

• 实验研究 •

pAD-TGF- β 1 对骨组织工程种子细胞转归的转基因保护

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【摘要】目的 探讨转化生长因子- β 1(TGF- β 1)真核表达载体质粒 pAD-TGF- β 1 基因转染对骨组织工程种子细胞骨髓基质干细胞(MSC)体外转归的转基因保护价值。**方法** 采用荧光显微镜下观察转染细胞的绿色荧光蛋白表达,噻唑蓝比色法检测细胞增殖情况,核酸原位末端标记法检测细胞凋亡指数。**结果** 荧光显微镜下证实质粒 pAD-TGF- β 1 确切表达于 MSC,并显著提高细胞增殖率,降低细胞凋亡指数。**结论** 通过 pAD-TGF- β 1 转基因修饰种子细胞 MSC,可有效标记 MSC 并明显改善 MSC 转归。

【关键词】 转化生长因子- β 1;基因转染;种子细胞

DOI:10.3969/j.issn.1673-7083.2011.02.020

Study on protection of pAD-TGF- β 1 gene transfection to the fate of seed cells in bone tissue engineering YI Cheng-qing, CAO Yun, MA Chun-hui, TENG Song-song, ZHANG Guo-qiao, ZHU Jin-hong, SONG Wang-sheng. Department of Orthopaedics, First People's Hospital, Shanghai Jiaotong University, Shanghai 200080, China

【Abstract】Objective To explore the protection of transforming growth factor- β 1(TGF- β 1) eukaryotic expression plasmid pAD-TGF- β 1 gene transfection to the fate of seed cells in bone tissue engineering. **Methods** Green fluorescent protein(GFP) expression in bone marrow stromal cells(MSCs) was observed in pAD-TGF- β 1 transfected MSCs by fluorescence microscope, and the proliferation potential and the apoptosis index(AI) value of MSCs were detected by methyl thiazolyl tetrazolium (MTT) and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) methods. **Results** pAD-TGF- β 1 was expressed in MSCs exactly, demonstrated by fluorescence microscope. Proliferation potential of transfected MSCs was enhanced, while AI value decreased significantly. **Conclusion** Transfecting cultured MSCs with pAD-TGF- β 1 can not only mark them effectively with GFP, but also promote their survival potential.

【Key words】 Transforming growth factor- β 1; Gene transfection; Seed cells

作为实现组织修复重建的功能主体,种子细胞的生活力和去向直接决定骨组织工程的效能。近年种子细胞活性的诱导与优化研究已取得瞩目成绩,然而对其转归行为,仍缺乏深入认识与有效干预。为此,我们应用转化生长因子- β 1(TGF- β 1)真核表达载体质粒 pAD-TGF- β 1 转基因修饰种子细胞骨髓基质干细胞(MSC),观察 MSC 转归特征,以初步寻求种子细胞转归干预的规律与方法。

1 资料与方法

本实验采用的 Dulbecco 改良 Eagle 培养基(DMEM)、胰蛋白酶、胎牛血清购自 Gibco 公司,维生素 C、牛血清白蛋白购自 Sigma 公司, β -甘油磷酸钠、四环素、地塞米松为国产分析纯试剂,Triton-X-100 溶液购自 Merck 公司,脂质体 Lipofectamine2000 购自 Invitrogen 公司,细胞凋亡原位检测试剂盒购自 Boehringer Mannheim 公司,pAD-TGF- β 1 质粒为本科室合成保存。

1.1 MSC 分离培养与成骨诱导

取 6~8 周龄新西兰白兔,无菌条件下自双侧股骨大转子用 18 号骨穿针抽取骨髓 2~3 ml,立即以 1000 rpm 离

心 10 min,0.01 M PBS 洗 3 次,去除上层组织液及脂肪,吸取细胞层,以成骨条件培养基(DMEM 培养液中加入地塞米松 10 nM、 β -甘油磷酸钠 10 mM、维生素 C 50 μ g/ml、青霉素 100 U/ml、链霉素 100 μ g/ml)重悬。细胞以 1×10^4 /ml 的密度接种于 25 ml 培养瓶中,置 37 $^{\circ}$ C、5%CO₂及饱和湿度条件下培养,5~7 d 后首次换液,以后每 3 天换液一次,细胞长满后用 0.25%胰蛋白酶消化,按所需细胞浓度传代。

1.2 pAD-TGF- β 1 转基因修饰 MSC

取第二代 MSC,以 2×10^5 /ml 的密度接种于 6 孔培养板(预置盖玻片),待细胞长至 50%~80%融合时,参照 Lipofectamine2000 转染试剂盒说明,转染 24 h 后荧光显微镜下观察绿色荧光蛋白(GFP)标记情况。

1.3 测定转基因对 MSC 增殖和凋亡影响

将转染组与对照组 MSC 分别以 1×10^4 /150 μ l 的密度接种于 96 孔培养板,待两组细胞生长至接近融合时,采用噻唑蓝(MTT)比色法测定光密度(OD)值。将转染组与对照组 MSC 细胞爬片置于 0.01 M PBS(pH 7.4)漂洗 3 次,4%多聚甲醛固定 30 min,0.01 M PBS(pH 7.4)漂洗,0.5%Triton-X-100 增加质膜通透性 30 min,按照核酸原位末端标记(TUNEL)试剂盒方法处理样本。光镜下每张玻片选择 5 个高倍视野,计算凋亡指数(AI):凋亡指数 = 阳性细胞数/总细胞数。

基金项目:国家自然科学基金面上项目(30700853)、上海市卫生局青年科研基金项目(044Y18)

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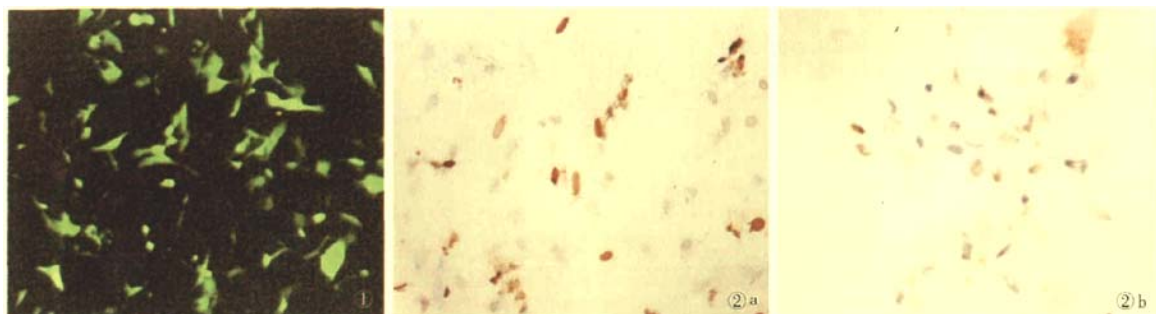


图1 MSC表达绿色荧光(荧光镜×200) pAD-TGF-β1转染MSC 24 h后绿色荧光分布于整个细胞,证实该质粒确切表达于MSC

图2 a.转染组MSC凋亡染色(光镜×100) b.对照组MSC凋亡染色(光镜×100) MSC细胞凋亡指数在pAD-TGF-β1转染组为 $5.72\% \pm 1.28\%$,对照组为 $17.34\% \pm 4.46\%$,转染组细胞凋亡水平显著下降

2 结果

2.1 pAD-TGF-β1转基因修饰MSC的表达

为确认pAD-TGF-β1在MSC中的表达,在体外以Lipofectamine2000介导转染,24 h后荧光显微镜见绿色荧光分布于整个细胞(见图1),证实质粒pAD-TGF-β1确切表达于MSC,所携带的GFP可作为靶细胞良好的示踪标记。

2.2 pAD-TGF-β1转基因修饰对MSC增殖的影响

MTT比色法测定OD值显示,实验组为 1.266 ± 0.080 ,对照组为 0.815 ± 0.064 ,两组有显著性差异;TGF-β1基因转染能显著提高MSC增殖能力。

2.3 pAD-TGF-β1对MSC转归的转基因保护

MSC在体外培养环境中受诱导环境影响与细胞间行为影响,可能发生转归不良。采用TUNEL法检测,可见转染后MSC凋亡指数下降(见图2),实验组为 $5.72\% \pm 1.28\%$,对照组为 $17.34\% \pm 4.46\%$,两组有显著性差异,证实pAD-TGF-β1转基因干预对于MSC转归具有保护效应。

3 讨论

组织工程学对于骨修复具有广泛的应用前景。其中,种子细胞是骨组织工程实现成骨效应的功能主体,其生存转归直接影响组织工程成骨潜力^[1]。

骨髓来源的基质干细胞是目前首选的种子细胞之一。受成骨环境诱导,MSC可定向分化为成骨细胞^[2]。

在生理性骨重建过程中,凋亡是成骨细胞最普遍的转归^[3]。成骨细胞凋亡率之高,以致于凋亡发生时期及发生程度对于成骨区内细胞数量产生巨大影响。骨组织工程是对生理性骨重建的模拟。然而,它并不能提供完全适宜的成骨微环境。种子细胞在一定程度上被剥夺生理状态下的营养支持和信号调控,细胞-细胞间、细胞-基质间联系受到干扰,活力和表型特征明显受制。研究^[4]显示,种子细胞部分发生凋亡,并可能出现去分化现象,丢失其成骨潜力。由于骨组织工程中植入种子细胞群的来源有限,这一现象可能是制约组织工程化骨的重要因素之一。

成骨细胞凋亡的发生受多种因素的影响。目前许多研究表明,成骨细胞具有多种基本生存因子。胰岛素样生长因子-I(IGF-I)、碱性成纤维细胞生长因子(bFGF)、TGF-β1和白细胞介素-6(IL-6)对成骨细胞均有促存活效应。其中,TGF-β1是一种多能生长因子,对成骨有确切的正向调控作用。TGF-β1可刺激成骨前体细胞克隆成纤维细胞集落形成单位(CFU-F)增殖,同时显著抑制体外培养的CFU-F凋亡发生率,并与组织分化阶段相关联。体外实验中,TGF-β1可明显抑制肿瘤坏死因子(TNF)、去血清或Fas诱导的成骨细胞凋亡。TGF-β1可阻止鸡胚胎骨髓板成骨区细胞凋亡。利用肝素颗粒携带骨形态发生蛋白-4、TGF-β1、TGF-β2植入鸡胚趾不同部位,发现TGF-β植入后可抑制间充质细胞凋亡而发生额外趾^[5-13]。

但是,对于MSC生存调控的报道很少见,其转归方向和调控机制如何尚缺乏明确阐述。由于成骨条件下MSC有向成骨细胞定向分化的能力,因此作为成骨细胞前体细胞,MSC可能存在与之近似的生存信号。大量文献^[4,9,14-16]提示,对于成骨细胞凋亡的调控存在双向效应,细胞生存还是凋亡,其转归取决于激活性信号与抑制性信号谁占优势。令人感兴趣的是,此时外源性细胞生存因子的介入,可以逆转细胞凋亡过程。

前期研究^[17,18]证实,TGF-β1转基因对MSC成骨能力有显著的上调效应,但对MSC生存能力的作用尚不得而知。目前示踪技术的发展,使得观察TGF-β1转基因保护对MSC转归的影响成为可能。GFP是从水母体内分离获得的一种发光蛋白,在现代细胞生物学和分子生物学研究领域的应用前景广泛,被喻为十分有用的活分子探针,GFP基因标记可用以追踪骨组织工程种子细胞的生物学行为。本研究利用示踪质粒pAD-TGF-β1转染MSC,比较MSC转基因前后的增殖与凋亡情况,观察TGF-β1转基因对MSC转归的保护作用,一方面证实示踪质粒可良好表达GFP,是研究MSC转归的有效示踪手

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本次研究结果表明,BMP-2及COX-2在创伤后HO形成中表达上调,在诱导和维持HO形成中起重要促进作用。依达拉奉作为一种新型自由基清除剂,可捕获羟自由基,减轻或防止肌肉组织损伤,下调BMP-2及COX-2表达,长期使用可明显抑制组织炎症反应,降低BMP-2及COX-2表达,对预防HO具有积极重要的意义。

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段;另一方面,通过转染组与对照组的比较,可见pAD-TGF- β 1转染MSC显著改善种子细胞的生存能力。本研究为骨组织工程种子细胞的体内示踪初步建立方法学基础,并确认TGF- β 1对于MSC转归的保护作用。其意义还在于可能进一步通过种子细胞的形态学和功能学追踪,寻找理想的监测与干预靶标,以指导组织工程技术介入的时机与架构方式,因而具有良好的可拓展性与科学意义。

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(收稿:2010-10-28;修回:2010-11-29)

(本文编辑:边倩)