的表达显著低于过表达组($P<0.001$)(图2B)。

2.3 *miR-218-5p*能有效抑制SHG-139s的干性特征

将稳定转染后的各组细胞行二次球体形成实验, 发现上调*miR-218-5p*组SHG-139s细胞的二次成球球体数明显低于空白组和阴性对照组, 更显著低于下调*miR-218-5p*组, 表明上调*miR-218-5p*后能显著抑制SHG-139s的二次成球率(图3)。干细胞标志物A2B5、CD133、Nestin、PLAGL2、ALDH1、SOX2行细胞免疫荧光化学染色及qRT-PCR的结果显示, 过表达*miR-218-5p*的细胞中A2B5、Nestin、PLAGL2、ALDH1、SOX2的表达显著降低, 而干扰组的表达显著升高。qRT-PCR实验显示, 上调*miR-218-5p*后能显著降低SHG-139s细胞中CD133的表达水平; 而细胞免疫荧光结果显示, CD133在各对照组中基本不表达($P>0.05$)(图4)。

2.4 *miR-218-5p*抑制SHG-139s的侵袭能力, 同时降低MMP9的表达

Transwell实验发现, 上调*miR-218-5p*后SHG-139s细胞透过Matrigel胶的细胞数量明显少于下调组, 因此过表达*miR-218-5p*的SHG-139s的侵袭能力明显较下调组SHG-139s的侵袭能力降低(图5A)。同时, qRT-PCR检测及免疫荧光染色显示, MMP9的表达在过表达*miR-218-5p*组也显著降低(图5B和图5C)。以上提示, *miR-218-5p*的表达与MMP9的表达呈负相关, *miR-218-5p*可能通过抑制MMP9的表达抑制SHG-139s的侵袭能力。



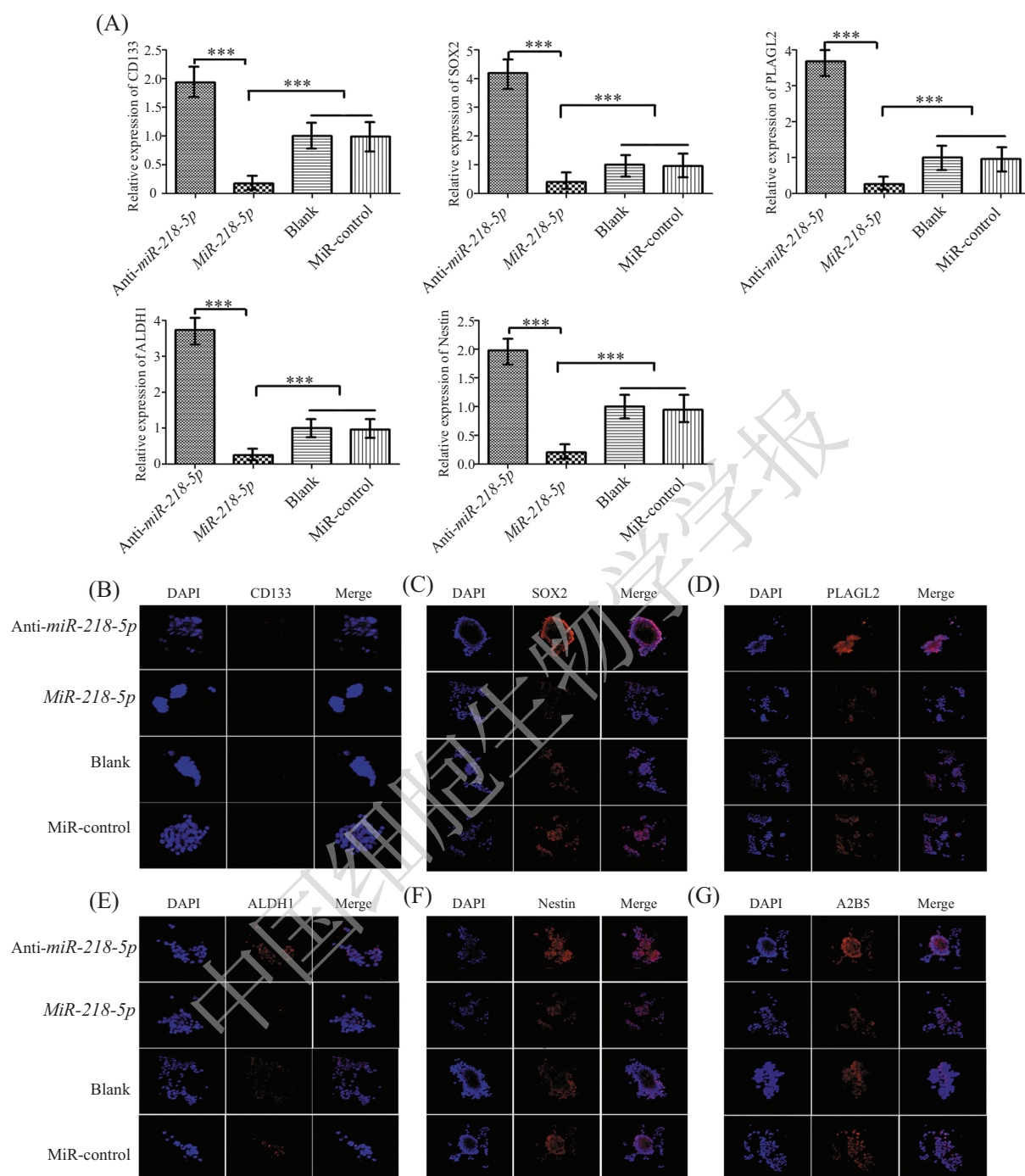
下调*miR-218-5p*的表达后SHG-139s干细胞球数显著高于空白组、阴性对照组及*miR-218-5p*上调组。*** $P<0.001$ 。

The number of neurospheres (SHG-139s) formed in the anti-*miR-218-5p* group was significantly higher than those in the blank group, miR-control group and *miR-218-5p* group. *** $P<0.001$.

图3 过表达及干扰*miR-218-5p*后SHG-139s细胞球体的形成
Fig.3 Sphere formation assay of SHG-139s after *miR-218-5p* up-regulation and down-regulation

3 讨论

细胞培养是肿瘤研究中最重要的一种方式之一, 到目前为止已经历了60余年^[6]。1984年, 本室培养出了我国第一株胶质瘤细胞株SHG-44后, 近年又成功培养出一株人脑胶质瘤细胞株SHG-139, 应用神经干细胞培养基(NSCM)成功获得其干细胞SHG-139s, 其干性标志物表达为A2B5⁺/CD133⁺^[4]。本研究将这株表达A2B5⁺/CD133⁺的人脑胶质瘤干细胞株作为研究对象, 探讨*miR-218-5p*对A2B5⁺/CD133⁺胶质瘤干细胞亚群的治疗作用。胶质瘤干细胞的研究日趋深入, 普遍认为, 由于GSCs的存在及其耐放化疗特性, 导致常规的治疗无法有效地清除GSCs, 因此要彻底治愈胶质瘤需彻底清除这群具有种子作用的GSCs^[7]。胶质瘤干细胞表达如Nestin、CD133、A2B5、Bmi-1、SOX2等干细胞标志物, 早先认为, GSCs均表达CD133, 但发现部分CD133阴性细胞也具有GSCs的生物学特性^[2-3], 因此推测干细胞的特性有可能并非由某一种标志物决定而是由多种标志物共同决定, 而且干细胞根据表达标志物的不同可分为不同的细胞亚群。目前针对A2B5⁺/CD133⁺干细胞亚群的研究极少, 2008年, Ogden等^[8]分别根据干细胞标志物CD133、A2B5采用流式细胞仪分选技术分选了25例新鲜胶质瘤组织中的细胞, 发

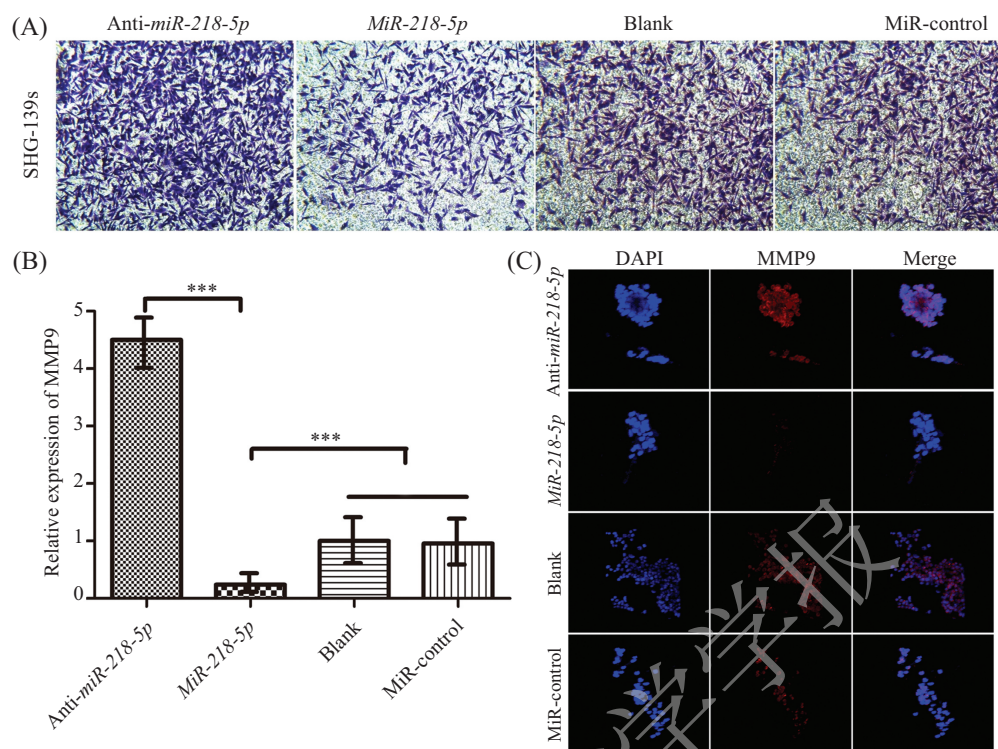


A: *miR-218-5p*干扰组的SHG-139s的干细胞标志物CD133、SOX2、PLAGL2、ALDH1、Nestin的表达水平显著高于空白组、阴性对照组及*miR-218-5p*上调组, 差异具有统计学意义。*** $P < 0.001$; B~G: 细胞免疫荧光-激光共聚焦实验检测*miR-218-5p*对SHG-139s干细胞标志物表达水平的影响, 下调*miR-218-5p*后SHG-139s的干性标志物(SOX2、PLAGL2、ALDH1、Nestin、A2B5)表达显著高于空白组、阴性对照组及*miR-218-5p*上调组, 但免疫荧光染色显示*miR-218-5p*表达的变化对CD133的表达无明显影响; DAPI复染的细胞核释放出蓝色荧光(60 $\times$)。

A: qRT-PCR results showing higher expression of glioma stem cell markers (CD133, Sox2, PLAGL2, ALDH1, Nestin) in the anti-*miR-218-5p* group compared with the blank and miR-control groups, and much higher expression than that in the *miR-218-5p* group. *** $P < 0.001$; B~G: confocal laser microscopy images of immunofluorescence staining confirming the results of the qRT-PCR analysis, with the exception of CD133, for which no significant differences were detected between the groups; DAPI staining of nuclei observed as blue fluorescence (60 $\times$).

图4 SHG-139s稳定转染后各组细胞中干细胞标志物的表达

Fig.4 Expression of stem cell markers in SHG-139s as stably transfected with recombinant lentiviruses



A: 上调*miR-218-5p*的表达能显著抑制SHG-139s细胞的侵袭能力(400×); B: qRT-PCR检测显示, 上调*miR-218-5p*后SHG-139s细胞中MMP9表达显著低于空白组、阴性对照组及*miR-218-5p*下调组, *** $P < 0.001$; C: 免疫荧光染色-激光共聚焦成像证实, 上调*miR-218-5p*后MMP9的荧光表达显著降低, DAPI复染的细胞核释放出蓝色荧光(60×)。

A: transwell experiment showing suppressed invasive ability of SHG-139s in the *miR-218-5p* group compared with the other groups (400×); B: qRT-PCR results showing lower MMP9 expression in the *miR-218-5p* group compared with the other groups, *** $P < 0.001$; C: confocal laser microscopy images of immunofluorescence staining showing lower MMP9 expression in the *miR-218-5p* group compared with the other groups; DAPI staining of nuclei observed as blue fluorescence (60×) .

图5 上下调*miR-218-5p*对SHG-139s侵袭能力及相关基质金属蛋白酶9表达的影响

Fig.5 The influence of up-regulated and down-regulated *miR-218-5p* expression on the invasive ability and MMP9 expression of SHG-139s

现CD133⁺的细胞全部包含在A2B5⁺的细胞中, 由此将胶质瘤细胞分为A2B5⁺/CD133⁺、A2B5⁺/CD133⁻和A2B5⁻/CD133⁻三个亚群, 且体内实验证实前两群细胞具有很强的成瘤能力, 而A2B5⁻/CD133⁻细胞则无成瘤能力。Tchoghandjian等^[9]也通过流式细胞仪分选A2B5⁺的胶质瘤干细胞球, 发现其中包含表达A2B5⁺/CD133⁺、A2B5⁺/CD133⁻和A2B5⁻/CD133⁻的三个亚群细胞, 其中前两群具有强侵袭性、潜在成球能力和致瘤性, 而A2B5⁻/CD133⁻亚群细胞则没有上述特征。因此我们认为, 胶质瘤的发生、发展与复发可能与A2B5⁺/CD133⁺和A2B5⁺/CD133⁻两个亚群细胞的存在有关。

本室近年培养出胶质瘤细胞SHG-139并成功转化表达A2B5⁺/CD133⁻的干细胞SHG-139s。基因芯片技术分析发现, 在表达A2B5⁺/CD133⁻的胶质瘤干细胞SHG-139s中存在着一些差异表达的lncRNA

和microRNA, 其中*miR-218-5p*在SHG-139s中表达非常低(结果未显示), 查阅文献发现其前体miR-218在胶质瘤中相对低表达, 起着抑癌基因的作用^[10-12], 因此, 本研究应用*miR-218-5p*探讨其对SHG-139s干性维持及侵袭性方面的调控作用。通过qRT-PCR实验证实, *miR-218-5p*在正常非瘤脑组织中的表达要显著高于胶质瘤组织, 在低级别胶质瘤组织中的表达要高于高级别胶质瘤组织, 本实验结果与以往报道的*miR-218-5p*的前体miR-218在胶质瘤中低表达是一致的^[10-12], *miR-218-5p*可能与其前体miR-218一样, 在胶质瘤中作为抑癌基因而发挥作用。此外, 重要的是, *miR-218-5p*在表达A2B5⁺/CD133⁻的SHG-139s干细胞中的表达要显著低于表达CD133⁺的U87s和SU2^[13-14], 这表明*miR-218-5p*可能在A2B5⁺/CD133⁻亚群干细胞致瘤性方面扮演着更加重要的角色, 我们为了探讨其对A2B5⁺/CD133⁻亚群干细胞特性及侵

袭性的影响进行了进一步的研究。

我们发现,上调*miR-218-5p*的表达能显著抑制SHG-139s的二次成球能力及降低干细胞标志物的表达,由此可见,*miR-218-5p*可能促进了SHG-139s的分化,低表达的*miR-218-5p*对SHG-139s干细胞特性的维持具有重要作用。这与Tu等^[15]报道的*miR-218*可靶向作用于*Bmi-1*基因从而促进胶质瘤干细胞U251s的分化相一致。因此,低表达的*miR-218-5p*也可能通过调控*Bmi-1*基因的表达来维持SHG-139s干细胞的特性,然而其机制仍有待于进一步研究。此外,我们还发现,上调SHG-139s中*miR-218-5p*的表达能显著降低其侵袭能力,且*miR-218-5p*的表达与基质金属蛋白酶MMP9的表达呈负相关,本实验结果与以往报道的有关*miR-218*可作用于信号通路LEF1/MMP9^[11]及IKK-B/NF- κ B/MMP9^[12]从而降低胶质瘤细胞的侵袭性是一致的。

本研究发现,高表达*miR-218-5p*能抑制SHG-139s细胞的干性维持及其侵袭能力,这与*miR-218*能促进胶质瘤细胞分化及抑制其侵袭性结果一致。虽然*miR-218-5p*在A2B5⁺/CD133⁻(SHG-139s)干细胞中的表达要比CD133⁺(U87s、SU2)干细胞中的表达更低,但*miR-218-5p*对各亚群胶质瘤干细胞的作用是否相同,或者是否对某亚群具有更强的调控作用,仍有待于更加深入的研究。从本研究中我们至少发现,*miR-218-5p*对表达A2B5⁺/CD133⁻的SHG-139s人脑胶质瘤干细胞的干性维持及侵袭性具有重要的作用,*miR-218-5p*靶向A2B5⁺/CD133⁻胶质瘤干细胞亚群的治疗将为临床胶质瘤的治疗提供科学依据,如果我们能更好地抑制该亚群干细胞,人脑胶质瘤有可能得到更好的治疗,患者将可能有更长的生存期。

参考文献 (References)

- 1 Wen PY, Kesari S. Malignant gliomas in adults. *N Engl J Med* 2008; 359(5): 492-507.
- 2 Wang J, Sakariassen PO, Tsinkalovsky O, Immervoll H, Boe SO, Svendsen A, *et al.* CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells. *Int J Cancer* 2008; 122(4): 761-8.
- 3 Beier D, Hau P, Proescholdt M, Lohmeier A, Wischhusen J, Oefner PJ, *et al.* CD133(+) and CD133(-) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. *Cancer Res* 2007; 67(9): 4010-5.
- 4 陈桂林, 李炎炎, 谢学顺, 陈金明, 吴庭枫, 李学涛, 等. 人源胶质瘤细胞株的建立及其生物特性的分析. *中华肿瘤杂志*(Chen Guilin, Li Yanyan, Xie Xueshun, Chen Jinming, Wu Tingfeng, Li Xuetao, *et al.* The establishment of a new human glioma cell line and its biological characteristic analysis. *Chinese Journal of Oncology*) 2015; 37(2): 84-90.
- 5 Lomonaco SL, Finniss S, Xiang C, Lee HK, Jiang W, Lemke N, *et al.* Cilengitide induces autophagy-mediated cell death in glioma cells. *Neuro Oncol* 2011; 13(8): 857-65.
- 6 Gey GO, Bang FB, Gey MK. Responses of a variety of normal and malignant cells to continuous cultivation, and some practical applications of these responses to problems in the biology of disease. *Ann N Y Acad Sci* 1954; 58(7): 976-99.
- 7 Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001; 414(6859): 105-11.
- 8 Ogden AT, Waziri AE, Lochhead RA, Fusco D, Lopez K, Ellis JA, *et al.* Identification of A2B5⁺/CD133⁻ tumor-initiating cells in adult human gliomas. *Neurosurgery* 2008; 62(2): 505-14; discussion 514-5.
- 9 Tchoghandjian A, Baeza N, Colin C, Cayre M, Metellus P, Beclin C, *et al.* A2B5 cells from human glioblastoma have cancer stem cell properties. *Brain Pathol* 2010; 20(1): 211-21.
- 10 Liu Y, Yan W, Zhang W, Chen L, You G, Bao Z, *et al.* MiR-218 reverses high invasiveness of glioblastoma cells by targeting the oncogenic transcription factor LEF1. *Oncol Rep* 2012; 28(3): 1013-21.
- 11 Song L, Huang Q, Chen K, Liu L, Lin C, Dai T, *et al.* miR-218 inhibits the invasive ability of glioma cells by direct downregulation of IKK-beta. *Biochem Biophys Res Commun* 2010; 402(1): 135-40.
- 12 Xia H, Yan Y, Hu M, Wang Y, Wang Y, Dai Y, *et al.* MiR-218 sensitizes glioma cells to apoptosis and inhibits tumorigenicity by regulating ECOP-mediated suppression of NF-kappaB activity. *Neuro Oncol* 2013; 15(4): 413-22.
- 13 Zhang B, Sun J, Yu SP, Chen C, Liu B, Liu ZF, *et al.* Rac1+ cells distributed in accordance with CD 133+ cells in glioblastomas and the elevated invasiveness of CD 133+ glioma cells with higher Rac1 activity. *Chin Med J (Engl)* 2012; 125(24): 4344-8.
- 14 Huang Q, Zhang QB, Dong J, Wu YY, Shen YT, Zhao YD, *et al.* Glioma stem cells are more aggressive in recurrent tumors with malignant progression than in the primary tumor, and both can be maintained long-term *in vitro*. *BMC Cancer* 2008; 8: 304.
- 15 Tu Y, Gao X, Li G, Fu H, Cui D, Liu H, *et al.* MicroRNA-218 inhibits glioma invasion, migration, proliferation, and cancer stem-like cell self-renewal by targeting the polycomb group gene *Bmi1*. *Cancer Res* 2013; 73(19): 6046-55.