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• 基础研究 •

lncRNA TUG1 对人牙髓干细胞成骨/成牙本质分化的影响

蒋雅欣¹, 张华¹, 孙凌寒¹, 李适廷², 冯浩¹

1. 西南医科大学附属口腔医院口腔颌面外科, 四川 泸州(646000); 2. 西南医科大学附属口腔医院牙体牙髓病科, 四川 泸州(646000)

【摘要】 目的 探讨长链非编码 RNA(long non-coding RNA, lncRNA)牛磺酸上调基因 1(taurine upregulated 1, TUG1)对人牙髓干细胞(human dental pulp stem cells, hDPSCs)增殖及成骨/牙本质向分化的影响。**方法** 分离培养 hDPSCs, 流式细胞术检测细胞表面抗原 CD44、CD45、CD73、CD90、CD133、STRO-1, 碱性磷酸酶(alkaline phosphatase, ALP)和茜素红染色鉴定其分化能力。hDPSCs 成骨诱导 0、7、14 d 收集 RNA, qRT-PCR 检测 TUG1 的表达水平。构建携带 sh-TUG1 的慢病毒载体 pSLenti-U6-shRNA(TUG1)-CMV-EGFP-F2A-Puro-WPRE, 并通过感染 hDPSCs 及嘌呤霉素筛选建立稳定沉默 TUG1 的 hDPSCs 细胞系, CCK-8 检测 hDPSCs 增殖能力, ALP 和茜素红染色及定量检测 hDPSCs 的早期 ALP 活性和晚期矿化结节的形成, qRT-PCR 和 Western blot 检测成牙本质及成骨分化相关的基因牙本质涎磷蛋白(dentin sialophosphoprotein, DSPP)、牙本质基质蛋白-1(dentine matrix protein 1, DMP-1)、Runt 相关转录因子 2(runt-related transcription factor 2, Runx2)、骨钙素(osteocalcin, OCN)、骨桥蛋白(osteopontin, OPN)基因及蛋白表达变化。**结果** 成功分离、培养和鉴定 hDPSCs, hDPSCs 成骨向分化过程中 TUG1 的表达明显增加($P < 0.05$)。沉默 TUG1 后 hDPSCs 的增殖能力下降($P < 0.05$), ALP 活性降低, 矿化结节形成减少; 成牙本质分化基因 DSPP、DMP-1 以及成骨分化基因 Runx2、OCN、OPN 表达也显著降低($P < 0.05$)。**结论** 沉默 TUG1 可抑制 hDPSCs 的增殖及成骨/成牙本质分化。

【关键词】 长链非编码 RNA; 基因沉默; 牛磺酸上调基因 1; 人牙髓干细胞; 成骨分化; 成牙本质分化; 矿化结节; 碱性磷酸酶; 牙本质涎磷蛋白; 牙本质基质蛋白-1; Runt 相关转录因子 2; 骨钙素; 骨桥蛋白

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Effect of lncRNA TUG1 on osteogenic/odontogenic differentiation of human dental pulp stem cells JIANG Yaxin¹, ZHANG Hua¹, SUN Linghan¹, LI Shiting², FENG Hao¹. 1. Department of Oral and Maxillofacial Surgery, The Affiliated Stomatology Hospital of Southwest Medical University, Luzhou 646000, China; 2. Department of Dentistry and Endodontics, The Affiliated Stomatology Hospital of Southwest Medical University, Luzhou 646000, China
Corresponding author: FENG Hao, Email: Pixar1991@163.com, Tel: 86-13684039525

【Abstract】 Objective To explore the effects of long noncoding-RNA (lncRNA) taurine upregulated gene 1 (TUG1) on the proliferation and osteogenic/odontoblast differentiation of human dental pulp stem cells (hDPSCs). **Methods** hDPSCs were isolated and cultured. The surface antigens CD44, CD45, CD73, CD90, CD133 and STRO-1 were detected by flow cytometry. Alkaline phosphatase (ALP) staining and alizarin red staining were used to identify the ability of cells to differentiate. RNA was collected on Days 0, 7 and 14 of the osteogenic induction of hDPSCs, and qRT-PCR was

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【作者简介】 蒋雅欣, 硕士研究生, Email: 526176061@qq.com

【通信作者】 冯浩, 博士, Email: Pixar1991@163.com, Tel: 86-13684039525

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used to detect the relative expression of TUG1. The hDPSCs were stably transfected with a lentiviral vector containing the TUG1-silenced pSLenti-U6-shRNA(TUG1)-CMV-EGFP-F2A-Puro-WPRE to silence TUG1. The ability of hDPSCs to proliferate was assessed with the CCK-8 method. ALP and alizarin red staining and quantitative detection were used to detect the ALP activity and formation of mineralized nodules of hDPSCs. The expression levels of dentin sialophosphoprotein (DSPP), dentin matrix protein-1 (DMP-1), Runt-associated transcription factor 2 (Runx2), osteocalcin (OCN) and osteopontin (OPN) genes and proteins were measured by qRT-PCR and Western blot. **Results** The hDPSCs were successfully isolated and cultured, and TUG1 expression was significantly increased during osteogenic differentiation ($P < 0.05$). The hDPSCs proliferation was suppressed after silencing TUG1 ($P < 0.05$). After osteogenic induction, ALP and alizarin red staining showed that ALP activity and mineralized nodules were suppressed by silencing TUG1. The expression levels of the odontogenic differentiation gene DSPP and DMP-1 and the osteogenic differentiation gene Runx2, OCN and OPN were also significantly decreased ($P < 0.05$). **Conclusion** Knocking down TUG1 can inhibit the proliferation and osteogenic/odontogenic differentiation of hDPSCs.

【Key words】 long non-coding RNA; gene silencing; taurine upregulated 1; human dental pulp stem cells; osteogenic differentiation; odontoblast differentiation; mineralized nodules; alkaline phosphatase; dentin sialophosphoprotein; dentine matrix protein 1; runt-related transcription factor 2; osteocalcin; osteopontin

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人牙髓干细胞(human dental pulp stem cells, hDPSCs)源自于迁移的神经嵴细胞,于2000年首次从人牙髓中分离和鉴定,具有高度增殖、自我更新和多向分化等间充质干细胞特性^[1]。其取材方便、易于扩增保存、活性高,能在特定条件下分化为多种组织细胞,是干细胞治疗中极有潜力的细胞来源^[2],在再生医学领域中具有广阔的应用前景。牛磺酸上调基因1(taurine upregulated 1, TUG1)是一种长链非编码RNA,位于第22号染色体上,最早由学者Young等^[3]从小鼠视网膜细胞中发现,被认为是视网膜细胞发育和光感受器形成的重要参与者。有学者发现TUG1可促进骨髓间充质干细胞^[4]以及牙周膜细胞^[5]成骨向分化。但TUG1在hDPSCs的增殖及成骨/成牙本质向分化过程中的表达水平和功能作用尚未见报道。本研究旨在通过感染慢病毒构建沉默TUG1的hDPSCs,探究TUG1对hDPSCs的增殖及成骨/成牙本质分化的影响。

1 材料和方法

1.1 主要材料和仪器

α -MEM培养基(Procell,中国),青霉素-链霉素-胰酶细胞消化液、碱性磷酸酶(alkaline phosphatase, ALP)显色试剂盒(Beyotime,中国),胎牛血清(vivacell,意大利),地塞米松、维生素C、茜素红染

色液(Solarbio,中国), β -甘油磷酸钠(Macklin,中国),I型胶原酶(Biofroxx,德国),CD44、CD45、CD73、CD90、CD133、STRO-1抗体(Ebioscience,美国),甘油醛-3-磷酸脱氢酶(glyceraldehyde-3-phosphate dehydrogenase, GAPDH)抗体(Abcam,英国),兔抗人DPSS抗体(Abcam,英国),兔抗人DMP-1抗体(Abcam,英国),兔抗人Runx2抗体(Abcam,英国),兔抗人OCN抗体(Abcam,英国),兔抗人OPN抗体(Abcam,英国),山羊抗兔二抗(Proteintech,美国),4%多聚甲醛(Biosharp,中国),CCK-8试剂盒(Dojindo,日本),氯化十六烷吡啶(Solarbio,中国),BCA蛋白浓度测定试剂盒(Beyotime,中国),ALP测定试剂盒(南京建成,中国),SteadyPure快速RNA提取试剂盒、Evo M-MLV反转录预混型试剂盒、SYBR Green Pro Taq HS预混型qPCR试剂盒(Accurate,中国)。

1.2 hDPSCs的分离培养和鉴定

本研究已取得西南医科大学附属口腔医院伦理委员会批准(批号:20220531002)。牙髓组织来源于西南医科大学附属口腔医院颌面外科,采用酶消化组织块法提取hDPSCs。患者及家属知情同意后,拔除因正畸要求拔除的前磨牙,于无菌条件下劈开牙齿,取出牙髓,立即用含青霉素-链霉素的PBS梯度冲洗,剪碎牙髓组织,加入I型胶原酶

(3 g/L), 15 min后加入完全培养基, 低速离心后弃上清液, 将消化后的组织块接种于培养瓶底后倒置培养瓶, 加入完全培养基, 于37℃、体积分数5% CO₂条件下培养, 4 h组织块贴壁后翻转培养瓶, 每隔3 d换液, 细胞长至瓶底80%后传代。取第3代hDPSCs流式细胞仪检测表面标志物CD44、CD45、CD73、CD90、CD133、STRO-1。取第3代hDPSCs于6孔板, 成骨诱导7 d进行ALP染色, 成骨诱导21 d进行茜素红染色观察。

1.3 qRT-PCR检测hDPSCs中TUG1的表达

取第3代hDPSCs于6孔板, 分别于成骨诱导0、7、14 d提取细胞总RNA, 测得RNA浓度, 逆转录获得cDNA, 实时定量扩增, 检测成骨过程中TUG1的表达变化, 实验重复3次。

1.4 慢病毒转染

慢病毒 sh-TUG1 载体 pSLenti-U6-shRNA (TUG1)-CMV-EGFP-F2A-Puro-WPRE 由上海和元技术股份有限公司提供, sh-TUG1 序列为: 5'-GCTTGCTTCTATTCTGAATCCTTT-3'; 取第3代的hDPSCs接种至6孔板中, 待细胞长至50%时进行转染, 根据转染说明书, 慢病毒滴度为3.78×10⁸ TU/mL, 通过转染预实验测定其感染复数为40; 加入5 mg/L聚凝胺提高转染效率, 12 h后换完全培养基继续培养。48 h后使用荧光显微镜观察绿色荧光蛋白的表达, 确认感染成功后加入4 mg/L嘌呤霉素筛选稳定转染株。根据病毒转染情况将细胞分为3组: ①空白对照组(NC组): 未转染处理的hDPSCs; ②阴性对照组(sh-NC组): 感染无序列质粒载体慢病毒的hDPSCs; ③实验组(sh-TUG1组): 感染sh-TUG1慢病毒的hDPSCs。

1.5 CCK-8检测细胞增殖活性

将NC组、sh-NC组和sh-TUG1组细胞分别接种于96孔板, 每孔2×10³个。在1、3、5、7 d进行CCK-8检测。去除原培养基, 添加CCK-8混合工作液, 在避光条件下孵育1 h, 酶标仪在450 nm处测定吸光度值, 实验重复3次。

1.6 ALP染色及定量检测

将NC组、sh-NC组和sh-TUG1组细胞分别接种于6孔板中, 待细胞贴壁后更换为成骨诱导培养基。成骨诱导7 d, 按ALP显色试剂说明书配置染液, 染色15 min, 在倒置显微镜下观察。将成骨诱导7 d的细胞裂解, BCA蛋白浓度测定试剂盒测定总蛋白质浓度, ALP定量试剂盒检测样本内细胞

ALP活性, 实验重复3次。

1.7 茜素红染色及定量检测

将NC组、sh-NC组和sh-TUG1组细胞分别接种于6孔板中, 待细胞贴壁后更换为成骨诱导培养基。成骨诱导21 d, 用0.2%茜素红染液染色30 min, 倒置显微镜下观察。超纯水清洗3次, 加入氯化十六烷基吡啶溶液, 避光30 min, 将上清液置于96孔板中, 酶标仪在562 nm处测定吸光度值, 实验重复3次。

1.8 qRT-PCR检测成牙本质及成骨分化相关基因mRNA水平

按试剂盒步骤分别提取成骨诱导7 d时3组细胞总RNA, 测得RNA浓度, 去除基因组DNA, 逆转录获得cDNA, 实时定量扩增, 检测成牙本质及成骨分化相关的基因牙本质涎磷蛋白(dentin sialophosphoprotein, DSPP), 牙本质基质蛋白-1(dentine matrix protein 1, DMP-1), Runt相关转录因子2(runt-related transcription factor 2, Runx2), 骨钙素(osteocalcin, OCN)、骨桥蛋白(osteopontin, OPN)的基因表达, 实验重复3次。目的基因的相对表达量使用2^{-ΔΔCT}方法计算得出。引物序列见表1。

表1 qRT-PCR引物序列
Table 1 Primer sequences for qRT-PCR

Gene	Primers sequence (5'-3')
TUG1	F: TAGCAGTTCCTCCCAATCCTTG R: CACAAATTCCTCATTCCTCC
DSPP	F: GCAGTGATGAATCTAATGGC R: CTGATTTGCTGCTGTCTTGAC
DMP-1	F: TCACCTCCAAAAGAAATCCTG R: GACTGGAACATACACAGCCAT
Runx2	F: CGCCACCACTCACTACCACAC R: TGGATTTAATAGCGTGCTGCC
OCN	F: TAGTGAAGAAGACCCAGGCGCT R: ATAGGCCTTCCTGAAAGCCGA
OPN	F: AAGCCTGACCCATCTCAGAA R: CATCGTCATGGCTTTCATTG
GAPDH	F: AATTCCACGGCAGTCAAGGC R: AACATACTCAGCACCAGCATCACC

TUG1: taurine upregulated 1. DSPP: dentin sialophosphoprotein. DMP-1: dentine matrix protein 1. Runx2: runt-related transcription factor 2. OCN: osteocalcin. OPN: osteopontin

1.9 Western blot检测hDPSCs成牙本质及成骨分化相关蛋白含量

在成骨诱导7 d时, 提取3组细胞蛋白并测定

其浓度, SDS-PAGE 凝胶电泳, 转膜 90 min, 5% 脱脂奶粉室温封闭 1 h, 加一抗, (DSPP, 1:500; DMP-1, 1:1 000; RUNX2, 1:2 000; OCN, 1:1 000; OPN, 1:1 000; GAPDH, 1:10 000), 4 °C 振荡过夜。TBST 润洗 3 遍后加入二抗 (1:10 000), 37 °C 孵育 1 h, 显影成像, Image J (National Institute of Mental Health, 美国) 软件分析结果, 实验重复 3 次。

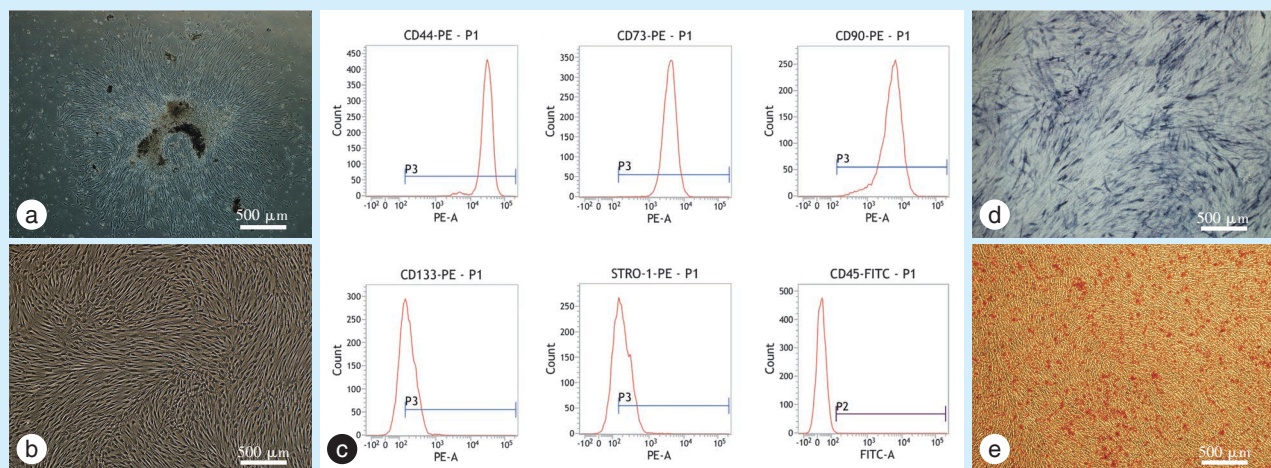
1.10 统计学分析

用 SPSS 19.0 分析数据, 计量资料的组间比较采用单因素方差分析, $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 hDPSCs 的分离培养和鉴定

组织块培养 5~7 d 后显微镜下可见细胞沿组织块边缘爬出 (图 1a), 呈放射状生长; 传至第 3 代时观察细胞呈长梭形, 胞核明显, 呈旋涡状生长 (图 1b)。流式细胞术检测细胞高表达间充质来源的表面标志物 CD44、CD73、CD90、CD133、STRO-1, 低表达造血组织来源的表面标志物 CD45 (图 1c)。ALP 和茜素红染色显示成骨诱导后的 ALP 活性强 (图 1d) 且具有矿化结节生成能力 (图 1e)。



a: hDPSCs were observed around the tissue mass; b: the third generation hDPSCs arranged in vortex shape and radial shape; c: the expression rates of CD44, CD73, CD90, CD133, STRO-1 and CD45 in hDPSCs were respectively 99.99%, 99.96%, 99.99%, 61.99%, 65.78%, 0.12%; d: alkaline phosphatase staining; e: alizarin red staining. hDPSCs: human dental pulp stem cells

Figure 1 Cultivation of hDPSCs and identification

图 1 hDPSCs 的培养及鉴定

2.2 TUG1 在 hDPSCs 成骨分化过程中上调

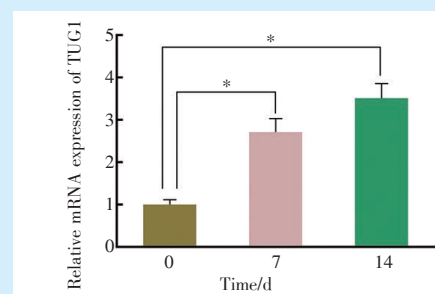
hDPSCs 成骨诱导 0、7、14 d, 分别收集细胞提取总 RNA 进行 qRT-PCR 检测 TUG1 含量相对变化, 结果显示, TUG1 的含量在成骨诱导过程中逐渐增加, 相较于 0 d, 成骨诱导 7 d ($P = 0.001$)、14 d ($P < 0.001$) 时, TUG1 mRNA 表达明显升高, 差异有统计学意义 (图 2)。

2.3 构建沉默 TUG1 的 hDPSCs

荧光显微镜观察慢病毒感染 hDPSCs 48 h 后, 细胞表达绿色荧光 (图 3a、3b), 表明慢病毒成功感染 hDPSCs, qRT-PCR 证实感染慢病毒后 TUG1 沉默效率高于 60% ($P < 0.001$) (图 3c)。

2.4 沉默 TUG1 抑制 hDPSCs 的增殖

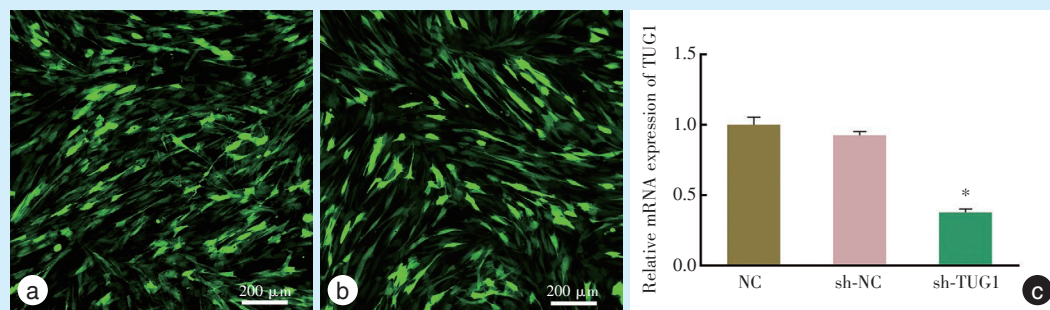
通过 CCK-8 法检测细胞的增殖能力 (图 4), 与 NC 组相比, sh-NC 组 hDPSCs 的增殖能力未发生明



*: compared with day 0, $P < 0.05$. The mRNA expression of TUG1 increased significantly on the 7th day and the 14th day after osteogenic induction. TUG1: taurine upregulated 1. hDPSCs: human dental pulp stem cells

Figure 2 Relative mRNA expression of TUG1 in hDPSCs after osteogenic induction by qRT-PCR

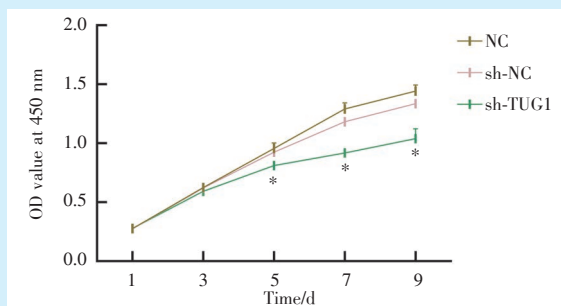
图 2 qRT-PCR 检测 hDPSCs 成骨诱导后 TUG1 mRNA 相对表达量



a & b: the expression of green fluorescent protein in group sh-NC(a) and group sh-TUG1(b) indicated successful transfection of lentivirus into hDPSCs; c: the expression of TUG1 in hDPSCs transfected with lentivirus decreased significantly. *: compared with the sh-NC group and NC group, $P < 0.05$. TUG1: taurine upregulated 1. hDPSCs: human dental pulp stem cells

Figure 3 Verification of TUG1 silencing in hDPSCs after lentivirus transfection

图3 TUG1沉默hDPSCs的慢病毒转染验证



In the first 3 days, there was no significant difference in the cell proliferation level of each group. From the 3rd day to 9th day, the proliferation in the sh-TUG1 group was significantly inhibited. *: compared with NC group, $P < 0.05$. TUG1: taurine upregulated 1. hDPSCs: human dental pulp stem cells

Figure 4 Proliferation of hDPSCs after TUG1 silencing

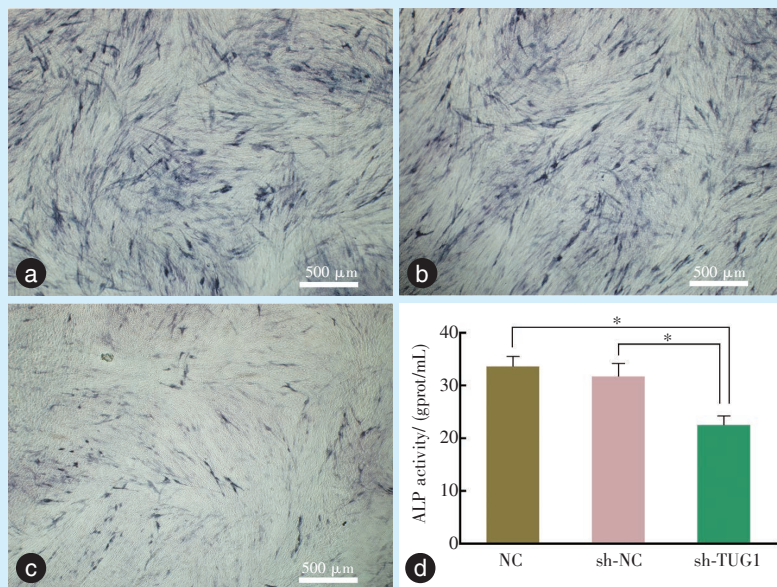
图4 沉默TUG1后hDPSCs增殖活性

显变化 ($P > 0.05$), 在 5 d ($P = 0.0010$)、7 d ($P = 0.0011$)、9 d ($P = 0.0040$) 时, sh-TUG1 组 hDPSCs 的增殖能力减弱 ($P < 0.05$)。

2.5 沉默TUG1后抑制hDPSCs的ALP活性及矿化结节的形成

成骨诱导培养 7 d, ALP 染色及定量检测结果显示 sh-TUG1 组着色较 NC 组 ($P = 0.002$) 和 sh-NC 组 ($P = 0.006$) 浅, 而 NC 组和 sh-NC 组间无明显差异 (图 5), 说明沉默 TUG1 后 hDPSCs 早期 ALP 活性减弱。

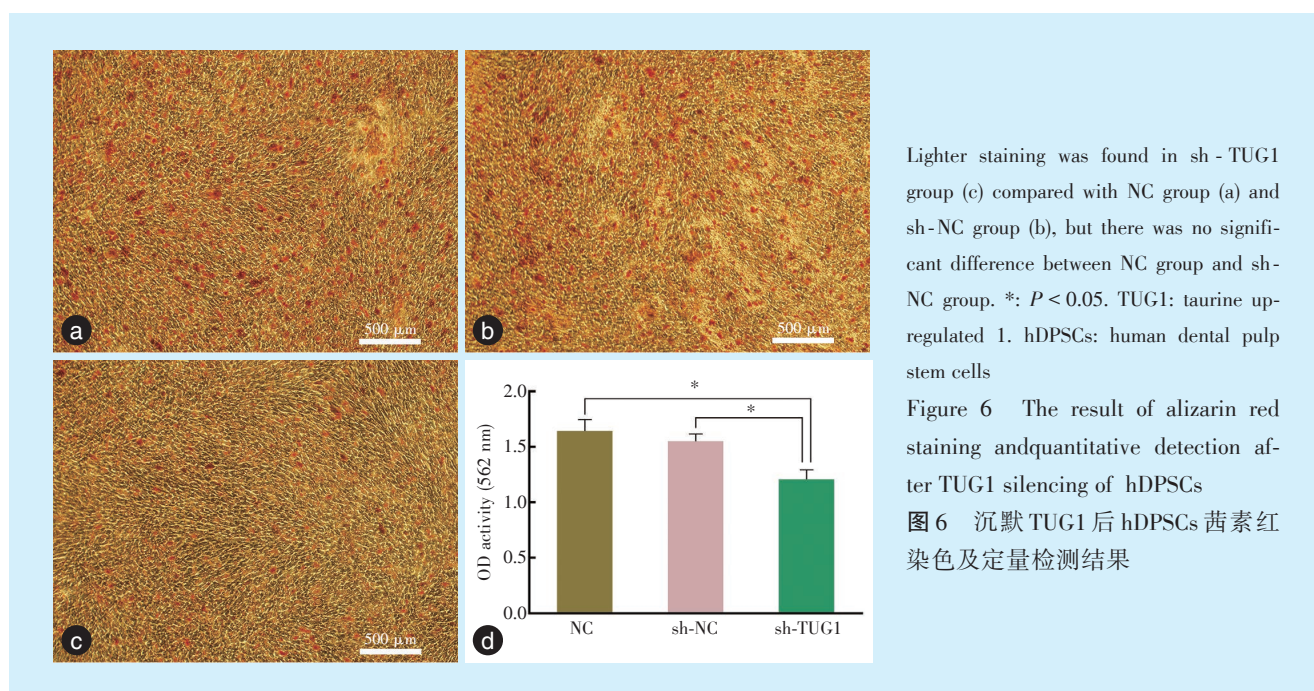
成骨诱导培养 21 d, 茜素红染色及定量检测结果显示 NC 组 ($P = 0.005$) 和 sh-NC 组 ($P = 0.005$) 矿化结节形成能力明显高于 sh-TUG1 组, 而 NC 组和 sh-NC 组间无明显差异 (图 6)。说明沉默 TUG1 后 hDPSCs 晚期矿化结节形成能力减弱。



Lighter staining was found in sh-TUG1 group (c) compared with NC group (a) and sh-NC group (b), but there was no significant difference between NC group and sh-NC group. *: $P < 0.05$. TUG1: taurine upregulated 1. hDPSCs: human dental pulp stem cells. ALP: alkaline phosphatase

Figure 5 The result of alkaline phosphatase and quantitative detection after TUG1 silencing of hDPSCs

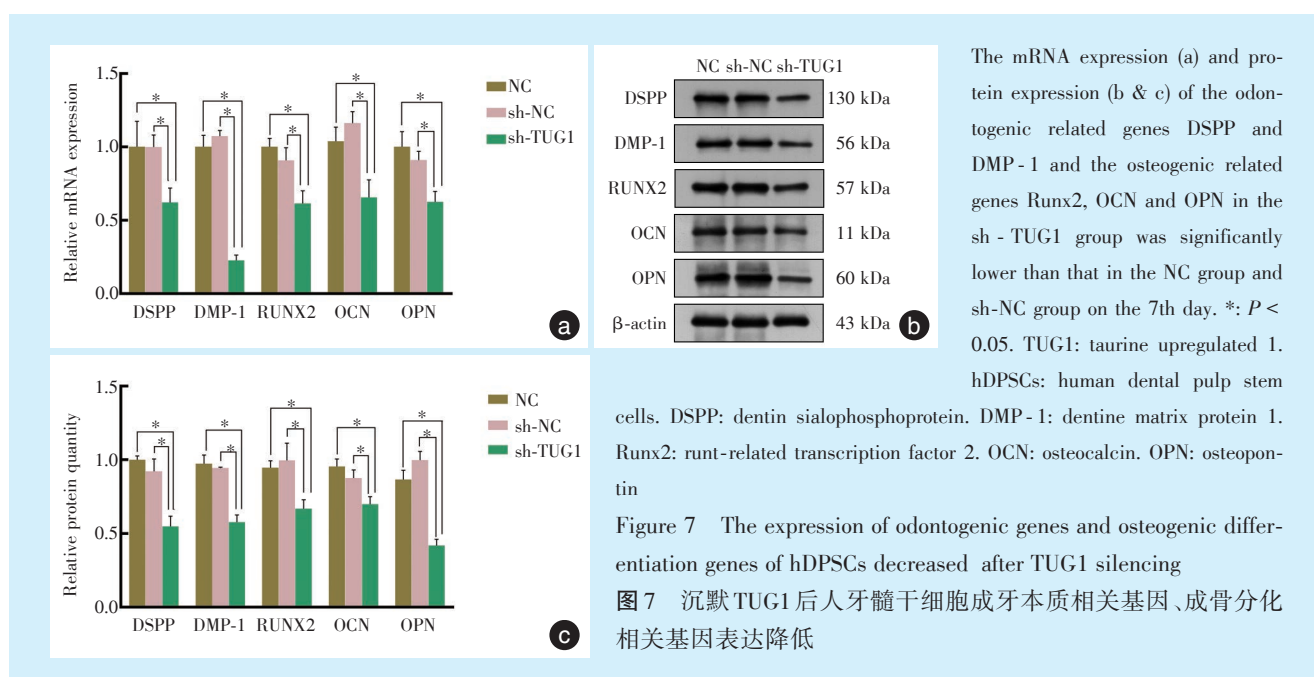
图5 沉默TUG1后hDPSCs碱性磷酸酶染色及定量检测结果



2.6 沉默TUG1后hDPSCs成骨/成牙本质相关基因和蛋白表达水平下调

成骨诱导7 d, qRT-PCR和Western blot技术检测各组细胞中成牙本质分化相关基因(DSPP、DMP-1)以及成骨分化相关基因(Runx2、OCN、OPN)的

mRNA和蛋白含量变化(图7),和NC组相比,sh-NC组细胞内成牙本质及成骨分化相关基因的mRNA和蛋白含量未发生明显变化($P > 0.05$);相较NC组和sh-NC组,sh-TUG1组成牙本质及成骨分化相关基因的mRNA和蛋白表达降低($P < 0.05$)。



3 讨论

hDPSCs具有多向分化潜能,可以在特定条件下分化为牙本质^[2]、牙周组织^[6]、骨^[7]、神经组织^[8]和皮肤组织^[9]等多种组织细胞,可用于牙本质及骨

组织再生等研究领域,目前已成为组织再生与工程领域的研究热点。

lncRNA是一类长度超过200 bp、不编码蛋白质的RNA^[10]。近年来,高通量测序技术的进步提

高了对细胞中 lncRNA 及其复杂信号网络的认识。lncRNA 可参与多种生物学过程,如染色质调控和基因表达^[11]。在胚胎干细胞中 lncRNA 具有调节细胞周期、维持细胞的多向分化潜能的作用,也可能参与细胞的更新和分化^[12]。此外,大量研究已证明 lncRNA 在间充质干细胞骨形成调控中的不同作用,例如,lncRNA XIXT 通过 miRNA-30a-5p 上调 RUNX2,从而诱导 hBMSCs 成骨缓解骨质疏松。lncRNA HOTAIRM1 通过调节 JNK/AP-1 信号介导的 RUNX2 表达来促进成骨分化^[13-15]。

TUG1 是一个 7.1 kb 的 lncRNA,首次发育中的小鼠视网膜细胞中发现,对牛磺酸处理反应上调^[3]。研究发现 TUG1 在人类多种癌症中表达上调,促进癌细胞增殖、迁移和侵袭,抑制细胞凋亡,是一个新发现的致癌基因。近年来研究发现沉默 TUG1 会显著抑制主动脉瓣钙化疾病下破骨细胞的形成,且 TUG1 通过 miR-204-5p 上调 RUNX2 基因表达,促进主动脉瓣钙化疾病中的成骨细胞分化^[16]。Teng 等^[17]发现 TUG1 在骨质疏松症中下调,影响骨髓间充质干细胞的成骨分化。Lu 等^[4]进一步研究发现 TUG1 通过调控 AMPK/mTOR/自噬通路促进骨髓间充质干细胞的成骨分化。Hao 等^[18]发现 TUG1 促进了成骨前体细胞的增殖,且通过 miR-545-3p 上调 CNR2 促进成骨前体细胞的成骨分化。Liu 等^[19]发现沉默 TUG1 后成骨经典通路 Wnt/ β -catenin 通路重要标志物 Runx2、Frizzled-2、Axin2 和 β -catenin 表达下调。以上这些研究表明 TUG1 在干细胞成骨分化过程中起着重要作用。但 TUG1 在 hDPSCs 成骨/成牙本质向分化过程中的表达水平和作用尚未见报道。

本研究成功分离并鉴定 hDPSCs,发现 TUG1 在 hDPSCs 成骨分化中明显增加。为验证 TUG1 对 hDPSCs 增殖和成骨/成牙本质分化的调控作用,利用慢病毒感染技术体外成功构建沉默 TUG1 的 hDPSCs。发现沉默 TUG1 后 hDPSCs 增殖被抑制。通过 ALP 和茜素红染色及定量检测发现在成骨诱导条件下,沉默 TUG1 抑制 hDPSCs 的早期 ALP 活性和晚期矿化结节形成能力。通过 qRT-PCR 检测验证了 TUG1 抑制 hDPSCs 内 DSPP、DMP-1、Runx2、OCN 和 OPN 等基因的表达。DSPP 是牙本质中最丰富的非胶原蛋白^[20],DMP-1 为成牙本质细胞分化早期的标志^[21],均在牙本质的形成及矿化中起到重要作用。Runx2 是成骨分化早期标志^[22-23]。Sheng 等^[24]发现 lncRNA TUG1 通过 RUNX2 促进骨

肉瘤的发展,Du 等^[25]发现沉默 lncRNA TUG1 通过 Runx2/ANPEP 增强氧化低密度脂蛋白处理的人脐静脉内皮细胞的增殖和迁移,促进动脉粥样硬化血管损伤的修复。OPN 和 OCN 分别是成骨分化中晚期和晚期的标志^[26-27]。Teng 等^[17]发现与健康对照组相比,骨质疏松患者血浆中 TUG1 表达明显降低,miR-23b 表达明显增加。此外,miR-23b 表达增加可抑制 RUNX2、骨钙素和骨桥蛋白的表达。在本实验中发现沉默 TUG1 抑制了 hDPSCs 向成牙本质细胞及成骨细胞的分化。

本实验探讨了 TUG1 在 hDPSCs 成骨过程中的表达水平以及对增殖和成骨/成牙本质分化的影响,但 TUG1 对 hDPSCs 增殖和分化的调控机制及体内实验仍有待进一步研究。

【Author contributions】 Jiang YX performed the experiments and wrote the article. Sun LH collected the data. Zhang H assisted in the data statistics. Li ST assisted in the experimental design. Feng H designed the experiments and reviewed the article. All authors read and approved the final manuscript as submitted.

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