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·基础研究·

盘状蛋白结构域受体1调控胶质瘤细胞增殖和凋亡的机制研究

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[摘 要] **目的** 探究盘状蛋白结构域受体1(DDR1)对胶质瘤细胞增殖和凋亡的调控作用和机制。**方法** 通过qRT-PCR和Western blot检测人胶质瘤细胞和正常胶质细胞中DDR1的表达量。用慢病毒感染法构建稳定过表达和敲降DDR1的胶质瘤细胞U251,利用CCK-8法检测稳定细胞系的增殖能力,并用流式细胞仪检测细胞凋亡能力。利用Western blot检测稳定细胞系中相关通路的激活情况,探究DDR1的调控机制。**结果** qRT-PCR和Western blot结果显示,人胶质瘤细胞U251中DDR1的表达量高于人胶质瘤细胞U87细胞和正常胶质细胞HEB,因此,选择U251细胞系进行后续实验。CCK-8实验显示,过表达DDR1能够促进胶质瘤细胞的增殖,敲降DDR1则抑制了胶质瘤细胞的增殖。流式细胞结果显示,过表达DDR1能够抑制胶质瘤细胞的凋亡,敲降DDR1则促进胶质瘤细胞凋亡。Western blot结果显示,过表达DDR1激活了PI3K/AKT通路,敲降DDR1抑制了PI3K/AKT通路的激活。**结论** DDR1能够通过激活PI3K/AKT通路增强胶质瘤细胞的增殖能力并抑制其凋亡,促进了胶质瘤细胞的发展。因此,DDR1可能成为治疗胶质瘤的一个潜在靶点。

[关键词] 胶质瘤;盘状蛋白结构域受体1;增殖;凋亡;PI3K/AKT通路
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Mechanism of disidin domain receptor 1 regulating the proliferation and apoptosis of glioma cells

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Abstract: Objective To explore the regulatory effect and mechanism of disidin domain receptor 1 (DDR1) on the proliferation and apoptosis of glioma cells. **Methods** The expression levels of DDR1 in human glioma cells and normal glial cells were detected by qRT-PCR and Western blot. Glioma cells U251 with stable overexpression and knockdown of DDR1 were constructed by lentivirus infection, the proliferation ability of the stable cell line was detected by CCK-8 assay, and the apoptosis ability was detected by flow cytometry. Western blot was used to detect the activation of related pathways in stable cell lines and explore the regulatory mechanism of DDR1. **Results** The results of qRT-PCR and Western blot showed that the expression level of DDR1 in human glioma cell U251 was higher than those in human glioma cell U87 and normal glial cell HEB. Therefore, the U251 cell line was selected for subsequent experiments. CCK-8 assay showed that overexpression of DDR1 promote the proliferation of glioma cells, while knockdown of DDR1 inhibit the proliferation of glioma cells. The results of flow cytometry showed that overexpression of DDR1 inhibit the apoptosis of glioma cells, while knockdown of DDR1 induce the apoptosis of glioma cells. Western blot results showed that overexpression of DDR1 activate the PI3K/AKT pathway, while knockdown of DDR1 inhibit the activation of the PI3K/AKT pathway. **Conclusion** DDR1 can enhance the proliferation ability of glioma cells and inhibit their apoptosis by activating the PI3K/AKT pathway, promoting the development of glioma cells. Therefore, DDR1 may become a potential target for the treatment of glioma.

Keywords: glioma; disidin domain receptor 1; proliferation; apoptosis; PI3K/AKT pathway

胶质瘤(glioblastoma multiforme,GBM)是一种源自神经组织的恶性原发性脑肿瘤,约占所有原发性脑肿瘤的50%。胶质瘤发病率高、预后差,患者的平均存活期15~20个月[1-4],生存时间为3~5年的胶质瘤患者仅占5%[3,5]。由于胶质瘤细胞增殖能力强,手术难以彻底清除病灶,导致其复发率和病死率居高不下[6]。因此,深入探讨胶质瘤的发生发展机制,探究新的治疗策略和靶点对提高胶质瘤的治愈率具有重要临床意义。

盘状蛋白结构域受体1(disidin domain receptor 1,DDR1)属于非整合素胶原受体家族,具有酪氨酸激酶活性,是一种被广泛研究的酪氨酸激酶受体(disk-shaped receptor tyrosine kinase, DDRs),主要表达于正常上皮细胞,参与细胞外基质重塑及上皮细胞与基底基质的相互作用[7]。研究表明,DDR1异常表达与包括癌症在内的多种疾病密切相关,其广泛分布于肺、结

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肠、胰腺和乳腺等正常上皮组织中,在肺癌、肝癌、乳腺癌等快速生长的肿瘤中显著上调,且通过调控肿瘤细胞与胶原基质的相互作用促进癌症进展,其过表达可加速体内肿瘤生长,促进癌症进展[8-17]。尽管已有研究证实DDR1参与胶质瘤的发生发展[18],但其具体调控机制仍不明确。

基于此,本研究拟通过抑制DDR1表达,探讨其对胶质瘤细胞增殖与凋亡的调控作用及潜在机制,以期为胶质瘤治疗提供新的靶点与理论依据。

1 材料与方法

1.1 材料

人胶质瘤细胞系U87、U251和正常胶质细胞株HEB均购自中科院上海细胞库,细胞培养液购自美国Hyclone公司,胎牛血清购自美国Gibco公司,兔抗人DDR1抗体购自美国Abcam公司。

1.2 细胞培养

人胶质瘤细胞系U87、U251或正常胶质细胞株HEB均使用含10%胎牛血清的DMEM培养液,置于37 ℃、5%CO_2的细胞培养箱中培养。当细胞汇合率为80%~90%时传代培养。

1.3 慢病毒感染U251细胞制备稳定过表达和敲降DDR1的胶质瘤细胞系

将处于对数生长期的U251细胞接种于24孔板,汇合率达到30%时进行病毒感染,将细胞分为NC组、DDR1组、shcon组及shDDR1组,并加入5 μg/mL的polybrene,分别转染NC、DDR1、shcon及shDDR1。待T25瓶中细胞的汇合率达到50%时,加入5 μg/mL嘌呤霉素进行筛选。连续筛选2周,提取细胞总蛋白,利用Western blot实验鉴定HSP90的表达量,构建成功的稳定细胞系扩养备用。

1.4 qRT-PCR检测实验

将各组细胞培养至对数生长期(80%汇合率)时,使用TRIzol法提取各组细胞总RNA。经质量检测(A260/A280比值1.8~2.0,RIN>7.0)后逆转录为cDNA。使用特异性引物(DDR1正向引物5′-CTGGACCTCAACGTGCTACT-3′,反向引物5′-GCTCAGGTTGTTGGTGATGG-3′;GAPDH正向引物5′-GGAGCGAGATCCCTCCAAAAT-3′,反向引物5′-GGCTGTTGTCATACTTCTCATGG-3′)进行SYBR Green法扩增,反应条件:95 ℃ 30 s预变性;95 ℃ 5 s,60 ℃ 30 s,共40个循环。每组设3个复孔。采用$2^{-\Delta\Delta Ct}$法计算相对表达量,以NC组为校准样本,分析各组DDR1的表达量。

1.5 Western blot

提取HEB、U87、U251细胞以及各组稳定细胞系中的总蛋白并进行蛋白定量后,按照设计的上样顺序和上样量加样进行凝胶电泳分离,然后将目的蛋白转移至PVDF膜,再用TBST润洗,缓冲液封闭1 h后,加入一抗孵育液,4 ℃孵育过夜。然后用TBST洗膜3次,每次10 min,再加入二抗孵育液,室温孵育1 h。二抗孵育结束后,重复TBST洗膜3次,每次10 min。最后将超敏发光液A、B液等体积混合,润洗膜后于曝光仪中曝光。

1.6 CCK-8细胞增殖实验

将各组处于对数生长期的U251稳定细胞系细胞消化,用培养液重悬制成细胞悬液,对细胞进行计数并调整细胞浓度,以1×10^4个/孔的细胞密度接种于96孔板,置于37 ℃、5%CO_2的细胞培养箱中培养。分别在培养24 h、48 h、72 h、96 h、120 h后用CCK-8法检测细胞活力。于96孔板每孔加入110 μL含CCK-8的培养液(1∶10),继续在培养箱中孵育4 h,分别于450 nm波长处测量光密度(optical density, OD)值。

1.7 细胞凋亡检测实验

待各组稳定细胞系汇合率达80%时,给予凋亡诱导剂(1 μmol/L STS)或对照处理,培养6~12 h后消化细胞并用PBS重悬细胞,调整细胞浓度为1×10^6/mL。参照试剂盒说明书(BD Biosciences #556547)对细胞进行Annexin V-FITC/PI双染,取100 μL细胞悬液加入流式管,加入5 μL Annexin V-FITC和5 μL PI(50 μg/mL),避光孵育15 min,随后加入400 μL 1×Binding Buffer混匀。最后,用流式细胞仪进行检测,激发波长为488 nm。

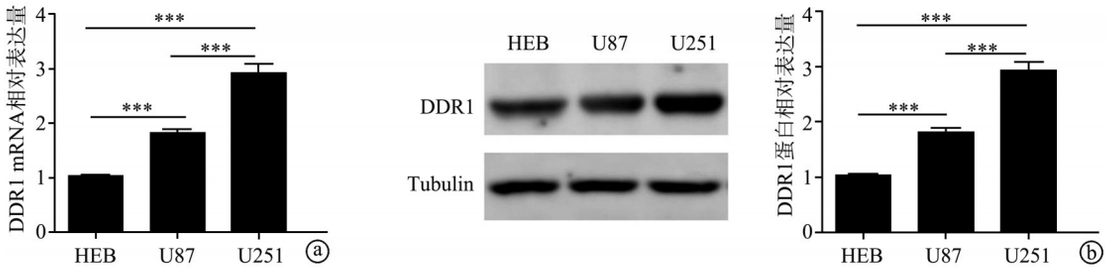
1.8 统计学方法

使用SPSS 13.0和GraphPad Prism 5.0软件进行统计学分析,计量数据以均数±标准差($\bar{x}\pm s$)表示,2组间比较采用独立样本t检验,$P<0.05$为差异具有统计学意义。本研究实验结果均来源于重复3次的独立实验。

2 结果

2.1 胶质瘤细胞中DDR1的表达情况

qRT-PCR和Western blot结果显示,与正常胶质细胞HEB相比,人胶质瘤细胞U87、U251中DDR1 mRNA和蛋白表达水平均显著升高(图1),且胶质瘤细胞U251中DDR1的表达水平高于U87细胞,因此,选择U251细胞进行后续实验。

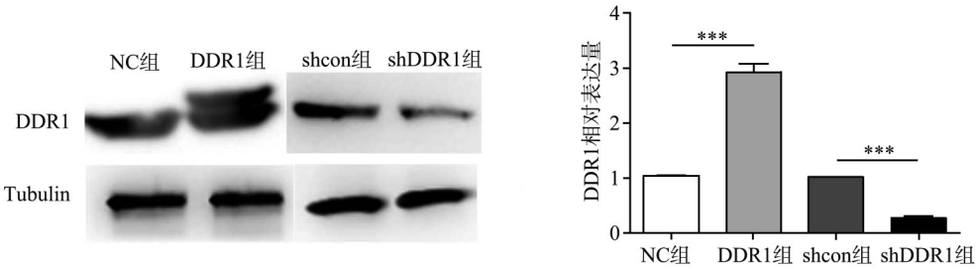


a:qRT-PCR检测DDR1 mRNA表达;b:Western blot检测DDR1蛋白表达 ***:P<0.001

图1 人正常胶质细胞和胶质瘤细胞中DDR1 mRNA和蛋白的表达

2.2 构建稳定过表达和敲降DDR1的胶质瘤细胞系U251

Western blot结果显示,与NC组相比,DDR1组细胞中DDR1蛋白表达量均明显上调(P<0.05);而与shcon组相比,shDDR1组细胞中DDR1蛋白表达量均显著降低(P<0.05),见图2。表明稳定过表达和敲降DDR1的U251细胞系构建成功,可用于后续实验。

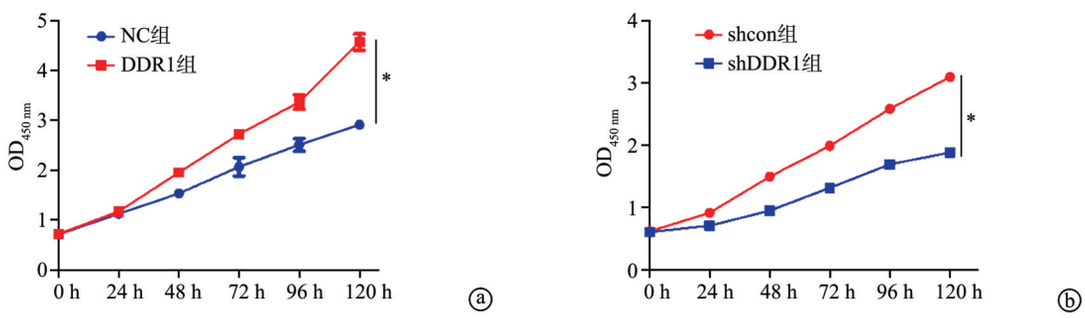


***:P<0.001

图2 稳定过表达和敲降DDR1的胶质瘤细胞中DDR1的表达

2.3 DDR1能够促进胶质瘤细胞的增殖

CCK-8法检测结果显示,DDR1组较NC组细胞增殖能力增强(P<0.05),shDDR1组较shcon组细胞增殖能力减弱(P<0.05),见图3。因此,过表达DDR1能够促进胶质瘤细胞的增殖,抑制DDR1的表达能够抑制胶质瘤细胞的增殖。



a:过表达DDR1的胶质瘤细胞系的增殖能力;b:敲降DDR1的胶质瘤细胞系的增殖能力 *:P<0.05

图3 稳定过表达和敲降DDR1的胶质瘤细胞系的增殖能力

2.4 DDR1能够抑制胶质瘤细胞的凋亡

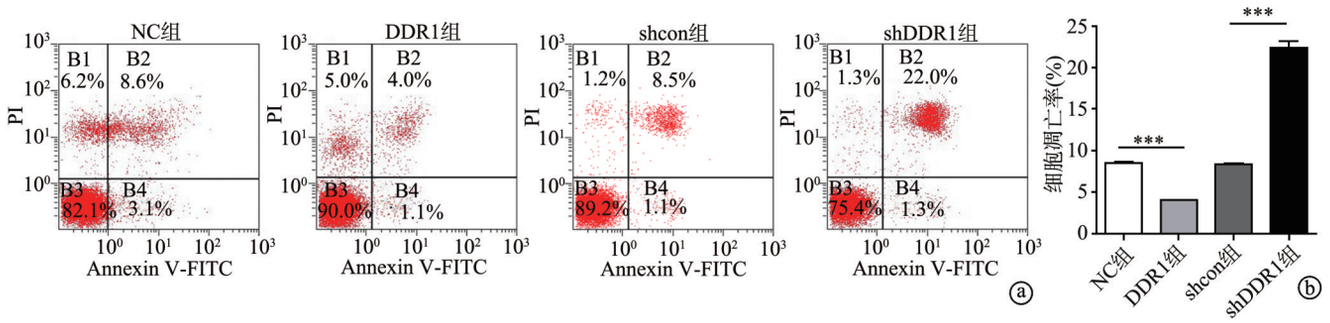
流式细胞结果显示,DDR1组U251细胞凋亡率较NC组低(P<0.05),shDDR1组U251细胞凋亡率较shcon组高(P<0.05),见图4。因此,过表达DDR1能够抑制胶质瘤细胞的凋亡,而抑制DDR1的表达能够促进胶质瘤细胞的凋亡。

2.5 DDR1能够激活PI3K/AKT通路

Western blot结果显示,DDR1组细胞中p-AKT和p-PI3K的表达量相比NC组显著升高(P<0.05),shDDR1组细胞中p-AKT和p-PI3K的表达量相比shcon组显著降低(P<0.05)。所以,过表达DDR1可激活PI3K/AKT通路,敲降DDR1则抑制PI3K/AKT通路的激活(图5)。

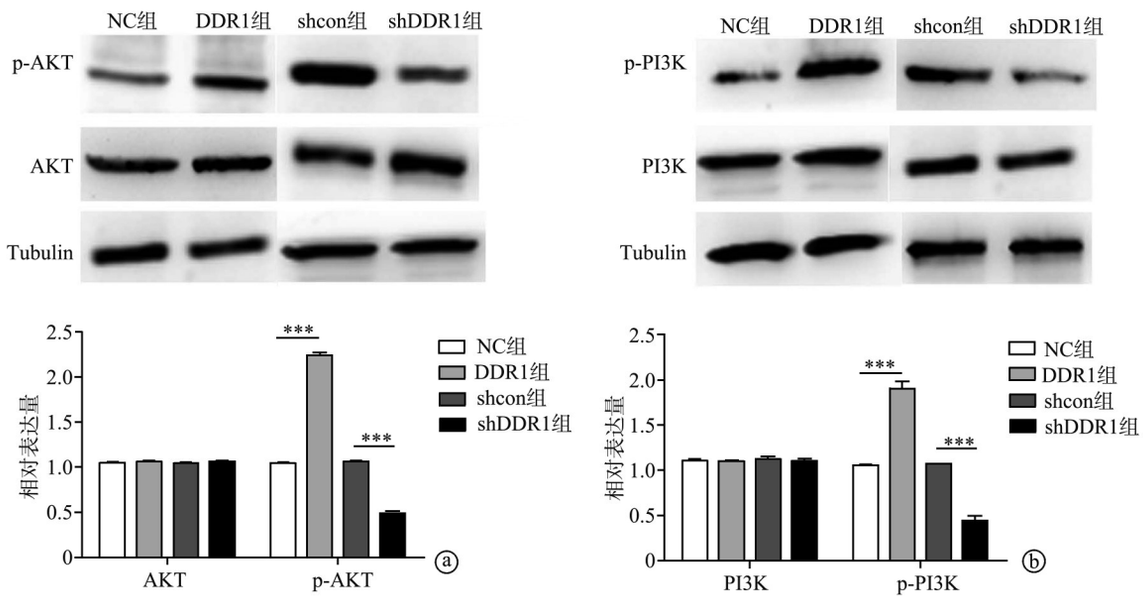
3 讨论

目前诸多研究已证实DDR1的异常高表达与多种肿瘤的发生发展密切相关[19-23]。DDR1在脑肿瘤组织和血清样本中表达显著升高,其表达量与实体瘤大小呈正相关[24]。DDR1能够调控胶质母细胞瘤的耐药性,影响其治疗和预后[25]。本研究发现,过表达DDR1



a:各组胶质瘤细胞的凋亡情况;b:各组胶质瘤细胞凋亡情况定量分析　***:$P<0.001$

图4　各组稳定胶质瘤细胞系的凋亡情况



a:各组稳定细胞系中AKT通路的激活情况;b:各组稳定细胞系中PI3K通路的激活情况　***:$P<0.001$

图5　过表达或敲降DDR1的胶质瘤细胞系中PI3K/AKT通路的激活情况

能够促进胶质瘤细胞的增殖并抑制其凋亡,而敲降DDR1则抑制了胶质瘤细胞的增殖并促进其凋亡。同时,过表达DDR1能够激活PI3K/AKT通路,敲降DDR1则能够抑制PI3K/AKT通路的激活。因此,DDR1能够通过激活PI3K/AKT通路增强胶质瘤细胞的增殖能力,并抑制胶质瘤细胞的凋亡,从而调控胶质瘤的发展进程。

DDRs主要表达于上皮细胞,其通过与胶原蛋白结合发生磷酸化,进而激活多种信号通路[26]。作为DDR家族成员之一,DDR1的激活或过表达可诱导部分下游信号通路活化,如Notch通路、PI3K/AKT通路等[27-28],从而对肿瘤细胞的发生、增殖、迁移、侵袭以及肿瘤微环境起到重要的调控作用。其中,PI3K/AKT通路是细胞内重要的信号传导途径,该通路接受多种上游调控信号后,可调控细胞生长、分化及细胞骨架重组。近年研究显示,PI3K/AKT通路在多种肿瘤中出现异常活化[29]。该通路被证实参与调控乳腺癌的进程及药物抵抗[27],并在胰腺癌、消化系统肿瘤中异常激活,推动肿瘤进展。越来越多的证据表明,PI3K/AKT通路在多种恶性肿瘤中过度激活,参与调控肿瘤的发生发展过程。AKT的表达量改变或激活可能是人类多种肿瘤发生发展的关键环节。研究发现,PI3K/AKT信号通路激活可增强胶质瘤细胞对刺激诱导凋亡的抵抗力[30]。本研究则证实DDR1能够通过激活PI3K/AKT通路调控胶质瘤细胞的增殖和凋亡,提示DDR1可能成为胶质瘤治疗的潜在靶点。尽管抑制DDR1活性已成为分子靶向治疗的潜在策略,但其详细调控机制尚未完全阐明。深入探索DDR1在各类肿瘤中的作用及下游信号通路,有助于开发DDR1抑制剂与通路调节剂的联合治疗方案。

综上,本研究聚焦DDR1对胶质瘤发展的调控作用与机制,通过功能学实验证实了DDR1对胶质瘤细胞增殖和凋亡的影响,并进一步证明了DDR1/PI3K/AKT轴在胶质瘤发生发展中的关键作用,为靶向DDR1的胶质瘤治疗提供了新靶点与理论依据。

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