.实验研究.

下调RNA甲基化酶NSUN2表达对脑胶质瘤U87细胞增殖、侵袭、迁移的影响

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【摘要】**目的** 探讨下调NOP2/Sun RNA甲基转移酶家族成员2(NSUN2)表达对脑胶质瘤细胞增殖、侵袭、迁移的影响。**方法** 体外培养正常胶质细胞HEB、胶质瘤细胞系(A172、U251、U87),免疫印迹法检测NSUN2蛋白表达水平;用shNSUN2慢病毒(sh−NSUN2组)及shRNA scramble慢病毒(sh−CON组)感染U87细胞下调NSUN2表达,用CCK−8法检测细胞增殖活力,Transwell实验检测细胞侵袭和迁移。**结果** 与正常胶质细胞HEB相比,胶质瘤细胞系A172、U251、U87的NSUN2蛋白表达量均明显增高(P<0.05)。与sh−CON组比较,sh−NSUN2组NSUN2蛋白表达水平、细胞增殖活力、侵袭能力和迁移能力均明显降低(P<0.05)。**结论** 胶质瘤NSUN2呈高表达,下调其表达明显抑制胶质瘤细胞增殖、侵袭和迁移。

【关键词】胶质瘤;U87细胞;细胞增殖;细胞侵袭;细胞迁移;RNA甲基转移酶

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Effects of down−regulated expression of RNA methylase NSUN2 on proliferation, invasion, and migration of glioma U87 cells

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【**Abstract**】**Objective** To investigate the effect of down−regulating the expression of NOP2/Sun RNA methyltransferase family member 2 (NSUN2) on proliferation, invasion, and migration of glioma cells. **Methods** HEB and glioma cell lines (A172, U251, U87) were cultured in vitro, and NSUN2 protein expression levels were detected using the western blotting. U87 cells were infected with shNSUN2 lentivirus (sh−NSUN2 group) and shRNA scramble lentivirus (sh−CON group) to dowm−regulate NSUN2 expression. Cell proliferation activity was detected using the CCK−8 method, and cell invasion and migration were detected using the Transwell assay. **Results** Compared with HEB cells, expressions of NSUN2 protein in A172, U251, and U87 cells were significantly increased (P<0.05). Compared with the sh−CON group, NSUN2 protein expression level, cell proliferation activity, invasion ability, and migration ability of the sh−NSUN2 group were significantly decreased (P<0.05). **Conclusions** NSUN2 is highly expressed in gliomas, and down−regulation of NSUN2 can inhibit the proliferation, invasion, and migration of glioma cells.

【**Key words**】Glioma; U87 cell; Cell proliferation; Cell invasion; Cell migration; RNA methyltransferase

胶质瘤是常见的颅内原发性肿瘤,尽管近年来脑胶质瘤的治疗取得了长足进展,但是其5年生存率未见明显提高[1, 2]。5−甲基胞嘧啶(5−methylcytidine,M5C)在tRNA、rRNA和mRNA中均存在,作为一种可逆的表观遗传学修饰,RNA M5C修饰在RNA稳定性调控、蛋白结合及转录调控中发挥重要作用[3]。研究显示,RNA M5C修饰在肿瘤发生、发展及免疫治疗中有潜在价值[4]。NOP2/Sun RNA甲基转移酶家族成员2(NOP2/Sun RNA methyltransferase family member 2, NSUN2)是M5C RNA甲基转移酶,可通过影响肿瘤细胞增殖、侵袭、凋亡、免疫浸润而参与肝细胞癌[5]、黑素素瘤[6]、鼻咽癌[7]等进展。本文探讨NSUN2对脑胶质瘤细胞增殖、侵袭和迁移的影响。

1 材料与方法

1.1 细胞培养　胶质瘤细胞系(A172、U251、U87)和正常胶质细胞HEB(上海研匠生物科技有限公司),用含10%胎牛血清的DMEM高糖培养基(上海语纯生物科技有限公司)培养,细胞融合度达90%时进行传代。

1.2 免疫印迹法检测细胞NSUN2蛋白表达水平　收集HEB、胶质瘤细胞系(A172、U251、U87),加入细胞裂解液和蛋白酶抑制剂(上海研匠生物科技有限公司),冰上裂解30 min后离心10 min,用BCA试剂盒(上海语纯生物科技有限公司)进行蛋白定量。随后加入上样缓冲液,并煮沸5 min,用SDS−PAGE分离

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蛋白并将其转移至PVDF膜上，5%脱脂牛奶封闭1 h。加入NSUN2抗体（1:500，美国Sigma公司）和内参GAPDH抗体（1:1 000，美国Sigma公司）孵育过夜。洗膜3次后，加入羊抗兔IgG抗体（1:5 000，美国Sigma公司）孵育1 h，洗膜3次，最后进行ECL显色。

1.3 U87细胞转染及分组 慢病毒构建包装服务由上海吉玛公司提供，用HEK293T细胞包装shNSUN2慢病毒（sh-NSUN2组）及shRNA scramble慢病毒（sh-CON组），并感染U87细胞，48 h后进行传代，用嘌呤霉素筛选法筛选稳定低表达NSUN2基因的细胞，免疫印迹法检测转染效率。

1.4 CCK-8法检测U87细胞增殖活力 严格按照CCK-8试剂盒（上海炎怡生物科技有限公司）进行操作。取对数生长期U87细胞，以1×10^4个/孔密度接种于96孔板。待细胞贴壁后，培养24、48、72 h，加入10 μl CCK-8溶液。用酶标仪检测光密度值（optical density，OD）。实验重复4次。

1.5 Transwell实验检测U87细胞侵袭和迁移 严格按照Transwell试剂盒（上海多沃生物科技有限公司）进行操作。迁移实验：将细胞浓度调整为1.5×10^5/ml，取200 μl细胞悬液加入Transwell上室；向下室加入600 μl培养液，培养2 h后弃去培养液，用棉签擦去上室中未迁移细胞，加入甲醇固定10 min；随后用0.1%结晶紫染色15 min，显微镜下观察细胞形态并计数细胞。侵袭实验：用细胞培养液将Matrigel基质胶稀释15倍，随后加入Transwell上室，37 ℃培养4 h，后续步骤同迁移实验。

1.6 统计学方法 应用SPSS 20.0软件处理；计量资料以$\bar{x}\pm s$表示，采用单因素方差分析和t检验；$P<0.05$为差异有统计学意义。

2 结 果

2.1 胶质瘤细胞NSUN2的表达 与正常胶质细胞HEB相比，胶质瘤细胞系A172、U251、U87的NSUN2蛋白表达量均明显增高（$P<0.05$，图1）。

2.2 下调NSUN2表达对U87细胞增殖、侵袭、迁移的影响 与sh-CON组比较，sh-NSUN2组NSUN2蛋白表达水平、细胞增殖活力、侵袭能力和迁移能力均明显降低（$P<0.05$，图2）。

3 讨 论

脑胶质瘤，特别是高级别脑胶质瘤，侵袭性高、术后易复发、预后较差[8~10]，寻找脑胶质瘤的生物学标志物对胶质瘤的诊治、早期预测预后具有重要临床意义。NSUN2最早被发现是tRNA甲基转移酶。近年来，研究发现NSUN2还可以甲基化rRNA、非编码RNA和部分小分子RNA[11]。另外，NSUN2被证实是M5C RNA甲基转移酶[11,12]。有研究发现，NSUN2在mRNA上的甲基化可以调控靶基因表达，参与肿瘤发生、发展[13~15]。黄奕芝等[16]发现，NSUN2可能通过影响甲基化嘌呤代谢及嘧啶代谢通路中的磷酸核糖焦磷酸合成酶2 mRNA，促进核苷酸代谢，影响肝细胞癌进展。Chen等[17]采用重亚硫酸盐测序和单细胞测序技术发现，NSUN2以M5C依赖方式促进肝癌源性生长因子mRNA的稳定性，并促进肿瘤细胞增殖和侵袭。本研究发现，胶质瘤细胞系A172、U251、U87的NSUN2蛋白表达量均高于正常胶质细胞，沉默NSUN2表达，明显抑制细胞增殖、侵袭和迁移。但是本研究也存在一些局限性，例如未检测NSUN2下游靶基因甲基化水平变化，未分析NSUN2的调控机制。

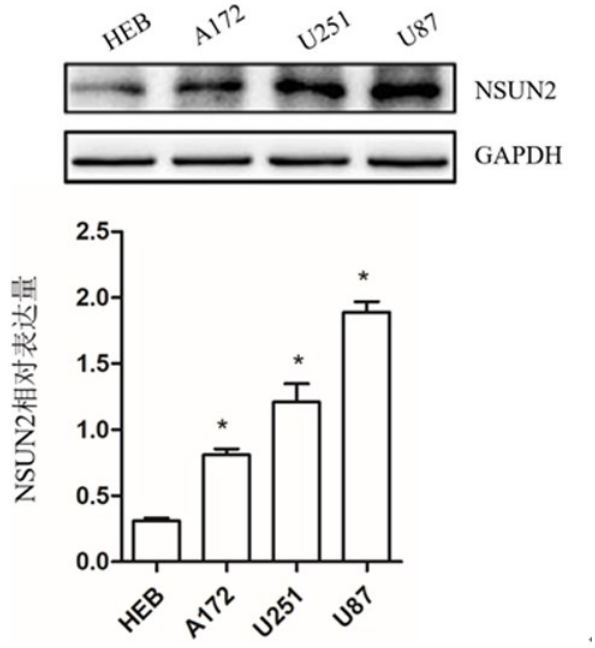

图1 免疫印迹法检测胶质瘤细胞NSUN2表达
与HEB组比较，* $P<0.05$

总之，胶质瘤NSUN2呈高表达，下调其表达明显抑制胶质瘤细胞增殖、侵袭和迁移。

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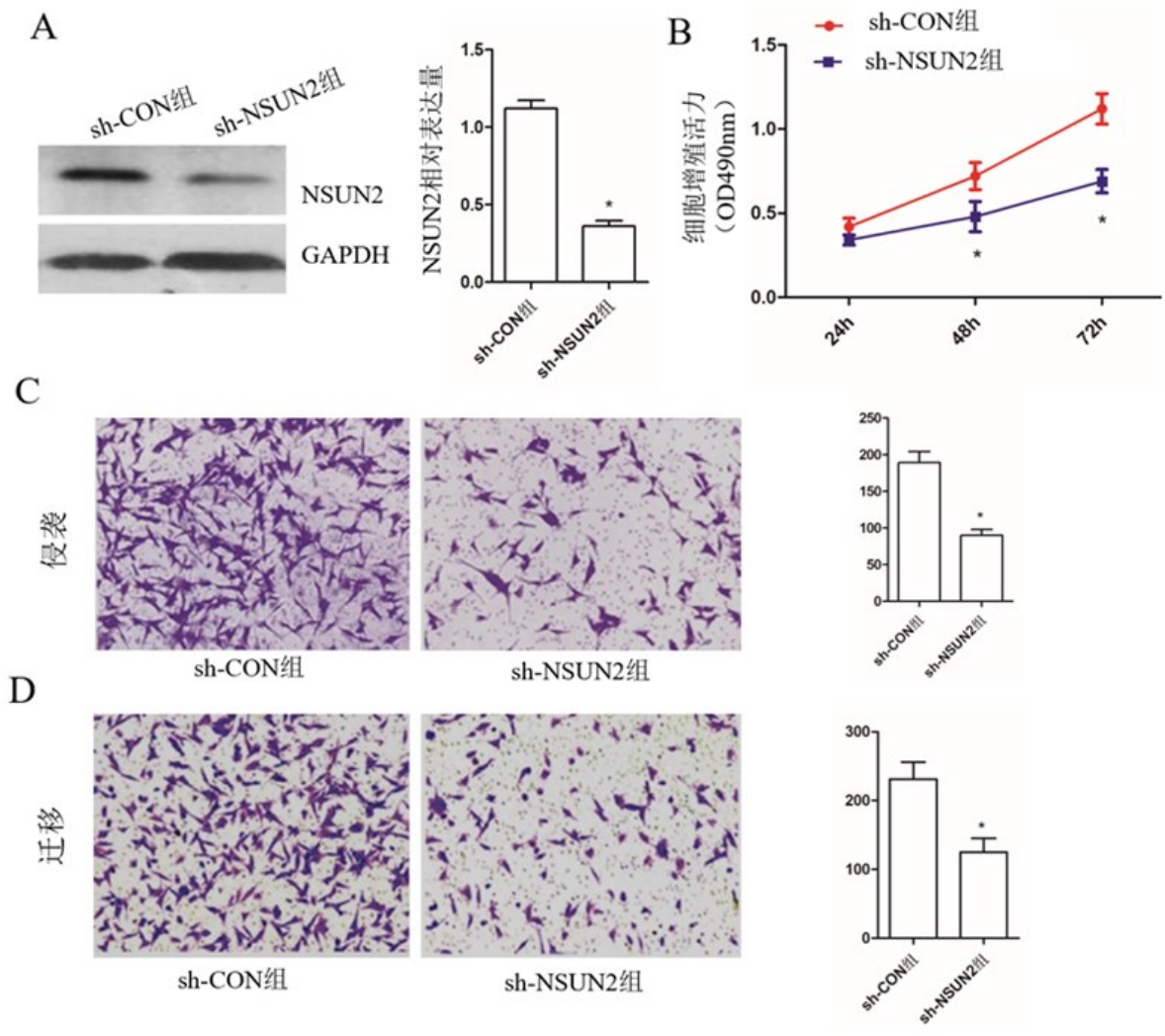

图2 下调NSUN2表达对U87细胞增殖、侵袭、迁移的影响

A. 免疫印迹法检测NSUN2蛋白表达水平；B. CCK-8法检测细胞增殖活力；C. Transwell检测细胞侵袭；D. Transwell检测细胞迁移；与sh-CON组比较，* $P<0.05$

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