·论 著·

脑胶质瘤干细胞衍生的外泌体lncRNA HOXA-AS2促进脑胶质瘤的增殖、迁移、侵袭和干细胞特性

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［**摘要**］ **背景与目的：**外泌体是介导肿瘤微环境中肿瘤细胞与受体细胞间相互作用的重要信使。然而，细胞外泌体长链非编码RNA（long non-coding RNA，lncRNA）在脑胶质瘤干细胞（glioma stem cell，GSC）和脑胶质瘤细胞的细胞间通信中的作用尚不清楚。本研究探究外泌体衍生的lncRNA对脑胶质瘤增殖、迁移、侵袭和干细胞特性的影响。**方法：**从中国脑胶质瘤基因组图谱（the Chinese Glioma Genome Atlas，CGGA）和癌症基因组图谱（the Cancer Genome Atlas，TCGA）数据库下载包含低级别脑胶质瘤（low-grade glioma，LGG）和高级别脑胶质瘤（high-grade glioma，HGG）lncRNA表达数据的数据集，识别LGG和HGG组织之间的差异表达lncRNA（differentially expressed lncRNA，DelncRNA），并分析HOXA-AS2水平与胶质瘤患者总生存期（overall survival，OS）之间的关系。从人胶质瘤细胞系SHG44中分离GSC，用流式细胞术检测CD133$^+$富集的细胞，再用蛋白质印迹法（Western blot）检测干细胞相关蛋白（CD133、SOX2和OCT4）的表达水平。提取和识别SHG44-GSC衍生的外泌体，并用PKH26细胞膜染料进行荧光标记；再将转染了Cy3标记HOXA-AS2的SHG44-GSC与SHG44细胞进行间接共培养；后用实时荧光定量聚合酶链反应（real-time fluorescence quantitative polymerase chain reaction，RTFQ-PCR）检测SHG44-GSC和SHG44-GSC衍生外泌体中HOXA-AS2的水平。使用pLVX-IRES-PURO HOXA-AS2慢病毒质粒和含靶向HOXA-AS2质粒的慢病毒shRNA进行慢病毒转染。采用细胞计数试剂盒-8（cell counting kit-8，CCK-8）和transwell实验检测SHG44-GSC衍生的外泌体HOXA-AS2对SHG44细胞增殖和侵袭能力的影响。**结果：**HOXA-AS2在胶质瘤中呈现高表达，且与患者较差的OS相关（$P<0.01$）。SHG44-GSC中CD133$^+$细胞比例明显高于SHG44细胞（$P<0.000\ 1$），SHG44-GSC中干细胞相关蛋白（CD133、SOX2和OCT4）的表达水平明显高于亲代SHG44细胞（$P<0.000\ 1$），并且SHG44-GSC中HOXA-AS2水平显著升高（$P<0.000\ 1$）。PKH26标记的外泌体被SHG44细胞吸收，且SHG44细胞中可观察到Cy3标记的HOXA-AS2；HOXA-AS2 OE转染的SHG44-GSC细胞（SHG44-GSC/HOXA-AS2 OE）和SHG44-GSC/HOXA-AS2 OE衍生的外泌体（SHG44-GSC/HOXA-AS2 OE-Exo）中HOXA-AS2水平显著升高（$P<0.01$），在与SHG44-GSC/HOXA-AS2 OE细胞共培养的SHG44细胞中HOXA-AS2水平显著升高（$P<0.01$）。SHG44-GSC/HOXA-AS2 OE-Exo可显著促进SHG44细胞增殖、迁移和侵袭。**结论：**来自SHG44-GSC的外泌体HOXA-AS2能显著促进胶质瘤细胞增殖、迁移、侵袭和干细胞特性，提示HOXA-AS2可能是脑胶质瘤潜在的治疗靶点。

［**关键词**］脑胶质瘤；肿瘤干细胞样细胞；外泌体；长链非编码RNA；HOXA-AS2

DOI: 10.19401/j.cnki.1007-3639.2022.12.010

中图分类号：R739.41 **文献标志码：**A **文章编号：**1007-3639(2022)12-1218-11

Glioma stem cell-derived exosomal lncRNA HOXA-AS2 promoted proliferation, migration, invasion and stemness in glioma LIAO Xinghe[1], LIU Zhantao[2], LIU Minghui[1] ［1. Department of Integrated Therapy, Fudan University Shanghai Cancer Center, Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China; 2. Department of Neurosurgery, Guangdong Provincial People's Hospital Ganzhou Hospital (Ganzhou Municipal Hospital), Ganzhou 341000, Jiangxi Province, China］

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［**Abstract**］ **Background and purpose:** Exosomes are important messengers that mediate the crosstalk between cancer cells and recipient cells in the tumor microenvironment; however, the role of extracellular exosomal long non-coding RNA (lncRNA) in the cell-cell communications of glioma stem cells (GSCs) and glioma cells remains unclear. This study investigated the effects of exosome-derived lncRNAs on proliferation, migration, invasion and stemness in glioma. **Methods:** The datasets containing low-grade glioma (LGG) and high-grade glioma (HGG) lncRNA expression data were downloaded from the Chinese Glioma Genome Atlas (CGGA) and the Cancer Genome Atlas (TCGA) databases. Differentially expressed lncRNA (DelncRNA) between LGG and HGG tissues was identified, and the relationship between HOXA-AS2 levels and overall survival (OS) of glioma patients was analyzed. GSCs were isolated from human glioma cell line SHG44, and $CD133^+$ enriched cells were detected by flow cytometry. The expression levels of stem cell-related proteins (CD133, SOX2 and OCT4) were detected by Western blot. Exosomes derived from SHG44-GSCs were extracted and identified, and labeled with PKH26 cell membrane dye. Then SHG44-GSCs transfected with Cy3-labeled HOXA-AS2 were indirectly co-cultured with SHG44 cells. The levels of HOXA-AS2 in SHG44-GSCs and SHG44-GSC-derived exosomes were detected by real-time fluorescence quantitative polymerase chain reaction (RTFQ-PCR). The plVX-IRES-PURO HOXA-AS2 lentivirus plasmid and lentivirus shRNA containing targeting HOXA-AS2 plasmid were used for lentivirus transfection. The effect of HOXA-AS2 derived from SHG44-GSC on the proliferation and invasion of SHG44 cells was detected by cell counting kit-8 (CCK-8) and transwell assays. **Results:** HOXA-AS2 was highly expressed in gliomas, and was associated with worse OS in patients ($P<0.01$). The proportion of $CD133^+$ cells were significantly higher in SHG44-GSC than in SHG44 cells ($P<0.000\ 1$). The expression levels of stem cell related proteins (CD133, SOX2 and OCT4) in SHG44-GSC cells were significantly higher compared with parental SHG44 cells ($P<0.000\ 1$). HOXA-AS2 level was significantly increased in SHG44-GSC cells ($P<0.000\ 1$). PKH26-labeled exosomes were absorbed by SHG44 cells, and Cy3-labeled HOXA-AS2 could be observed in SHG44 cells. HOXA-AS2 levels were significantly increased in HOXA-AS2 OE transfected SHG44-GSCs cells (SHG44-GSC/HOXA-AS2 OE) and SHG44-GSC/HOXA-AS2 OE-derived exosomes (SHG44-GSC/HOXA-AS2 OE-Exo) ($P<0.01$). In addition, HOXA-AS2 levels were significantly increased in SHG44 cells co-cultured with SHG44-GSC/HOXA-AS2 OE cells ($P<0.01$). HOXA-AS2 could be transferred from SHG44-GSC to SHG44 cells through exosomes. Functionally, SHG44-GSC/HOXA-AS2 OE-Exo significantly promoted the proliferation, migration and invasion of SHG44 cells. **Conclusion:** Exosomal HOXA-AS2 derived from SHG44-GSCs significantly promoted glioma cell proliferation, migration, invasion and stemness, suggesting that HOXA-AS2 may be a potential therapeutic target for glioma.

［**Key words**］ Gliomas; Cancer stem-like cells; Exosomes; Long non-coding RNA; HOXA-AS2

脑胶质瘤是原发性脑瘤中发病率最高的肿瘤，因其独特的生物学特性[1-2]导致患者治疗难度大、致残和致死率高及预后差[3-4]。因此，探索新的诊断标志物和治疗方法显得极其重要和紧迫。

长链非编码RNA（long non-coding RNA，lncRNA）在脑胶质瘤的发生和发展过程中发挥着重要作用[5-6]。目前已经发现HOXA集群反义RNA2（HOXA-AS2）与胶质瘤相关[7-8]。然而HOXA-AS2调节胶质瘤细胞增殖的机制仍不清楚。肿瘤干细胞样细胞（cancer stem-like cell，CSC）具有自我更新和形成大块肿瘤的能力[9-10]。CSC与非CSC可以互相转化[11]，既往研究已经发现lncRNA参与了这一过程[12]。然而目前HOXA-AS2与胶质瘤干细胞（glioma stem cell，GSC）的关系尚未确定。外泌体可以通过传递细胞内的物质（包括蛋白质、DNA、miRNA、lncRNA和mRNA）来介导细胞间的相互作用[13]。此外，肿瘤源性外泌体与肿瘤发生及肿瘤微环境有关[14]。然而，含有HOXA-AS2的GSC来源外泌体是否对胶质瘤细胞增殖、迁移和干细胞特性有影响尚不清楚。因此，本研究对上述问题进行探究。

1 材料和方法

1.1 细胞培养和实验试剂

人皮质星形胶质细胞系HA1800、人胶质瘤细胞系T98G、SHG44、U251MG和HEK-293T细胞均购自中国科学院典型培养物保藏委员会细胞库。胎牛血清（fetal bovine serum，FBS）、DMEM培养基、TRIzol®试剂、免疫磁珠分选

试剂盒、RIPA缓冲液、ECL检测试剂、死亡细胞凋亡试剂盒、Step-One Plus实时荧光定量聚合酶链反应（real-time fluorescence quantitative polymerase chain reaction，RTFQ-PCR）系统均购自美国Thermo Fisher Scientific公司。EntiLink™第1链cDNA试剂盒、EnTurbo™SYBR-Green PCR SuperMix系统购自武汉科鹿生物科技有限公司。FACS Calibur流式细胞仪购自美国BD公司。CD133兔单抗（1∶1 000）、SOX2兔单抗（1∶1 000）、OCT4兔单抗（1∶1 000）、cleaved caspase 3兔单抗（1∶1 000），CD9兔单抗（1∶1 000）、CD63兔单抗（1∶1 000）、CD81兔单抗（1∶1 000）、TSG101兔单抗（1∶1 000）和β-actin小鼠单抗（1∶1 000）等所有一抗和相应二抗均购自英国Abcam公司。PKH26细胞膜染料、transwell小室购自美国Merck公司。pLVX-IRES-PURO HOXA-AS2慢病毒质粒和含靶向HOXA-AS2质粒购自美国克隆泰克实验室。细胞计数试剂盒-8（cell counting kit-8，CCK-8）购自上海碧云天生物技术研究所。Cell-Light EdU Apollo567体外试剂盒购自广州锐博生物科技有限公司。

1.2 临床数据获取

从中国脑胶质瘤基因组图谱（the Chinese Glioma Genome Altas，CGGA; http://www.cgga.org.cn）和癌症基因组图谱（the Cancer Genome Atlas，TCGA; https://portal.gdc.cancer.gov/）数据库下载了包含低级别脑胶质瘤（low-grade glioma，LGG）和高级别脑胶质瘤（high-grade glioma，HGG）lncRNA的表达数据。本研究从CGGA数据库中选取312例患者信息，从TCGA数据库中选取975例患者信息，包括性别、年龄、生存时间及lncRNA表达情况等。

1.3 方法

1.3.1 识别差异表达lncRNA（differentially expressed lncRNA，DelncRNA）

从CGGA和TCGA数据库下载了包含LGG和HGG lncRNA表达数据的数据集。用R语言识别LGG和HGG组织之间的DelncRNA。调整后$P<0.05$和|log2（差异倍数）|＞2的lncRNA认定为DelncRNA。使用维恩图包对来自两个数据集（CGGA和TCGA）的重叠DelncRNA进行了识别。此外，我们通过CGGA和TCGA数据集来确定HOXA-AS2水平与胶质瘤患者总生存期（overall survival，OS）之间的关系。

1.3.2 细胞培养

人皮质星形胶质细胞系HA1800，人胶质瘤细胞系T98G、SHG44、U251MG和HEK-293T细胞在含10%FBS的DMEM培养基中培养，细胞均在37 ℃、CO_2体积分数为5%的培养箱中培养。

1.3.3 RTFQ-PCR检测

使用TRIzol®试剂分离出总RNA，再使用EntiLink™第1链cDNA试剂盒合成cDNA，然后使用EnTurbo™SYBR-Green PCR SuperMix和Step-One Plus RTFQ-PCR系统进行RTFQ-PCR。温度条件如下：在95 ℃预温育3 min，然后在95 ℃温育10 s，58 ℃温育30 s，72 ℃温育30 s，循环40次。以β-actin作为lncRNA HOXA-AS2的内参照物。使用标准$2^{-\Delta\Delta Cq}$方法计算表达水平。引物序列如下：β-actin有义链为5’-GTCCACCGCAAATGCTTCTA-3’，反义链为5’-TGCTGTCACCTTCACCGTTC-3’；HOXA-AS2有义链为5’-GGAAGGACACGTTTCTATGCC-3’，反义链为5’-ACTTGGATTCTGACGGCTCAC-3’。

1.3.4 流式细胞术筛选GSC

在本研究中，我们使用FACS Calibur流式细胞仪和之前描述的免疫磁珠分选试剂盒分选$CD133^+$细胞。SHG44细胞在DMEM/F12培养基［包括碱性成纤维细胞生长因子（basic fibroblast growth factor，bFGF）和表皮生长因子（epidermal growth factor，EGF）］中培养，在37 ℃、CO_2体积分数为5%的培养箱中培养。细胞与CD133抗体珠复合物一起温育，之后再加入分选液重新悬浮细胞，洗脱$CD133^+$细胞，随后将分离的$CD133^+$细胞加入CD133-PE抗体，用流式细胞仪进行分选。

1.3.5 蛋白质印迹法（Western blot）检测

利用RIPA缓冲液裂解细胞以获得蛋白质，用二辛可宁酸（bicinchoninic acid，BCA）

法测定蛋白质浓度，蛋白质（30 μg/lane）用10%十二烷基硫酸钠聚丙烯酰胺凝胶电泳（sodium dodecylsulphate polyacrylamide gel electrophoresis，SDS-PAGE）凝胶分离，转移到PVDF膜（美国Millipore公司）上，然后用5%脱脂牛奶在含有吐温-20三乙醇胺缓冲盐溶液（tris-buffered saline Tween，TBST）中堵塞膜。随后将膜与一抗在4 ℃下温育过夜，然后与相应的二抗（1∶5 000）在室温下温育。ECL检测试剂用于检测蛋白带。所用的一抗如下：CD133兔单抗（1∶1 000，货号397903，Biolegend），SOX2兔单抗（1∶1 000，货号ab92494）、OCT4兔单抗（1∶1 000，货号ab19857）、TSG101兔单抗（1∶1 000，货号ab125011）购自英国Abcam公司，cleaved caspase 3兔单抗（1∶1 000，货号AF7022）、CD63兔单抗（1∶1 000，货号AF5117）购自美国Affbiotech公司，CD9兔单抗（1∶1 000，货号20597-1-AP）、CD81兔单抗（1∶1 000，货号27855-1-AP）购自美国Proteintech公司，β-actin兔单抗（1∶1 000，货号TDY051）购自天德悦（北京）生物科技有限责任公司。以β-actin作为对照。

1.3.6 外泌体的分离和表征

从GSC中收集上清液，使用超离心法分离外泌体，用BCA法测定外泌体蛋白浓度，外泌体的识别使用纳米粒子跟踪分析（nanoparticle tracking analysis，NTA）和透射电镜（transmission electron microscope，TEM）测定。

1.3.7 外泌体摄取

从GSC中提取的外泌体（20 μg）用PKH26细胞膜染料进行荧光标记，胶质瘤细胞与荧光标记的外泌体共培养48 h，使用荧光显微镜观察胶质瘤细胞外泌体的内化情况。

1.3.8 共培养系统

用Cy3标记的HOXA-AS2转染SHG44-GSC，转染的SHG44-GSC接种于transwell® 聚酯透性支架上，SHG44细胞接种于下层腔室，温育24 h后，用共聚焦显微镜对SHG44细胞成像。

1.3.9 慢病毒转染

获取pLVX-IRES-PURO HOXA-AS2慢病毒质粒和含靶向HOXA-AS2质粒的慢病毒shRNA后，用慢病毒质粒和包装质粒（pLP/VSVG、pLP1和pLP2）转染HEK-293T细胞72 h，然后收集含病毒的上清液，将适量的慢病毒转导至细胞内72 h。

1.3.10 CCK−8检测

在相应处理后，胶质瘤细胞被接种到96孔板（5 000个细胞/孔）中，37 ˚C培养48 h，随后将CCK-8试剂10 μL分别加入孔中，37 ˚C温育2 h，然后使用酶标仪在450 nm处对每个孔的吸光度（*D*）进行评估。

1.3.11 5−乙炔基−2'−嘧啶核苷（EdU）染色

使用Cell-Light EdU Apollo567体外试剂盒测定细胞增殖，细胞用50 μmol/L EdU温育后，用Apollo染料溶液染色，随后使用荧光显微镜观察EdU阳性细胞。

1.3.12 Transwell小室分析

使用孔径为8 μm的transwell小室分析细胞迁移和侵袭能力，将细胞（1×10^5）重悬浮于200 μL无血清培养液中，加入上室，下室填充含10%FBS的DMEM（700 μL），温育24 h后，用1%结晶紫对下膜表面的细胞进行30 min的染色，随后用荧光显微镜（购自日本Olympus公司）捕获染色细胞，侵袭实验的条件与迁移实验相同，但transwell板的上室用人工基底膜预覆盖。

1.3.13 细胞凋亡检测

细胞凋亡的检测使用细胞凋亡试剂盒。细胞与Annexin Ⅴ-FITC和碘化丙啶（propidium iodide，PI）在暗室中温育30 min，然后用流式细胞术检测细胞凋亡。

1.4 统计学处理

数据统计分析采用SPSS 22.0及R 4.0.1软件。两组之间的比较用非配对Student's *t*检验进行分析，多组间比较采用单因素方差分析和Tukey事后检测法。连续性变量数据以$\bar{x}\pm s$表示。使用COX回归分析计算风险比（hazard ratio，HR）。采用双侧检验。$P<0.05$为差异有统计学意义。

2　结　　果

2.1　胶质瘤中DelncRNA的识别

从CGGA数据集中鉴定出312个DelncRNA（图1A），并从TCGA数据集中鉴定出975个DelncRNA（图1B）。在两个数据集中识别了11个重叠的DelncRNA，包括RP11-366L20.2、HOXB-AS1、RP4-792G4.2、RP11-93B14.5、HOTAIRM1、AC002454.1、CRNDE、RP11-834C11.5、HOXA-AS2、AGAP2-AS1和CTD-3049M7.1，并用维恩图表示（图1C）。在CGGA和TCGA数据集中，胶质瘤患者中高水平的HOXA-AS2与较差的OS相关（图1D~E，P均＜0.01）。此外，我们发现与HA1800细胞相比，T98G、SHG44和U251 MG细胞中HOXA-AS2的表达量更高（HA1800 *vs* T98G：HR＝−1.690，95% CI：−2.259～−1.121，P＜0.000 1；HA1800 *vs* SHG44：HR＝−3.430，95% CI：−3.999～−2.861，P＜0.000 1；HA1800 *vs* U251MG：HR＝−0.961，95% CI：−1.530～−0.392，P＜0.01，图1F）。上述结果表明HOXA-AS2在胶质瘤中表达升高，并且与OS呈负相关。

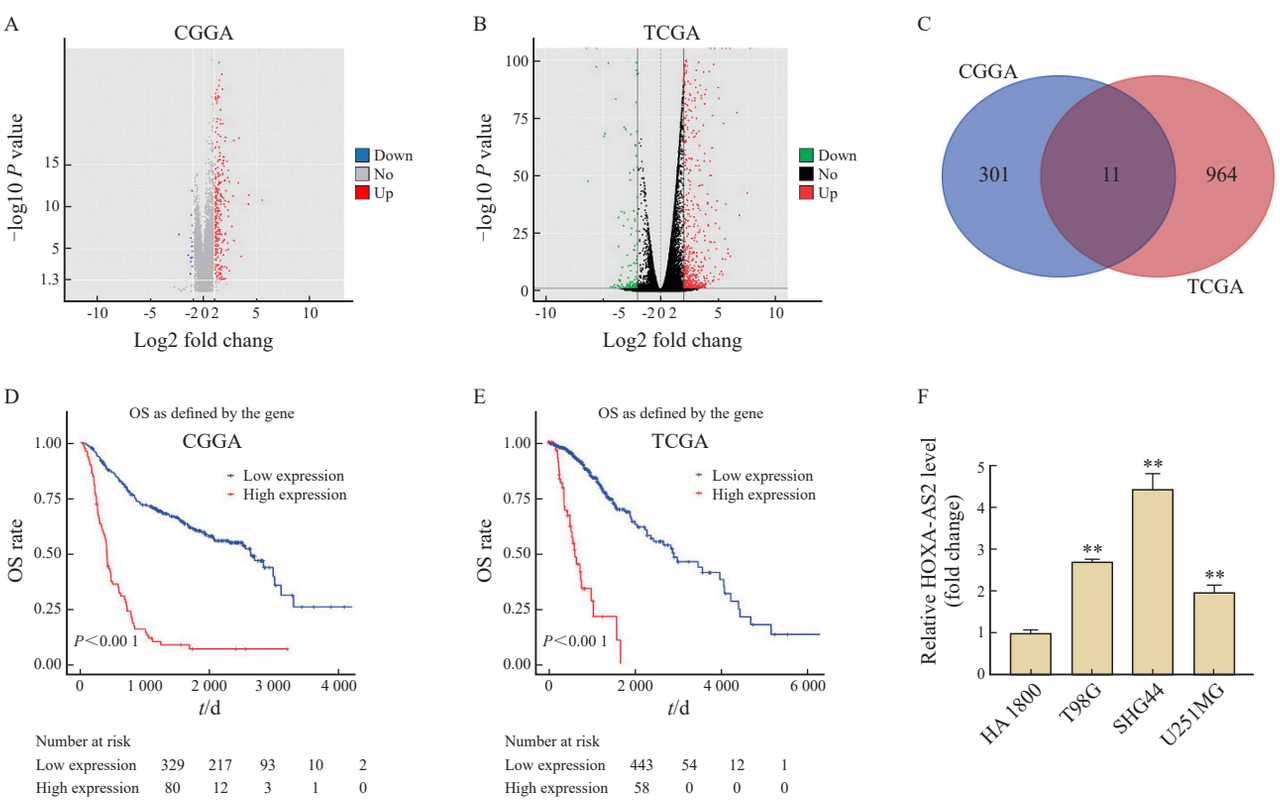

图1　胶质瘤中DelncRNA的识别

Fig. 1　Identification of DElncRNAs in glioma

A, B: Volcano plot of DelncRNAs in CGGA and TCGA; C: 11 overlapping DelncRNAs were identified using R language; D: The correlation between HOXA-AS2 level and OS rate in patients with glioma in the CGGA datasets; E: The correlation between HOXA-AS2 level and OS rate in patients with glioma in the TCGA datasets; F: RTFQ-PCR showing HOXA-AS2 levels in T98G, SHG44 and U251 MG cells. **: P＜0.01, compared with HA1800 group.

2.2　HOXA–AS2在GSC中表达上调

本研究发现，SHG44-GSC中CD133＋细胞的比例明显高于SHG44细胞（81.6% *vs* 23.8%；HR＝0.988，95% CI：53.63～73.04，P＜0.000 1，图2A）。此外，与亲代SHG44细胞相比，SHG44−GSC中CD133（HR＝−0.520，95% CI：−0.717 6～−0.321 8，P＜0.000 1）、SOX2（HR＝−0.482，95% CI：−0.479 4～−0.283 6，

P＜0.000 1）和OCT4（HR＝−0.466，95% CI：−0.664 3～−0.268 5，P＜0.000 1）的表达水平明显升高（图2B）。而且，HOXA-AS2水平在SHG44-GSC中也显著升高（图2C；HR＝0.996，95% CI：2.377～2.833，P＜0.000 1）。

2.3 SHG44–GSC通过外泌体将HOXA–AS2转移到SHG44细胞

SHG44-GSC衍生的外泌体呈圆形和杯状，直径50～150 nm，表达外泌体标志物CD9、CD63、CD81和TSG101（图3A~3B）。此外，本研究发现PKH26标记的外泌体被SHG44细胞吸收（图3C）。本研究还发现在SHG44细胞中观察到Cy3标记的HOXA-AS2（图3D）。

本研究发现，HOXA-AS2 OE转染的SHG44-GSC细胞（SHG44-GSC/HOXA-AS2 OE）和SHG44-GSC/HOXA-AS2 OE衍生的外泌体（SHG44-GSC/HOXA-AS2 OE-Exo）中HOXA-AS2水平显著升高（P＜0.01，图4A、4B和4C）。然而，在HOXA-AS2 shRNA1转染的SHG44-GSC细胞（SHG44-GSC/HOXA-AS2 shRNA1）和来自SHG44-GSC/HOXA-AS2 shRNA1（SHG44-GSC/HOXA-AS2 shRNA1-Exo）的外泌体中该水平却下降。在与SHG44-GSC/HOXA-AS2 OE细胞共培养的SHG44细胞中，HOXA-AS2水平显著升高，而与SHG44-GSC/HOXA-AS2 shRNA1共培养的SHG44细胞则表现出相反的结果（P＜0.01，图4D）。抑制SHG44-GSC释放外泌体后，SHG44-GSC/HOXA-AS2 OE或SHG44-GSC/HOXA-AS2 shRNA1对SHG44细胞中HOXA-AS2水平没有影响（P＞0.05，图4E）。SHG44-GSC/HOXA-AS2 OE-Exo增加了SHG44细胞中HOXA-AS2的水平（HR＝−2.723，95% CI：−2.920～−2.526，P＜0.000 1），而SHG44-GSC/HOXA-AS2 shRNA1-Exo则显示出相反的结果（HR＝0.648，95% CI：0.450 5～0.844 8，P＜0.000 1，图4F）。

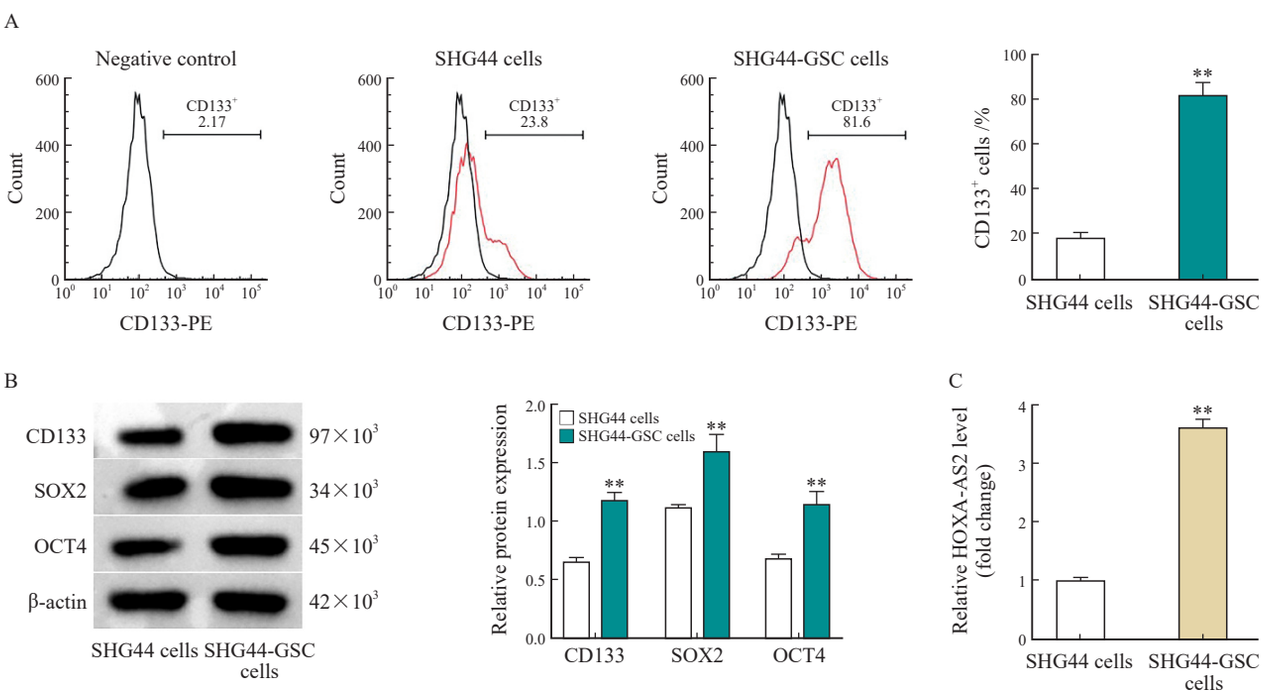

图2 HOXA–AS2在GSC中表达上调

Fig. 2 HOXA-AS2 is upregulated in GSC

A: Isolation of $CD133^+$ cells (SHG44-GSC) from SHG44 cells using flow cytometry. Meanwhile, the percentage of $CD133^+$ cells in the SHG44-GSC and SHG44 cells were detected; B: Western blot analysis of CD133, SOX2, OCT4 protein expressions in SHG44 cells and SHG44-GSC cells; C: RTFQ-PCR showing HOXA-AS2 levels in SHG44 cells and SHG44-GSC cells. **: P＜0.01, compared with SHG44 cell group.

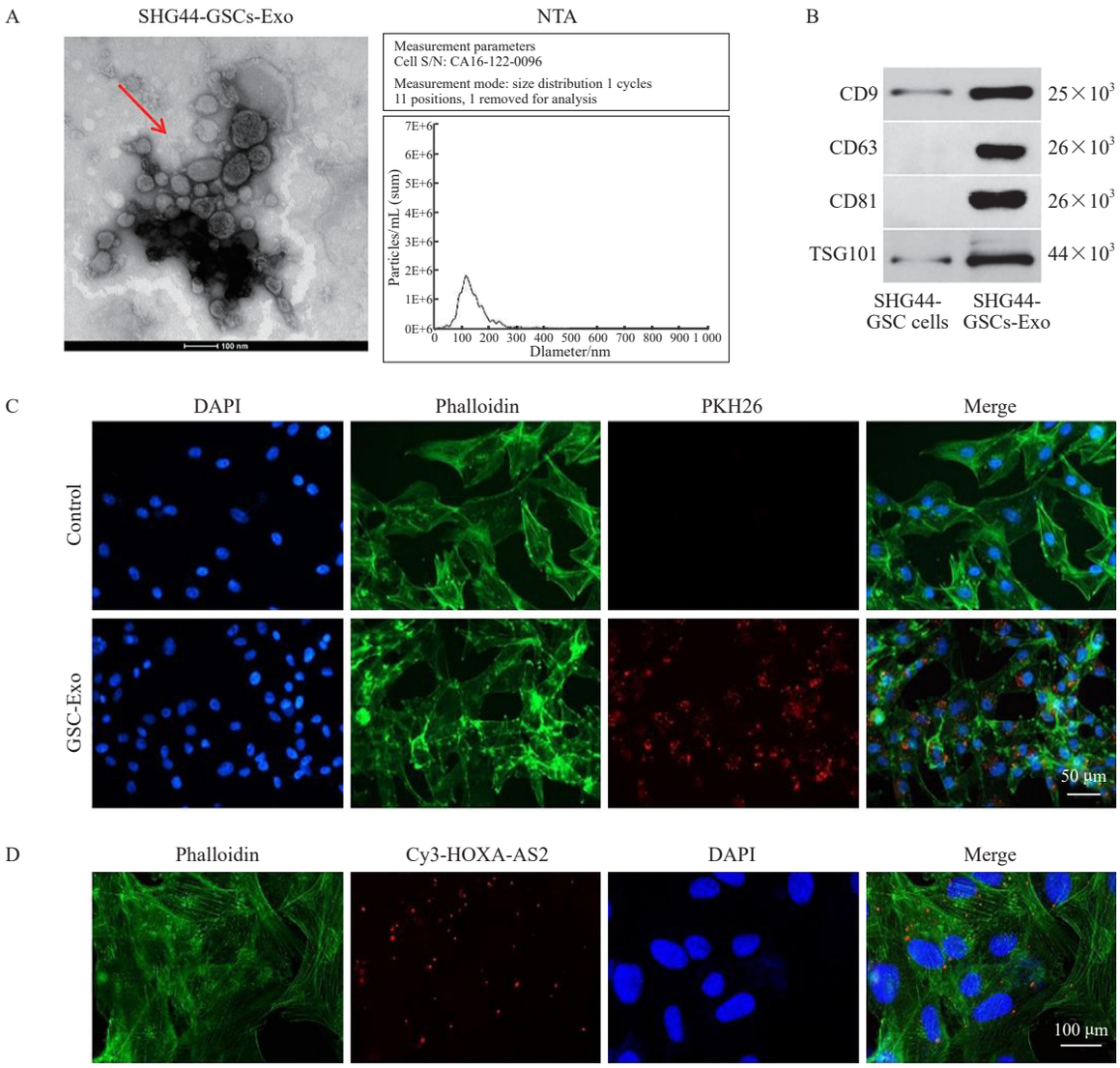

图3　外泌体颗粒表征

Fig. 3　Characterization of exosomal particles

A: NTA and TEM assays were used to identify exosomes; B: Exosome surface markers (CD9, CD63, CD81 and TSG101) detected by Western blot analysis; C: The exosomes absorbed by SHG44 cells were observed under confocal fluorescence microscope. Red color: Exosome; Green color: SHG44 cells; Blue color: Cell nucleus. Control group: SHG44 cells without the treatment of exosomes; D: Cy3-labeled HOXA-AS2 in SHG44 cells were observed under confocal fluorescence microscope. Red color: Cy3-labeled HOXA-AS2; Green color: SHG44 cells; Blue color: Cell nucleus.

2.4　SHG44-GSC来源的外泌体转染HOXA-AS2促进胶质瘤细胞增殖、迁移、侵袭和干细胞特性

本研究发现SHG44-GSC/HOXA-AS2 OE-Exo显著促进SHG44细胞增殖、迁移和侵袭（$P<0.01$），而SHG44-GSC/HOXA-AS2 shRNA1-Exo则对SHG44细胞增殖、迁移和侵袭无明显影响（图5A、5B和5C）。此外，SHG44-GSC/HOXA-AS2 shRNA1-Exo显著诱导SHG44细胞凋亡（$P<0.01$，图5D）。另外Western blot检测结果显示，SHG44-GSC/HOXA-AS2 OE-Exo显著降低SHG44细胞中cleaved caspase-3的表达，而SHG44-GSC/HOXA-AS2 shRNA1-Exo则表现出相反的结果（$P<0.01$，图5E）。

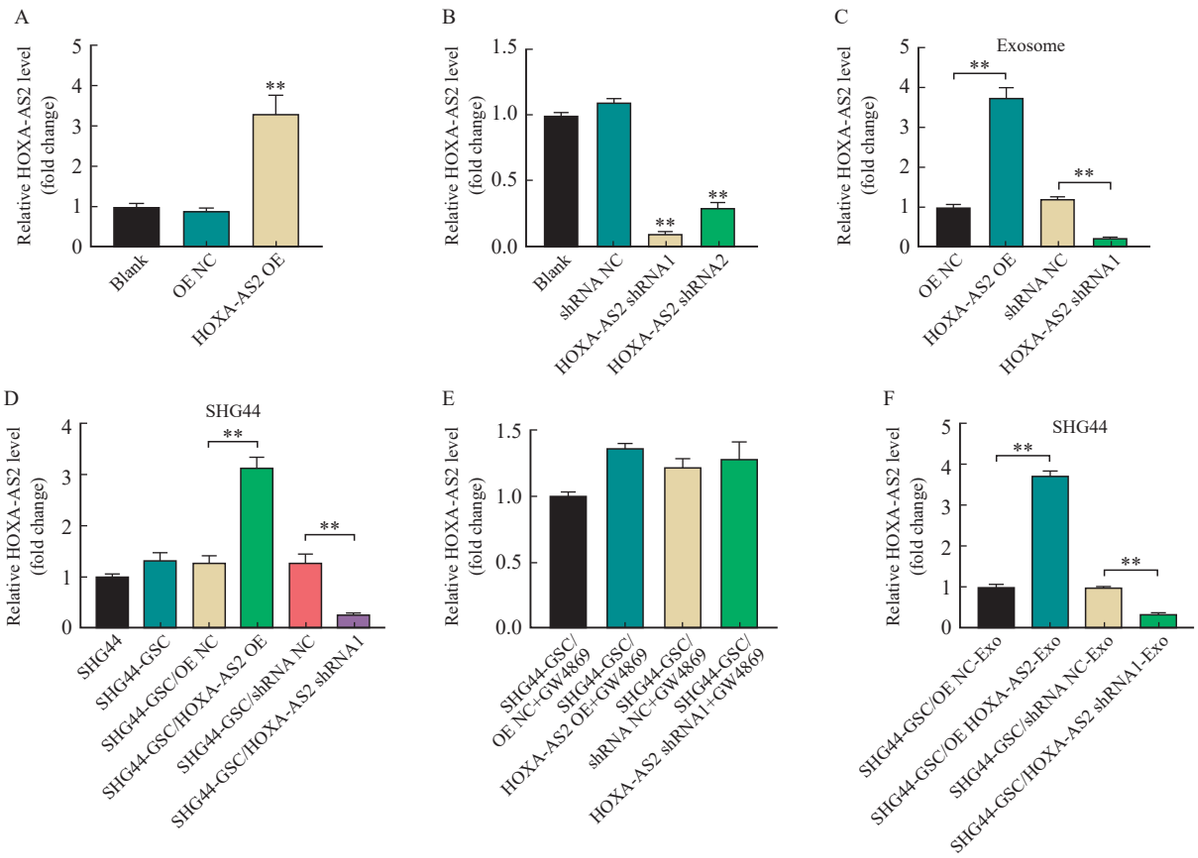

图4 SHG44–GSC通过外泌体将HOXA–AS2转移到SHG44细胞

Fig. 4 SHG44-GSC transfer HOXA-AS2 to SHG44 cells by exosomes

A: RTFQ-PCR showing HOXA-AS2 levels in SHG44-GSC transfected with HOXA-AS2 OE; B: RTFQ-PCR analysis of HOXA-AS2 level in SHG44-GSC transfected with HOXA-AS2 shRNA1 and HOXA-AS2 shRNA2; C: RTFQ-PCR analysis of HOXA-AS2 level in exosomes derived from SHG44-GSC that transfected with HOXA-AS2 shRNA1 and HOXA-AS2 shRNA2; D: RTFQ-PCR analysis of HOXA-AS2 level in SHG44 cells co-cultured with transfected SHG44-GSC; E: RTFQ-PCR showing HOXA-AS2 levels in SHG44 cells co-cultured with GW4869-treated transfected SHG44-GSC; F: RTFQ-PCR showing HOXA-AS2 levels in SHG44 cells treated with indicated exosomes. **: $P<0.01$, compared with each other.

3 讨 论

胶质瘤是中枢神经系统最常见的恶性肿瘤，由于胶质瘤的增殖能力极强，具有高度浸润性，导致患者的预后极差，其中HGG患者的中位生存时间仅有13个月[15]。因此进一步明确与胶质瘤侵袭的相关分子机制十分重要。随着分子生物学的发展，分子靶向治疗逐渐成为胶质瘤治疗的新方法，找到特异性的生物标志物是胶质瘤靶向治疗的关键[16]。

lncRNA是一组长度＞200个核苷酸的非编码RNA[17]，HOXA-AS2是lncRNA的一种，位于人染色体7p15.2，属于HOXA簇成员，以往研究发现HOXA-AS2可通过结合组蛋白去甲基酶1，使染色质发生重塑，从而影响肿瘤细胞增殖与侵袭[18]。既往的研究表明，过表达的HOXA-AS2可以促进视网膜母细胞瘤[19]、胰腺癌[20]、胆囊癌[21]细胞增殖、迁移和侵袭。目前有关HOXA-AS2在脑胶质瘤中的相关研究较少，虽然俞盛健等[22]通过建立风险模型发现HOXA-AS2可能是脑胶质瘤的预后风险lncRNA，但是并没有经过实验验证。本研究首次运用TCGA及CGGA数据库找到HOXA-AS2为共同的DelncRNA，并分析其表达情况，结果表明，高表达的HOXA-AS2与较差的OS相关，接着我们发现在胶质瘤细胞SHG44中的HOXA-AS2表达量明显高于HA1800细胞，此结果表明HOXA-AS2

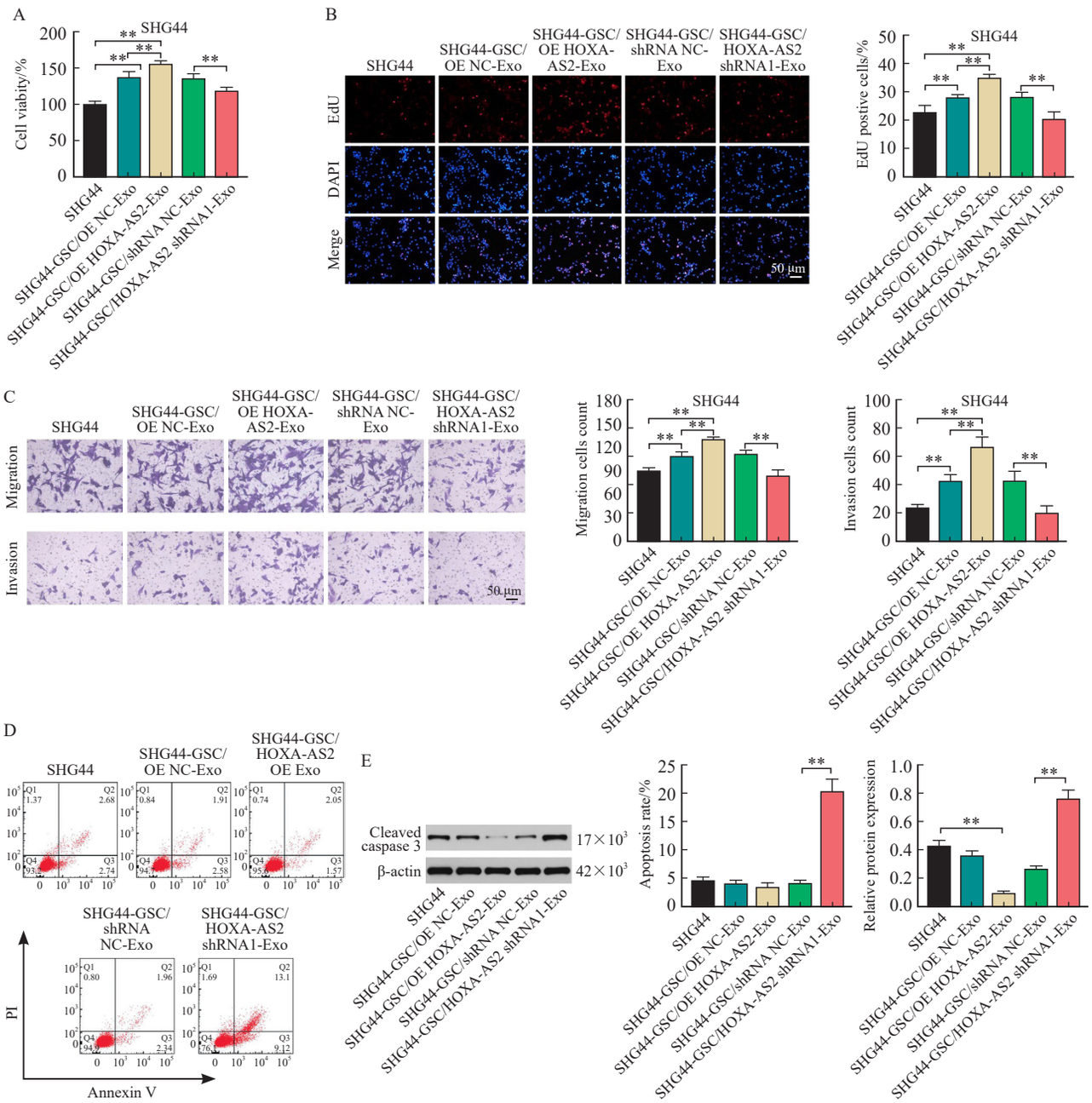

图5　SHG44–GSC来源的外泌体转染HOXA–AS2促进SHG44细胞的增殖、迁移、侵袭

Fig. 5　HOXA-AS2 transferred by SHG44-GSC-derived exosomes promoted proliferation, migration, invasion of SHG44 cells

A: CCK-8 assay of SHG44 cells incubated with indicated exosomes for 48 h; B: EdU staining assay of SHG44 cells incubated with indicated exosomes for 48 h; C: Transwell migration and invasion assays of SHG44 cells incubated with indicated exosomes for 24 h; D: Flow cytometry assay of SHG44 cells incubated with indicated exosomes for 48 h; E: Western blot showing cleaved caspase 3 expression in SHG44 cells incubated with indicated exosomes for 48 h. **: $P<0.01$, compared with each other.

在胶质瘤中高表达，且与较差的患者预后相关。但其具体机制不清楚，有学者[23]认为HOXA-AS2可通过表观遗传学介导Rho家族GTPase3的表达从而促进脑胶质瘤细胞的侵袭，也有学者[24]认为HOXA-AS2可通过microRNA（miRNA/miR）-373/EGFR轴促进胶质瘤的发展。

肿瘤干细胞具有自我更新和多向分化的能力，既往研究已经发现lncRNA参与了这一过程，如lncRNA H19的下调已被证明可以抑制胶质瘤细胞的致瘤性和干性[12]，然而目前HOXA-

AS2与GSC的关系在之前尚未确定，在本研究中，我们从SHG44细胞中分离GSC，用流式细胞术检测CD133$^+$富集的细胞，发现SHG44-GSC中CD133$^+$细胞的比例明显高于SHG44。并利用Western blot检测干细胞相关蛋白CD133、SOX2和OCT4的表达水平，发现SHG44-GSC中的上述干细胞相关蛋白表达水平较SHG44中高。并且HOXA-AS2在SHG44-GSC中的表达量也明显高于SHG44。说明lncRNA HOXA-AS2增强了胶质瘤的干细胞特性。

外泌体是大小为40～160 nm的细胞外囊泡，通过传递细胞内的lncRNA来介导细胞间的相互作用[25]。相关研究已证实外泌体在细胞之间传递lncRNA的作用，如CSC和非CSC[26-27]。以往研究已证实CSC可启动肿瘤生成，并促进多种人癌细胞侵袭和干细胞生长[28-30]。既往研究[31-32]表明，外泌体lncRNA在包括胶质瘤在内的许多癌症的发展中发挥着重要作用，与肿瘤的侵袭、转移等关系密切。Jiang等[33]指出GSC衍生的外泌体miR-944可抑制胶质瘤细胞生长和血管生成，且Conigliaro等[34]证实来自CSC样CD90$^+$细胞的外泌体lncRNA H19可促进内皮细胞的血管生成。另外，Sun等[35]发现GSC衍生的外泌体促进胶质瘤细胞增殖和干细胞特性。本研究首先分离出SHG44-GSC衍生的外泌体，接着用PKH26染液标记，发现标记的外泌体被SHG44细胞吸收，接着将转染了Cy3标记的HOXA-AS2的SHG44-GSC与SHG44细胞间接共培养，结果发现在SHG44细胞中观察到Cy3标记的HOXA-AS2。表明HOXA-AS2可通过外泌体从SHG44-GSC转移到SHG44细胞，且通过CCK-8检测及transwell法我们发现SHG44-GSC/HOXA-AS2 OE-Exo可显著促进SHG44细胞增殖、迁移和侵袭。

综上所述，本研究从组织表达、患者预后和细胞多个层面探究了来自SHG44-GSC的外泌体HOXA-AS2在胶质瘤细胞中的作用，发现其表达量升高，且与患者不良预后相关，可显著促进胶质瘤细胞增殖、迁移、侵袭和干细胞特性，提示HOXA-AS2可能是脑胶质瘤潜在的治疗靶点。但是本研究也有局限性，所以下一步可构建动物模型进行体内验证，并探索下游信号转导通路，以期在此基础上研发相应的小分子药物应用于临床。

利益冲突声明：所有作者均声明不存在利益冲突。

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（收稿日期：2022-09-25　修回日期：2022-12-01）