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## PRDM5过表达慢病毒载体的构建及稳定转染Neuro-2a细胞的建立

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**[摘要]** **目的:** 构建PR锌指区域蛋白(PRDM)5基因的过表达慢病毒载体, 建立稳定转染的小鼠神经瘤母细胞Neuro-2a, 为探讨PRDM5在缺血性脑卒中(IS)发病机制中的作用奠定基础。**方法:** 在NCBI上搜索PRDM5的序列并设计引物, PCR法扩增获取PRDM5基因序列, 将其与BamH I和Age I限制性内切酶双酶切后的慢病毒载体GV492进行连接, 构建GV492-PRDM5过表达重组质粒, 采用PCR法筛选并鉴定出的与目的基因片段长度相近的阳性克隆送生工生物工程(上海)股份有限公司测序。将测序正确的GV492-control质粒和GV492-PRDM5过表达重组质粒分别转染至人胚胎肾细胞HEK293T中, 转染48 h后离心收集慢病毒, 分别为GV492-control慢病毒和GV492-PRDM5过表达慢病毒, 采用慢病毒滴度测定法检测上述2种慢病毒滴度。将Neuro-2a细胞分为GV492-control组和GV492-PRDM5组, 分别使用GV492-control慢病毒和GV492-PRDM5过表达慢病毒感染Neuro-2a细胞, 慢病毒感染复数(MOI)为100, 感染72 h后使用嘌呤霉素( $10\text{ mg}\cdot\text{L}^{-1}$ )对成功感染GV492-control慢病毒和GV492-PRDM5过表达慢病毒的Neuro-2a细胞进行筛选, 通过荧光显微镜观察GV492-control组和GV492-PRDM5组Neuro-2a细胞的生长状态及绿色荧光蛋白的表达情况。采用实时荧光定量PCR(RT-qPCR)法检测2组Neuro-2a细胞中PRDM5 mRNA表达水平; Western blotting法检测2组Neuro-2a细胞中PRDM5蛋白表达水平。**结果:** PCR法, GV492-PRDM5重组质粒阳性转化子的长度约为684 bp, GV492-PRDM5过表达重组质粒基因序列与设计合成的PRDM5过表达序列一致; GV492-control慢病毒和GV492-PRDM5过表达慢病毒的滴度均为 $2.5\times 10^8\text{ TU}\cdot\text{mL}^{-1}$ 。荧光显微镜, GV492-control组和GV492-PRDM5组Neuro-2a细胞的生长状态均良好, 且能观察到绿色荧光蛋白的表达。RT-qPCR法, 与GV492-control组比较, GV492-PRDM5组Neuro-2a细胞中PRDM5 mRNA表达水平明显升高( $P<0.01$ )。Western blotting法, GV492-control组和GV492-PRDM5组Neuro-2a细胞在相对分子质量为75 000附近出现特异性条带; 与GV492-control组比较, GV492-PRDM5组Neuro-2a细胞中PRDM5蛋白表达水平升高( $P<0.01$ )。**结论:** 成功构建PRDM5过表达慢病毒载体, 建立了稳定转染的GV492-PRDM5-Neuro-2a细胞。

**[关键词]** PR锌指区域蛋白; 过表达慢病毒载体; 稳定表达; Neuro-2a细胞

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## Construction of PRDM5 over-expression lentivirus vector and establishment of stably transfected Neuro-2a cells

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**ABSTRACT Objective:** To construct the over-expressed lentivirus vector of PRDM5 gene and establish the Neuro-2a cells stably transfected PRDM5, and to provide the basis evidence for exploring the effect of PRDM5 in pathogenesis of ischemic stroke (IS). **Methods:** The sequence of PRDM5 was searched and designed based on NCBI. The PRDM5 gene was amplified by PCR and ligated with the lentiviral vector GV492 digested by *BamH* I and *Age* I restriction enzymes to form the GV492-PRDM5 over-expression recombinant plasmid. The positive clones with similar length and size to the target gene fragment were screened by PCR and sent to Shenggong Bioengineering (Shanghai) Co. Ltd. for identification. The correctly-sequenced GV492-control plasmid and GV492-PRDM5 over-expression recombinant plasmid were transfected into the HEK293T cells, respectively. After 48 h of transfection, the lentiviruses were collected by centrifugation, and they were GV492-control lentivirus and GV492-PRDMS over-expression lentivirus; the titers of these two lentiviruses were determined by lentiviral titer assay. The Neuro-2a cells were divided into GV492-control group and GV492-PRDM5 group, and then infected with GV492-control lentivirus and GV492-PRDM5 over-expression lentivirus, respectively, with a lentivirus multiplicity of infection (MOI) of 100. The Neuro-2a cells successfully infected with GV492-control lentivirus and GV492-PRDM5 over-expression lentivirus were screened with puromycin ( $10 \text{ mg} \cdot \text{L}^{-1}$ ) after 72 h of infection. The growth status and the expression of green fluorescence protein of Neuro-2a cells in GV492-control group and GV492-PRDM5 group were observed by fluorescence microscope. The expression levels of PRDM5 mRNA and PRDM5 protein in the Neuro-2a cells in two groups were detected by real-time fluorescence quantitative RCR (RT-qPCR) and Western blotting methods. **Results:** The PCR results showed that the length of the positive transformant of GV492-PRDM5 recombinant plasmid was about 684 bp, and the gene sequence of GV492-PRDM5 over-expression recombinant plasmid was consistent with the designed and synthesized PRDM5 over-expression sequence. The titers of GV492-control lentivirus and GV492-PRDM5 over-expression lentivirus were both  $2.5 \times 10^8 \text{ TU} \cdot \text{mL}^{-1}$ . The Neuro-2a cells in GV492-control group and GV492-PRDM5 group grew well, and the expressions of green fluorescence protein were found under fluorescence microscope. The RT-qPCR results showed that the expression level of PRDM5 mRNA in the Neuro-2a cells in GV492-PRDM5 group was significantly increased compared with GV492-control group ( $P < 0.01$ ). The Western blotting results showed that the specific bands appeared in the Neuro-2a cells in GV492-control group and GV492-PRDM5 group with a relative molecular weight of 75 000; compared with GV492-control group, the expression level of PRDM5 protein in the Neuro-2a cells in GV492-PRDM5 group was increased ( $P < 0.01$ ). **Conclusion:** The over-expression lentivirus vector of PRDM5 gene is successfully constructed, and the stably transfected GV492-PRDM5-Neuro-2a cells are established.

**KEYWORDS** PRDI-BF1 and RIZ domain proteins; Over-expression lentivirus vector; Stable expression; Neuro-2a cells

PR 锌指区域蛋白 (PRDI-BF1 and RIZ domain proteins, PRDM) 家族是拥有 17 个成员的 Kruppel

样锌指结构的保守亚家族, 其N端均有保守的PR结构域<sup>[1-4]</sup>。PR结构域在结构和功能上与SET结构域高度同源, 因此该家族成员可通过募集组蛋白修饰酶至转录启动子区域进行转录抑制或激活; 其C端连接数量不等的锌指结构 (PRDM11除外), 因此具有对DNA、RNA和蛋白的识别能力<sup>[3, 5]</sup>。PRDM5是PRDM家族成员之一, 其N端具有PR结构域, C端则含有16个锌指结构<sup>[6]</sup>。目前关于PRDM5的研究多数在肿瘤领域, PRDM5是肺癌、胃癌和宫颈癌等实体瘤的肿瘤抑制因子, 是白血病和黑色素瘤等肿瘤的促进因子<sup>[7-10]</sup>。关于PRDM5在神经系统领域作用的研究多集中于急性脊髓损伤 (acute spinal cord injury, ASCI), 如在经过过氧化氢损伤的原代脊髓神经元中, 过表达PRDM5可促进细胞炎症反应及凋亡<sup>[11]</sup>; 在缺氧处理的神经元细胞中, 微小RNA-495 (microRNA-495, miR-495) 通过靶向PRDM5上调抗凋亡蛋白的表达进而抑制细胞凋亡<sup>[12]</sup>; 在大鼠ASCI模型中, 迪乔治综合征关键区基因5 (DiGeorge syndrome critical region gene 5, DGCR5) 通过直接结合和负调节PRDM5进而减轻大鼠ASCI症状等<sup>[13]</sup>。在脂多糖诱导神经炎症的星形胶质细胞中, PRDM5可诱发星形胶质细胞凋亡<sup>[14]</sup>, 然而PRDM5在缺血性脑卒中 (ischemic stroke, IS) 的具体功能及调控机制仍未见相关报道。本研究成功构建PRDM5过表达慢病毒载体并包装为PRDM5过表达慢病毒, 通过体外感染小鼠神经瘤母细胞 (Neuro-2a) 并采用抗生素筛选获得稳定过表达PRDM5的Neuro-2a细胞, 为进一步探讨PRDM5参与IS的发病机制奠定基础, 为后续直接使用GV492-PRDM5-Neuro-2a细胞构建体外氧-糖剥夺/复氧 (oxygen and glucose deprivation/reoxygenation, OGD/R) 的脑缺血再灌注损伤模型提供条件。

## 1 材料与方法

**1.1 细胞、主要试剂和仪器** Neuro-2a细胞和人胚胎肾细胞HEK293T均购自武汉普诺赛生命科技有限公司; 慢病毒载体质粒GV492 (Ubi-MCS-3FLAG-CBh-gcGFP-IRES-puromycin) 购自上海吉凯基因化学技术有限公司, DMEM高糖培养基、Opti-MEM、MEM (含非必需氨基酸)、0.25% Trypsin-EDTA、胎牛血清 (foetal bovine serum, FBS) 和Lipofectamine 2000均购自美国Thermo Scientific公司, BamH I和Age I限制性核酸内切酶、Taq DNA

聚合酶和T4 DNA连接酶均购自美国NEB公司, 实时荧光定量PCR (real-time fluorescent quantitative PCR, RT-qPCR) 染料预混液购自北京康润诚业生物科技有限公司, Anti-PRDM5抗体购自美国SANTA CRUZ公司, Anti-GAPDH抗体均购自美国Abcam公司。倒置光学显微镜和倒置荧光显微镜购自日本Olympus公司, RT-qPCR仪 (型号: LightCycler480) 购自瑞士Roche公司, 凝胶成像系统 (型号: Azure C300) 购自美国Azure Biosystems公司。

### 1.2 Neuro-2a细胞和HEK293T细胞的培养

Neuro-2a细胞的生长培养基为MEM (含非必需氨基酸)+10%FBS+1%双抗, HEK293T细胞的生长培养基为DMEM高糖培养基+10%FBS+1%双抗, 正常条件下2种细胞平均每隔2~3d传代1次。

### 1.3 引物设计和合成

通过NCBI搜索PRDM5 (Gene ID: 70779) 序列, 结合引物设计原则和载体GV492阅读框克隆位点设计引物。PRDM5上游引物: 5'-AGGTCGACTCTAGAGGATCCCGC-CACCATGCTGGGCATGTACGTACC-3'; 下游引物: 5'-TCCTTGTAGTCCATACCGGTGCT-GTCAGTACCCCATGGATGTTG-3'。设计并合成PCR法鉴定引物, 上游引物: 5'-TCCACAAC-AGTGAGAGGACT-3'; 下游引物: 5'-CCTTAT-AGTCCTTATCATCGTC-3'。设计并合成PRDM5 qPCR引物, 上游引物: 5'-GATTGGCTACCTG-GACAGTGATG-3'; 下游引物: 5'-CACACTGT-GGACAGCAAAGTC-3'。GAPDH qPCR引物, 上游引物: 5'-AGGTCAATGAAGGGGTCGTT-3'; 下游引物: 5'-AAATGGTGAAGGTCGGTGTG-3'。上述引物均由生工生物工程 (上海) 股份有限公司合成。

### 1.4 PRDM5慢病毒载体的构建

将合成的PRDM5基因引物通过PCR法扩增PRDM5基因。PCR法反应体系 (20  $\mu$ L): DNA模板 1  $\mu$ L, 10 $\times$  PCR缓冲液 2  $\mu$ L, 上游引物 (5  $\mu$ mol $\cdot$ L<sup>-1</sup>) 2  $\mu$ L, 下游引物 (5  $\mu$ mol $\cdot$ L<sup>-1</sup>) 2  $\mu$ L, dNTP (2.5 mmol $\cdot$ L<sup>-1</sup>) 2  $\mu$ L, 高保真Taq酶PrimeSTAR 0.15  $\mu$ L, ddH<sub>2</sub>O加至20  $\mu$ L进行PCR反应。酶切体系 (20  $\mu$ L): GV492 (1 g $\cdot$ L<sup>-1</sup>) 1  $\mu$ L, 10 $\times$ NEB Buffer 2  $\mu$ L, BamH I 0.5  $\mu$ L, Age I 0.5  $\mu$ L, ddH<sub>2</sub>O加至总体积20  $\mu$ L, 置于37  $^{\circ}$ C恒温箱酶切过夜。将PCR法扩增

出的PRDM5基因连接至GV492载体上,连接体系(15  $\mu\text{L}$ ): PRDM5 DNA 8  $\mu\text{L}$ , GV492载体 2  $\mu\text{L}$ ,  $10\times\text{T4}$ 连接缓冲液 1.5  $\mu\text{L}$ , T4 DNA连接酶 1  $\mu\text{L}$ , ddH<sub>2</sub>O加至 15  $\mu\text{L}$ 。次日将连接产物进行转化,挑取单克隆进行酶切鉴定。

**1.5 PRDM5慢病毒包装** 于HEK293T细胞密度约为80%时,按照传代细胞与总细胞1:3的比例均匀铺于100 mm细胞培养皿中培养过夜。转染体系分为Mixture A和Mixture B体系, Mixture A: 750  $\mu\text{L}$  Opti-MEM+15  $\mu\text{L}$  Lipofactamine 2000; Mixture B: 750  $\mu\text{L}$  Opti-MEM+7.5  $\mu\text{g}$  GV492-control或GV492-PRDM5+3.75  $\mu\text{g}$  pHelper 1.0+3.75  $\mu\text{g}$  pHelper 2.0。将Mixture A和Mixture B混合物轻柔滴加到细胞培养皿中,放回培养箱继续培养4~6 h,换成15 mL的生长培养基后继续培养48 h,即可收集细胞培养基进行病毒浓缩。

**1.6 PRDM5慢病毒的滴度测定** 将HEK293T细胞以每孔 $5\times 10^4$ 个的密度铺至96孔细胞培养板内,次日准备5个EP管,每个EP管内均加入90  $\mu\text{L}$ 的无血清培养基。在第1个EP管中再加入10  $\mu\text{L}$ 病毒原液,第1个EP管即为1E+1  $\mu\text{L}$ ;从第1个EP管中吸取10  $\mu\text{L}$ 置于第2个EP管中,第2个EP管即为1E+0  $\mu\text{L}$ ;随后从第2个EP管中吸取10  $\mu\text{L}$ 置于第3个EP管中,第3个EP管即为1E-1  $\mu\text{L}$ 。继续相同的操作直到第5个EP管;随后在对应孔内分别加入上述含病毒的培养基,4 d后在荧光显微镜下观察细胞中绿色荧光表达情况。

**1.7 PRDM5过表达慢病毒感染Neuro-2a细胞及构建稳定细胞** 将细胞均匀铺在12孔细胞培养板中,选择感染复数(multiplicity of infection, MOI)为10、25、50和100,进行慢病毒感染Neuro-2a细胞实验。将Neuro-2a细胞分为GV492-control组和GV492-PRDM5组,分别将GV492-control慢病毒和GV492-PRDM5过表达慢病毒加入至Neuro-2a细胞生长培养基中,配置含慢病毒的细胞培养基进行Neuro-2a细胞感染,24 h换液继续培养48 h,期间在荧光显微镜下观察慢病毒的感染效率和细胞的生长状态。感染48 h后采用嘌呤霉素(10  $\text{mg}\cdot\text{L}^{-1}$ )对感染慢病毒的细胞进行筛选,持续筛选2周。

**1.8 RT-qPCR法检测2组Neuro-2a细胞中PRDM5 mRNA表达水平** 使用TRIzol法提取Neuro-2a细胞中RNA反转录为cDNA,进行RT-qPCR反应,检测2组Neuro-2a细胞中PRDM5 mRNA表

达水平。独立进行3次实验后,以GAPDH为内参,采用 $2^{-\Delta\Delta\text{Ct}}$ 法计算Neuro-2a细胞中PRDM5 mRNA表达水平。

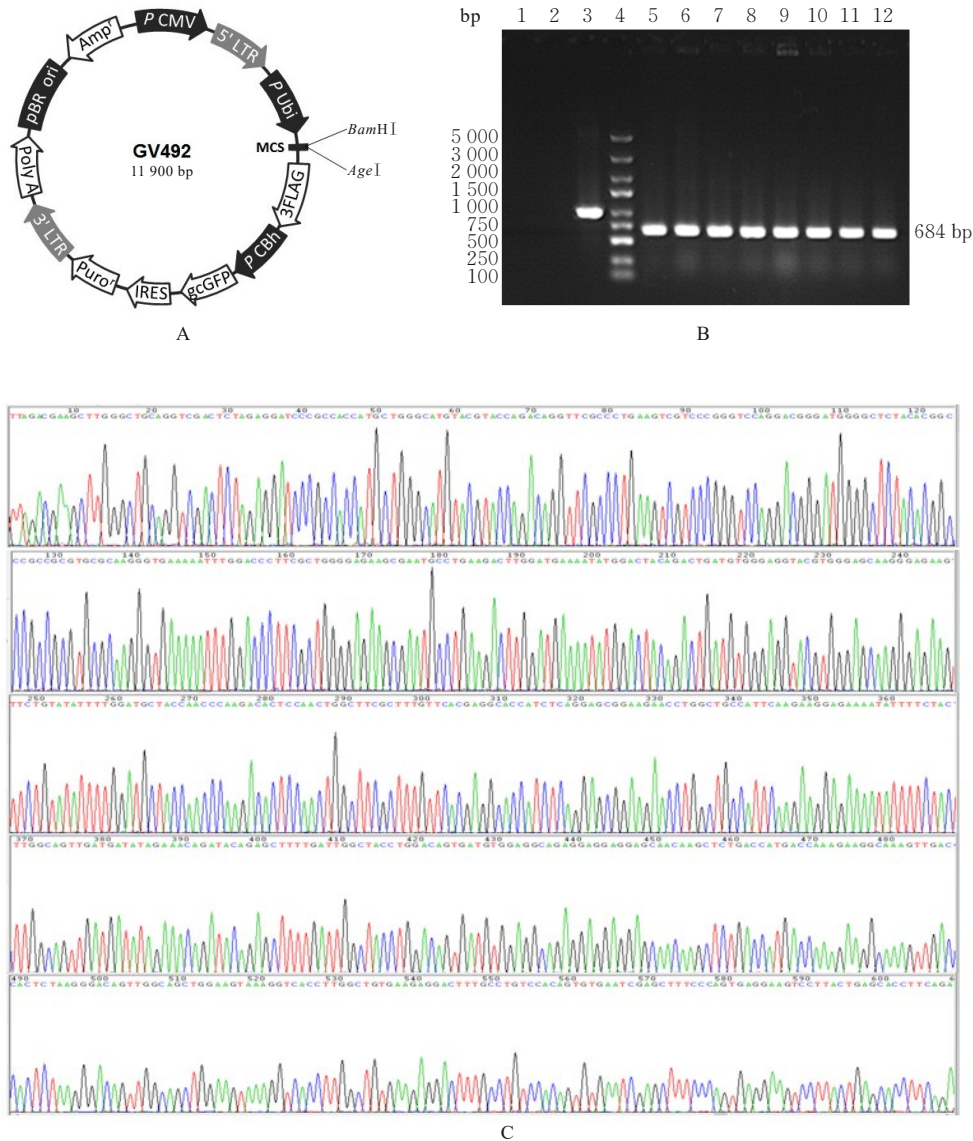
**1.9 Western blotting法检测2组Neuro-2a细胞中PRDM5蛋白表达水平** 使用RIPA(含蛋白酶抑制剂)裂解细胞收集蛋白,采用Western blotting法检测2组Neuro-2a细胞中PRDM5蛋白表达水平。电泳条件:50 V、30 min,100 V、90 min。转膜后将对应条带分别加入PRDM5或GAPDH一抗4  $^{\circ}\text{C}$ 孵育过夜,次日室温孵育二抗1 h进行条带曝光。独立进行4次实验后,以GAPDH为内参,采用Image J软件对条带进行灰度值分析,计算PRDM5蛋白表达水平。PRDM5蛋白表达水平=PRDM5蛋白条带灰度值/GAPDH蛋白条带灰度值。

**1.10 统计学分析** 采用Graphpad Prism 6.0统计软件进行统计学分析。2组Neuro-2a细胞中PRDM5 mRNA和蛋白表达水平均符合正态分布,以 $\bar{x}\pm s$ 表示,2组间样本均数比较采用两独立样本 $t$ 检验。以 $P<0.05$ 为差异有统计学意义。

## 2 结果

**2.1 PRDM5过表达慢病毒载体的构建** 通过NCBI搜索PRDM5(Gene ID: 70779)序列设计引物,携带gcGFP/Puromycin的慢病毒GV492载体通过限制性内切酶BamH I和Age I双酶切后,将PCR扩增的PRDM5基因连接至GV492载体上(图1A)。PCR法鉴定结果显示:GV492-PRDM5过表达重组质粒的基因片段长度约为684 bp,与预期结果一致(图1B)。将GV492-PRDM5质粒送生工生物工程(上海)股份有限公司测序,将测序成功的序列与设计的PRDM5序列比对,二者的DNA序列完全匹配(图1C),表明PRDM5序列成功克隆至GV492载体中,PCR法鉴定和测序结果提示GV492-PRDM5慢病毒载体构建成功。

**2.2 慢病毒感染及PRDM5过表达的Neuro-2a细胞的构建** GV492空载质粒和GV492-PRDM5重组质粒分别与辅助质粒共转染至HEK293T细胞,转染48 h后在荧光显微镜下可观察到绿色荧光,表明GV492-control慢病毒和GV492-PRDM5重组过表达慢病毒包装成功。将收集的病毒液进行滴度测定:GV492-control慢病毒和GV492-PRDM5过表达慢病毒的滴度均为 $2.5\times 10^8\text{ TU}\cdot\text{mL}^{-1}$ ;根据MOI=100取相应的病毒量感染Neuro-2a细胞,感



A: Construction of PRDM5 over-expression lentivirus vector; B: PCR identification results[Lane 1: Negative control (ddH<sub>2</sub>O); Lane 2: Negative control (GV492 empty plasmid); Lane 3: Positive control (GAPDH); Lane 4: Marker; Lane 5-12: 1 to 8 recombinants (GV492-PRDM5 over-expression recombinant plasmid)]; C: Sequencing results of GV492-PRDM5 over-expression lentivirus plasmid.

图1 PRDM5过表达慢病毒载体的构建和鉴定

Fig. 1 Construction and identification of PRDM5 over-expressed lentiviral vector

染72 h后加入嘌呤霉素(10 mg·L<sup>-1</sup>)进行筛选24 h, 随后继续使用半量的嘌呤霉素(5 mg·L<sup>-1</sup>)对细胞继续筛选2周, 在荧光显微镜下观察到GV492-control慢病毒和GV492-PRDM5过表达慢病毒感染Neuro-2a细胞生长状态良好且表达绿色荧光(图2), 表明GV492-control-Neuro-2a和GV492-PRDM5-Neuro-2a稳定表达细胞构建成功。

**2.3 2组 Neuro-2a 细胞中 PRDM5 mRNA 表达水平** 与GV492-control组(1.09±0.11)比较, GV492-PRDM5组Neuro-2a细胞中PRDM5 mRNA表达水平(2.33±0.15)明显升高(P<0.01)。

#### 2.4 2组 Neuro-2a 细胞中 PRDM5 蛋白表达水平

GV492-control组和GV492-PRDM5组Neuro-2a细胞中在相对分子质量约为75 000处均出现特异性条带, 提示Neuro-2a细胞中PRDM5蛋白表达成功。与GV492-control组(1.09±0.04)比较, GV492-PRDM5组细胞中PRDM5蛋白表达水平(1.81±0.18)明显升高(P<0.01)。见图3。

### 3 讨论

脑卒中包括出血性脑卒中和IS, 其中IS占80%以上<sup>[15]</sup>。IS是一种由于血栓阻塞导致血流不

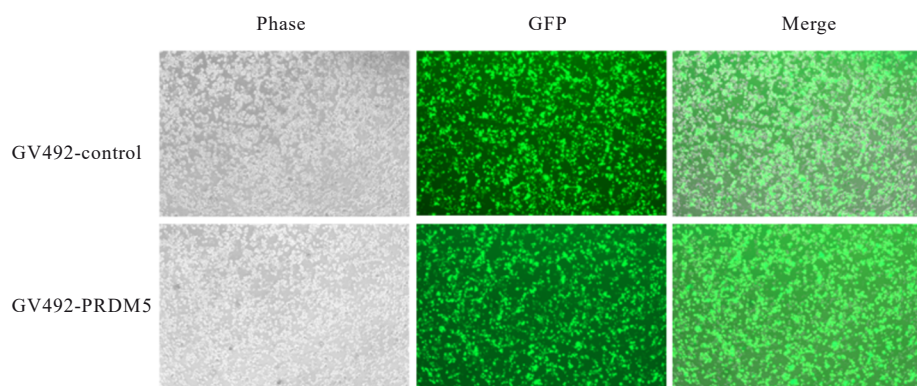
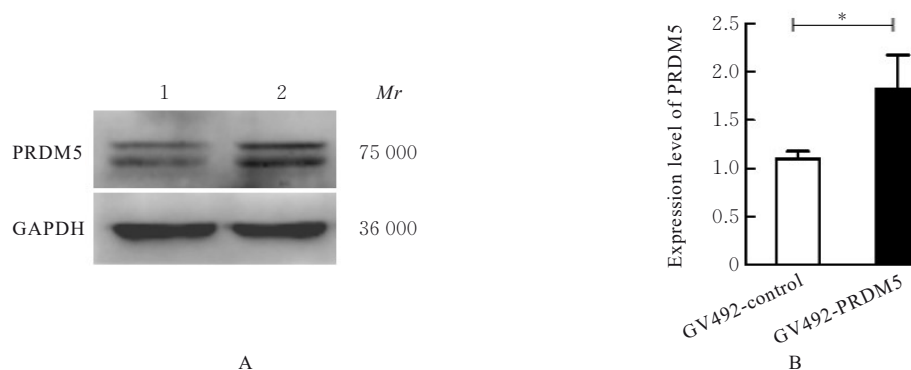


图2 荧光显微镜下观察 GV492-control 和 GV492-PRDM5 慢病毒感染后 Neuro-2a 细胞的形态表现及荧光表达( $\times 4$ )  
Fig. 2 Morphology and fluorescence expression of Neuro-2a cells after GV492-control and GV492-PRDM5 lentivirus infection observed under fluorescence microscope( $\times 4$ )



A: Electrophoregram (Lane 1: GV492-control group; Lane 2: GV492-PRDM5 group); B: Histogram. ( $*P < 0.01$  vs GV492-control group).

图3 2组 Neuro-2a 细胞中 PRDM5 蛋白表达情况

Fig. 3 Expressions of PRDM5 protein in Neuro-2a cells in two groups

畅而造成脑组织缺血缺氧坏死的神经系统疾病,其主要临床表现为肢体偏瘫、视野偏盲和感觉障碍等,严重可致患者意识障碍甚至死亡<sup>[16-17]</sup>。目前 IS 备受国内外专家关注,与其高患病率、高致残率、高死亡率和高医疗负担等存在密切关联。国际上 IS 最推荐的治疗方案是应用重组组织型纤溶酶原激活剂 (recombinant tissue plasminogen activator, rtPA) 进行静脉溶栓,但该治疗方法存在治疗时间窗短及出血风险大等弊端,因此有必要寻找一种更高效安全的治疗方法<sup>[18-19]</sup>。

PRDM5 可能在缺氧诱导脊髓损伤细胞模型的中枢神经系统病理生理中发挥重要作用<sup>[5]</sup>, miR-182 和 miR-7a 能够通过抑制 PRDM5 的表达进而抑制神经元的凋亡,减轻大鼠急性脊髓损伤<sup>[20]</sup>。敲除长链非编码 RNA BDNF 反义 RNA (long non-coding RNA BDNF antisense RNA, lncRNA BDNF-AS) 能够通过下调 miR-130b-5p 靶基因 PRDM5 抑制缺

氧诱导的急性脊髓损伤中神经细胞凋亡<sup>[21]</sup>。IS 常用的脑缺血再灌注损伤细胞模型即 OGD/R 也涉及到缺氧处理<sup>[22-25]</sup>,因此本文作者猜测 PRDM5 可能参与 IS 的发生发展。

通过人类免疫缺陷病毒 1 型 (human immunodeficiency virus type 1, HIV-1) 构建的慢病毒载体能够通过外源基因有效地整合到受体细胞的染色体上,从而使外源基因得到长期稳定表达<sup>[26]</sup>。本实验构建 GV492-PRDM5 过表达慢病毒载体,可使 PRDM5 得到长期稳定的表达。关于构建 PRDM5 过表达慢病毒载体及 PRDM5-Neuro-2a 细胞目前尚未见相关报道,因此本实验构建 PRDM5 稳定感染的细胞株具有一定的创新性,为后续直接使用 GV492-PRDM5 慢病毒稳转细胞系构建 OGD/R 脑缺血再灌注损伤模型提供参考,也为研究 PRDM5 在 IS 发生发展中作用提供条件。本研究 PCR 法鉴定结果和 DNA 测序结果证实 GV492-

PRDM5过表达慢病毒载体构建成功, 慢病毒感染 Neuro-2a 细胞后可在荧光显微镜下观察到绿色荧光, RT-qPCR法检测 2 组 Neuro-2a 细胞中 PRDM5 mRNA 的表达, 结果显示: GV492-PRDM5 组 Neuro-2a 细胞中 PRDM5 mRNA 表达水平较 GV492-control 组明显升高。Western blotting 法检测结果显示: 2 组 Neuro-2a 细胞中均有 PRDM5 蛋白的表达, GV492-PRDM5 组 Neuro-2a 细胞中 PRDM5 蛋白表达水平较 GV492-control 组明显升高。提示 GV492-PRDM5 的 Neuro-2a 细胞系构建成功, 并且可以升高 PRDM5 的表达水平。

综上所述, 本研究成功构建了 GV492-PRDM5 慢病毒表达载体, 建立了稳定过表达 GV492-PRDM5 的 Neuro-2a 细胞, 为进一步探讨 PRDM5 在 IS 的作用机制奠定了基础。

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所有作者声明不存在利益冲突。

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