[DOI]10.12016/j.issn.2096-1456.2022.03.003

·基础研究·

# CCDC134调控人牙髓干细胞成骨分化

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【摘要】 **目的**　探讨CCDC134(coiled-coil domain containing 134)对人牙髓干细胞(human dental pulp stem cells，hDPSCs)成骨分化功能的调控作用。**方法**　从牙髓组织中，分离培养hDPSCs，并分别以NC-CCDC134、shCCDC134、CCDC134慢病毒转染hDPSCs，分为空白对照组、阴性对照组、CCDC134下调组(shCCDC134)、CCDC134过表达组(CCDC134)。流式细胞术检测hDPSCs表面标志物Stro-1、CD105、CD34、CD45；甲苯胺蓝染色检测克隆形成；碱性磷酸酶(alkaline phosphatase，ALP)染色检测ALP表达；茜素红染色检测矿化结节形成；油红染色检测成脂能力；qPCR检测CCDC134、Runt相关转录因子2(Runt-related transcription factor 2，RUNX2)、骨钙素(osteocalcin，OCN)、骨形态发生蛋白-2(bone morphogenetic protein-2，BMP-2)、Smad家族成员1(mothers against decapentaplegic homolog 1，SMAD1)的mRNA水平表达；蛋白印迹法检测CCDC134、RUNX2、OCN、BMP-2、SMAD1蛋白表达水平。进一步以BMP-2信号激活剂(BMP-2)和抑制剂(Dorsomorphin)分别干预CCDC134下调及上调的hDPSCs(分组为：shCCDC134、shCCDC134+BMP-2、CCDC134、CCDC134+Dorsomorphin)，细胞聚合体移植于裸鼠皮下2个月，HE染色法检测新骨形成。**结果**　hDPSCs高表达间充质干细胞表面标志物，低表达造血干细胞表面标志物。与空白对照组相比，成骨诱导的hDPSCs中CCDC134的表达升高；与阴性对照组相比，shCCDC134组CCDC134的表达降低，CCDC134组的表达升高($P < 0.05$)；shCCDC134组的矿化结节减少、成骨相关基因和蛋白表达降低($P < 0.05$)，CCDC134组的指标升高($P < 0.05$)；shCCDC134组的BMP-2/SMAD1信号通路的相关表达降低，CCDC134组表达升高($P < 0.05$)。与shCCDC134组相比，shCCDC134+BMP-2组成骨相关基因和蛋白表达 升高、裸鼠皮下新骨形成增加($P < 0.05$)，与CCDC134组相比，CCDC134+Dorsomorphin组以上指标降低($P < 0.05$)。**结论**　CCDC134通过调控BMP-2/SMAD1信号通路促进hDPSCs成骨分化。

【关键词】 牙髓干细胞；　CCDC134；　成骨分化；　组织工程；　骨形成蛋白-2；　重组人Smad家族成员1；　Runt相关转录因子2；　骨钙素

微信公众号

【中图分类号】 R78　【文献标志码】 A　【文章编号】 2096-1456(2022)03-0169-09

【引用著录格式】 徐万田，董文睿，朱文胤. CCDC134调控人牙髓干细胞成骨分化[J]. 口腔疾病防治，2022，30(3)：169-177. doi：10.12016/j.issn.2096-1456.2022.03.003.

**CCDC134 regulates the osteogenic differentiation of human dental pulp stem cells**　XU Wantian，DONG Wenrui，ZHU Wenyin.　Department of the Third Outpatient, Nanjing Stomatological Hospital, Medical School of Nanjing University, Nanjing 210008, China
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【Abstract】 **Objective**　To study the regulatory effect of coiled-coil domain containing 134 (CCDC134) on the osteogenic differentiation of human dental pulp stem cells (hDPSCs). **Methods**　HDPSCs were isolated and cultured from dental pulp tissue and transfected with NC-CCDC134, shCCDC134 and CCDC134 lentiviruses. They were divided into the control group, negative control group, CCDC134 downregulation (shCCDC134) group and CCDC134 overexpression (CCDC134) group. Surface markers of hDPSCs (Stro-1, CD105, CD34, CD45) were detected by flow cytometry; colony formation was analyzed by toluidine blue staining; ALP expression was estimated by ALP staining; mineralized nodule

【收稿日期】2021-07-26；【修回日期】2021-09-05
【基金项目】江苏省自然科学基金项目(BK20200150)
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formation was evaluated by alizarin red staining; lipid droplet formation was examined by oil red staining; and gene and protein expression of CCDC134, Runt-related transcription factor 2 (RUNX2), osteocalcin (OCN), bone morphogenetic protein-2 (BMP-2), and mothers against decapentaplegic homolog 1 (SMAD1) was detected by qPCR and western blot, respectively. Further, a BMP-2 activator (BMP-2) and inhibitor (Dorsomorphin) were used to down-regulate and up-regulate CCDC134, respectively (shCCDC134, shCCDC134+BMP-2, CCDC134, CCDC134+Dorsomorphin), in hDPSCs. The hDPSC aggregates were subcutaneously transplanted into nude mice for 2 months, and new bone formation was detected by H&E staining. The BMP-2/SMAD1 signaling in each group was detected by qPCR. **Results** hDPSCs showed high expression of mesenchymal markers and low expression of hematopoietic markers. Compared with the control group, the expression of CCDC134 was increased in the osteogenic-induced hDPSCs ($P < 0.05$). Compared with the negative control group, the expression of CCDC134 was decreased in the shCCDC134 group, whereas it was increased in the CCDC134 group ($P < 0.05$). The mineralized nodules, osteogenic genes and proteins in the shCCDC134 group were decreased ($P < 0.05$), while they were increased in the CCDC134 group ($P < 0.05$). The expression of BMP-2/SMAD1 signaling decreased in the shCCDC134 group, while it increased in the CCDC134 group ($P < 0.05$). Compared to the shCCDC134 group, osteogenic genes and proteins increased in the shCCDC134+BMP-2 group, and subcutaneous new bone formation increased in nude mice ($P < 0.05$). The indexes of the CCDC134+Dorsomorphin group decreased compared with the CCDC134 group ($P < 0.05$). **Conclusion** CCDC134 promotes the osteogenic differentiation of hDPSCs by regulating the BMP-2/SMAD1 signaling pathway.

**【Key words】** dental pulp stem cells; CCDC134; osteogenic differentiation; tissue engineering; bone morphogenetic protein-2; recombinant human mothers against decapentaplegic homolog 1; Runt-related transcription factor 2; osteocalcin

**J Prev Treat Stomatol Dis, 2022, 30(3): 169-177.**

**【Competing interests】** The authors declare no competing interests.

This study was supported by the grants from Natural Science Foundation of Jiangsu Province (No. BK20200150).

颌面部骨缺损修复与再生是组织工程与再生医学的研究热点。选择合适的种子细胞，并通过外源性调控使其获得更好的成骨分化功能具有重要意义。

人牙髓干细胞(human dental pulp stem cells, hDPSCs)为牙源性间充质干细胞，具有自我更新能力、多向分化潜能，免疫原性低，可通过拔除的第三磨牙、正畸牙获得，来源较为充足，且与颌面部骨组织具有同源性，因此被视为颌面部骨缺损修复与再生的重要种子细胞[1]。

CCDC134(coiled-coil domain containing 134)是新发现的一种骨调控分子，其表达缺失可影响成骨细胞的骨向分化和骨基质形成，在成骨调控中发挥重要作用[2]。骨形态发生蛋白-2(bone morphogenetic protein-2, BMP-2)/Smad家族成员1(mothers against decapentaplegic homolog 1, SMAD1)信号通路是骨代谢中的重要通路，具有正向调控成骨分化的功能[3]。

本实验拟通过上调/下调CCDC134，观察其对hDPSCs成骨分化的影响，以及CCDC134与BMP-2/SMAD1信号通路的关系，为hDPSCs在颌面部骨缺损修复与再生中的应用提供参考依据。

## 1 材料和方法

### 1.1 主要试剂和仪器

α-MEM培养基、胰蛋白酶(Gibco，美国)；胎牛血清(四季青，中国)；成骨诱导液、细胞聚合体诱导液、ALP染液、茜素红染液(碧云天，中国)；Trizol reagent(Invitrogen，美国)；逆转录试剂盒(Toyobo，日本)；BMP-2、Dorsomorphin、HA/TCP(Sigma，美国)；慢病毒(锐博，中国)；人Stro-1抗体(ab214086，Abcam，英国)；CD105抗体(ab11414，Abcam，英国)；CD34抗体(ab187282，Abcam，英国)；CD45抗体(ab25386，Abcam，英国)；人CCDC134抗体(MAB7784-SP，R&D，美国)；RUNX2抗体(sc-390351，Santa Cruz，美国)；OCN抗体(sc-390877，Santa Cruz，美国)；BMP-2抗体(sc-137087，Santa Cruz，美国)、SMAD1抗体(sc-7965，Santa Cruz，美国)；GAPDH抗体(sc-47724，Santa Cruz，美国)；实时定量PCR仪(CFX96，Bio-Rad，美国)；流

式细胞仪(FACSCanto II,BD,美国)。

1.2 实验分组和方法

1.2.1 hDPSCs分离培养与鉴定 选取就诊患者中需要拔除的新鲜阻生第三磨牙或正畸减数牙,分离牙髓,并将牙髓组织切割为小碎块,采用组织块-酶消化法,配合有限稀释法,分离培养hDPSCs。以P3代细胞进行实验。

本实验获得南京大学医学院附属口腔医院伦理委员会批准(NJSH-2021NL-003)。所有阻生第三磨牙或正畸减数牙来源的患者,均已签署知情同意书。

对分离hDPSCs进行鉴定:①流式细胞仪检测表面标志物Stro-1、CD105、CD34和CD45;②hDPSCs克隆形成检测,将500个细胞接种于5 cm培养皿,14 d后固定细胞,甲苯胺蓝染色;③成骨诱导及碱性磷酸酶(alkaline phosphatase,ALP)染色检测、茜素红染色(详见1.2.4);④成脂诱导及油红染色检测成脂能力(详见1.2.5)。

1.2.2 慢病毒感染hDPSCs与实验分组 以$5 \times 10^4$个/mL的密度将hDPSCs接种于6孔培养板中,细胞生长融合至底面积的约80%时加入慢病毒感染。CCDC134低表达慢病毒(shCCDC134)和过表达慢病毒(CCDC134)的靶序列分别为5′-CTTCCAGAACCCATTTAAA-3′,5′-CAATGCACAGGGCTGCAGTCTAA-3′,病毒滴度 > $10^8$ PFU/mL。6 h后换液,72 h后收集感染细胞。

将hDPSCs分为4组,分别为空白对照组、阴性对照组、CCDC134下调组(shCCDC134)、CCDC134过表达组(CCDC134)。空白对照组细胞不感染任何慢病毒;阴性对照组细胞感染空白质粒慢病毒;shCCDC134组细胞感染CCDC134低表达慢病毒;CCDC134组细胞感染CCDC134过表达慢病毒。

1.2.3 BMP-2信号激活及抑制 hDPSCs感染了CCDC134低表达慢病毒或CCDC134过表达慢病毒后,诱导细胞聚合体形成,在细胞贴壁时将细胞分为4组,分别为:①shCCDC134组,加入等量溶剂;②shCCDC134+BMP-2组,加入BMP-2信号通路激活剂BMP-2(100 μM);③CCDC134组,加入等量溶剂;④CCDC134+Dorsomorphin组,加入BMP-2信号通路抑制剂Dorsomorphin(200 μM)。

1.2.4 成骨诱导及ALP染色检测、茜素红染色 将$1 \times 10^5$个细胞接种于12孔培养板中,待细胞贴壁后更换成骨诱导液,每2 d换液1次,14 d后固定细胞,ALP染液染色15 min,观察染色情况,28 d后固定细胞,茜素红染液染色10 min,PBS缓冲液洗涤染液,显微镜下观察矿化结节形成。

1.2.5 成脂诱导及油红染色检测成脂能力 将$1 \times 10^5$个细胞接种于12孔培养板中,待细胞贴壁后更换成脂诱导液,每2 d换液1次,7 d后,固定细胞,油红染液染色10 min,PBS缓冲液洗涤染液,显微镜下观察脂肪滴形成。

1.2.6 qPCR检测相关基因mRNA水平 Trizol法提取hDPSCs总RNA,逆转录获得cDNA(反应条件:37 ℃,5 min × 3;50 ℃,5 min × 3;98 ℃,5 min),实时定量扩增(反应条件:95 ℃,3 min;95 ℃,15 s;60 ℃,30 s;39个循环)。引物序列见表1。

**表1 引物序列**

Table 1 Primer sequences

| Gene | Forward (5′-3′) | Reverse (5′-3′) |
|---|---|---|
| CCDC134 | TGTTGGACCTTCGAGCCTA | CATGACATCAAGGATCTTGTACTG |
| RUNX2 | CCCGTGGCCTTCAAGGT | CGTTACCCGCCATGACAGTA |
| OCN | ATGAGAGCCCTCAGACTCCTC | CGGGCCGTAGAAGCGCCGATA |
| BMP-2 | CGGACTGCGGTCTCCTAA | GGAAGCAGCAACGCTAGAAG |
| SMAD1 | GTTTCAGGCGGTTGCTTA | ACACTTGTGGAGGAGGC |
| GAPDH | AGGTCGGAGTCAACGGATT | TCCTGGAAGATGGTG |

CCDC134: coiled-coil domain containing 134; RUNX2: Runt-related transcription factor 2; OCN: osteocalcin; BMP-2: bone morphogenetic protein-2; SMAD1: mothers against decapentaplegic homolog 1

1.2.7 Western blot检测相关蛋白水平 超声震荡联合反复冻融法提取总蛋白,BCA法测定蛋白浓度,取等量蛋白于SDS-PAGE凝胶电泳,转膜,5%脱脂牛奶封闭1 h,4 ℃孵育CCDC134、Runt相关转录因子2(Runt-related transcription factor 2,RUNX2)、骨钙素(osteocalcin,OCN)、BMP-2、SMAD1一抗工作液(1∶500)8 h,PBS洗涤,室温孵育二抗1 h,PBS洗涤,PVDF膜显影。

1.2.8 裸鼠皮下成骨实验 将BMP-2信号激活剂(BMP-2)和抑制剂(Dorsomorphin)分别于预后的

CCDC134下调及上调hDPSCs以 $1 \times 10^5$ 个/孔接种于12孔培养板中，待细胞贴壁后更换细胞聚合体诱导液，每2 d换液1次，待细胞聚合体形成后，将羟基磷灰石/磷酸三钙颗粒包裹于细胞聚合体中，移植于裸鼠皮下。2个月后，取材脱钙，行HE染色。

### 1.3　统计学方法

用SPSS 19.0统计分析数据，检验数据的正态性和方差齐性，两组间比较采用 $t$ 检验，多组间比较采用单因素方差分析。$P < 0.05$ 为有统计学差异。

## 2　结　果

### 2.1　hDPSCs的细胞鉴定

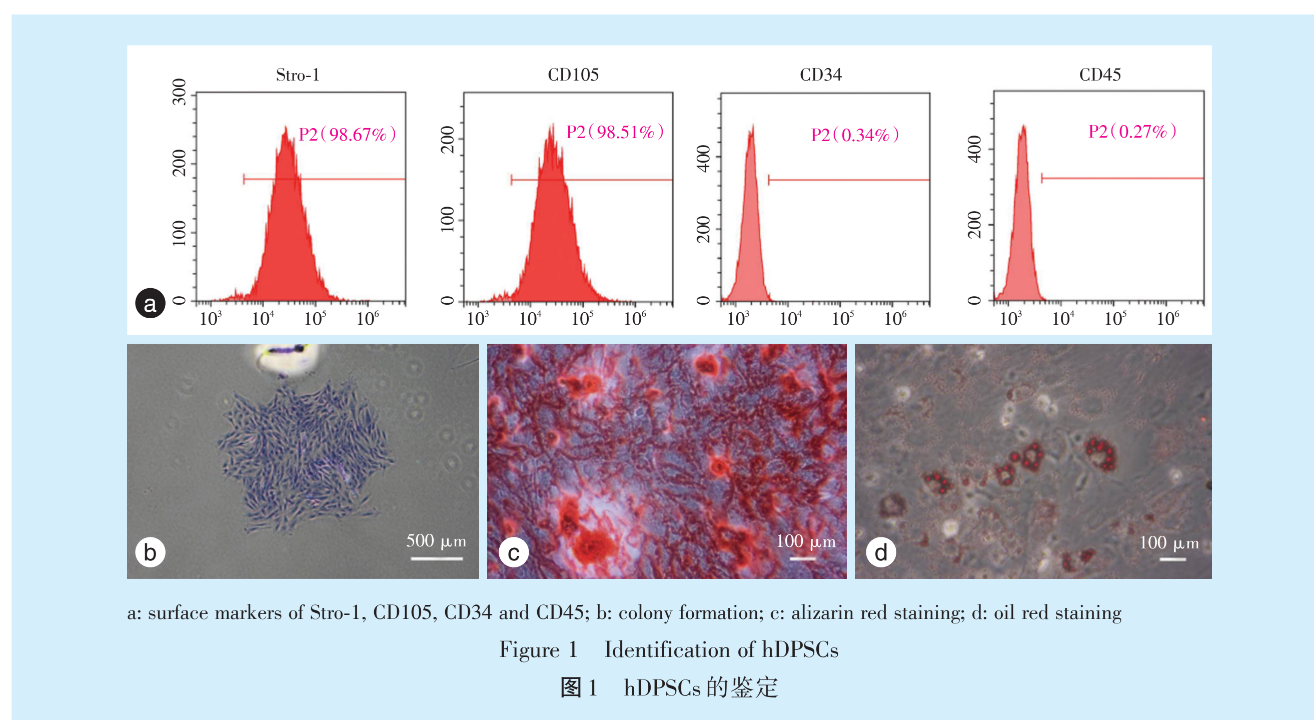
hDPSCs高表达间充质来源的表面标志物Stro-1(98.67%)和CD105(98.51%)，低表达造血系来源的表面标志物CD34(0.34%)和CD45(0.27%)。hDPSCs可形成细胞克隆，在成骨或成脂诱导条件下，可产生矿化结节或脂肪滴。以上结果说明hDPSCs分离培养成功，可进行后续实验。见图1。

### 2.2　成骨诱导条件下hDPSCs中CCDC134的mRNA和蛋白表达水平

成骨诱导条件下，hDPSCs中CCDC134的mRNA水平($P < 0.001$)和蛋白表达水平($P = 0.021$)的表达均上升。见图2。

### 2.3　慢病毒感染后hDPSCs中CCDC134的表达水平

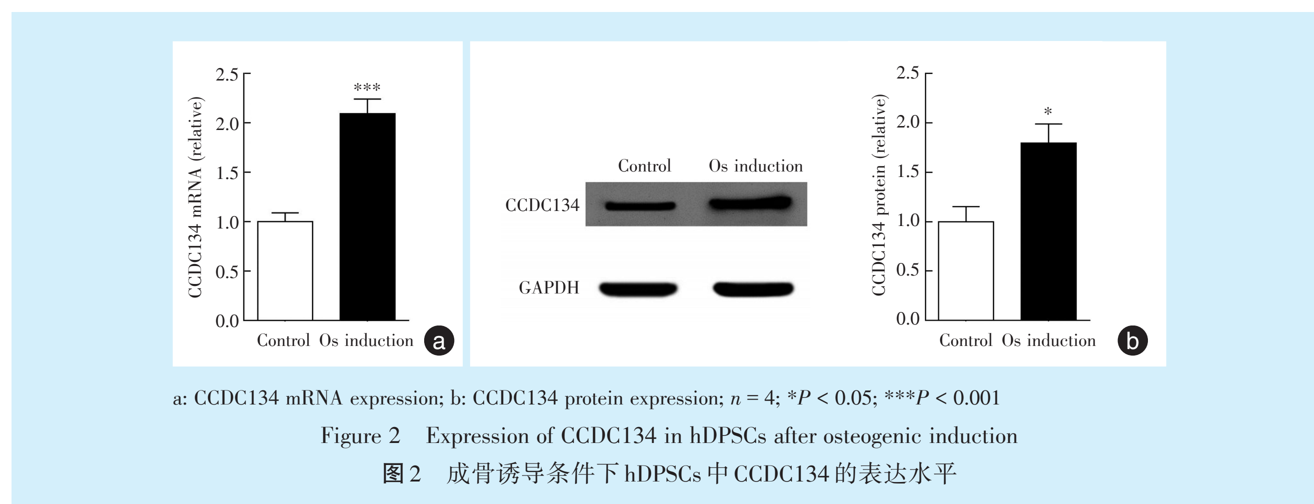
与空白对照组相比，阴性对照组的CCDC134的




a: surface markers of Stro-1, CD105, CD34 and CD45; b: colony formation; c: alizarin red staining; d: oil red staining

Figure 1　Identification of hDPSCs

图1　hDPSCs的鉴定

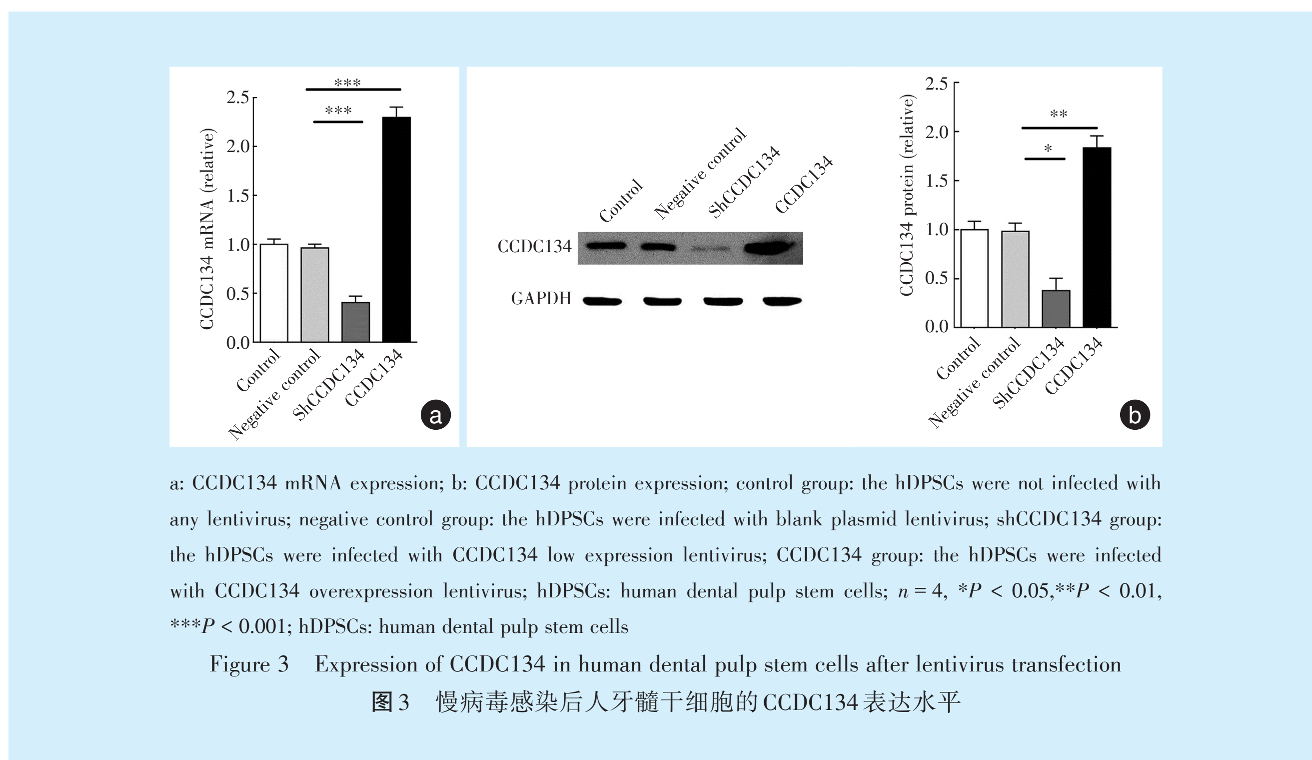



a: CCDC134 mRNA expression; b: CCDC134 protein expression; $n = 4$; $^*P < 0.05$; $^{***}P < 0.001$

Figure 2　Expression of CCDC134 in hDPSCs after osteogenic induction

图2　成骨诱导条件下hDPSCs中CCDC134的表达水平

mRNA和蛋白表达水平无明显变化($P = 0.364$)。与阴性对照组相比,shCCDC134组CCDC134的mRNA水平($P < 0.001$)和蛋白($P = 0.015$)水平表达显著降低,而CCDC134组的mRNA($P < 0.001$)和蛋白($P = 0.008$)表达量显著升高,见图3。




a: CCDC134 mRNA expression; b: CCDC134 protein expression; control group: the hDPSCs were not infected with any lentivirus; negative control group: the hDPSCs were infected with blank plasmid lentivirus; shCCDC134 group: the hDPSCs were infected with CCDC134 low expression lentivirus; CCDC134 group: the hDPSCs were infected with CCDC134 overexpression lentivirus; hDPSCs: human dental pulp stem cells; $n = 4$, *$P < 0.05$,**$P < 0.01$, ***$P < 0.001$; hDPSCs: human dental pulp stem cells

Figure 3 Expression of CCDC134 in human dental pulp stem cells after lentivirus transfection

图3 慢病毒感染后人牙髓干细胞的CCDC134表达水平

### 2.4 CCDC134对hDPSCs成骨分化功能的影响

与空白对照组相比,阴性对照组的ALP染色和矿化结节形成无明显差异($P > 0.05$);与阴性对照组相比,shCCDC134组的ALP染色($P < 0.001$)和矿化结节形成($P = 0.001$)显著降低;而CCDC134组的ALP染色($P < 0.001$)和矿化结节形成($P = 0.018$)显著增加。

与空白对照组相比,阴性对照组的成骨分子RUNX2和OCN的mRNA水平和蛋白表达水平也无明显变化($P > 0.05$);与阴性对照组相比,shCCDC134组的RUNX2和OCN的mRNA水平(RUNX2:$P = 0.001$,OCN:$P < 0.001$)和蛋白表达水平(RUNX2:$P < 0.001$,OCN:$P = 0.001$)均降低,差异具有统计学意义;而CCDC134组的RUNX2和OCN的的mRNA水平(RUNX2:$P < 0.001$, OCN:$P < 0.001$)和蛋白表达水平(RUNX2:$P = 0.001$,OCN:$P < 0.001$)均升高,差异具有统计学意义,见图4。

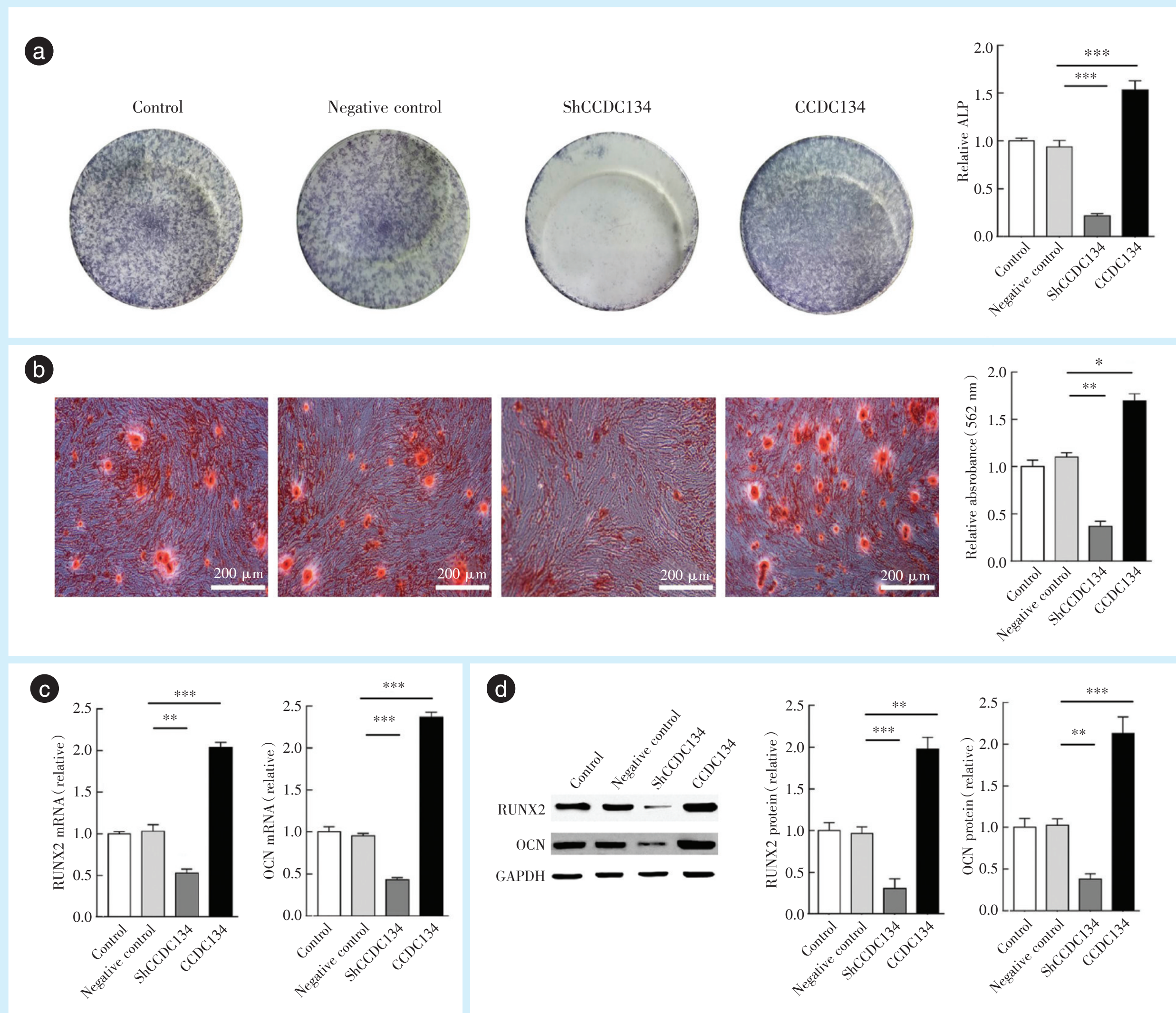
### 2.5 CCDC134通过BMP-2/SMAD1信号通路调控hDPSCs成骨分化功能

与空白对照组相比,阴性对照组的BMP-2和SMAD1的蛋白表达水平的表达无显著差异($P > 0.05$);与阴性对照组相比,shCCDC134组的BMP-2和SMAD1的蛋白表达水平(BMP-2:$P = 0.036$,SMAD1:$P = 0.039$)均降低,差异均具有统计学意义;而CCDC134组的BMP-2和SMAD1蛋白表达水平(BMP-2:$P < 0.001$,SMAD1:$P = 0.002$)均升高,差异具有统计学意义,见图5。

与shCCDC134组相比,shCCDC134+BMP-2组成骨分子RUNX2和OCN的mRNA水平(RUNX2:$P < 0.001$, OCN:$P < 0.001$)与蛋白表达水平(RUNX2:$P < 0.001$,OCN:$P < 0.001$)升高,裸鼠皮下异位成骨增加($P = 0.001$),差异具有统计学意义;与CCDC134组相比,CCDC134+Dorsomorphin组RUNX2和OCN的mRNA水平(RUNX2:$P < 0.001$,OCN:$P < 0.001$)与蛋白表达水平(RUNX2:$P = 0.001$,OCN:$P < 0.001$)均降低,裸鼠皮下异位成骨减少($P = 0.012$),差异具有统计学意义,见图6。

## 3 讨 论

hDPSCs是一类重要的牙源性种子细胞,研究表明hDPSCs具有成骨分化潜能[4-5],在骨组织修复与再生中发挥作用[6-10],因此,明确其成骨分化的




a: alkaline phosphatase; b: alizarin red staining; c: mRNA expressions of RUNX2 and OCN; d: protein expressions of RUNX2 and OCN; control group: the hDPSCs were not infected with any lentivirus; negative control group: the hDPSCs were infected with blank plasmid lentivirus; shCCDC134 group: the hDPSCs were infected with CCDC134 low expression lentivirus; CCDC134 group: the hDPSCs were infected with CCDC134 overexpression lentivirus; hDPSCs: human dental pulp stem cells; RUNX2: Runt-related transcription factor 2; OCN: osteocalcin; $n$ = 4, *$P$ < 0.05,**$P$ < 0.01,***$P$ < 0.001

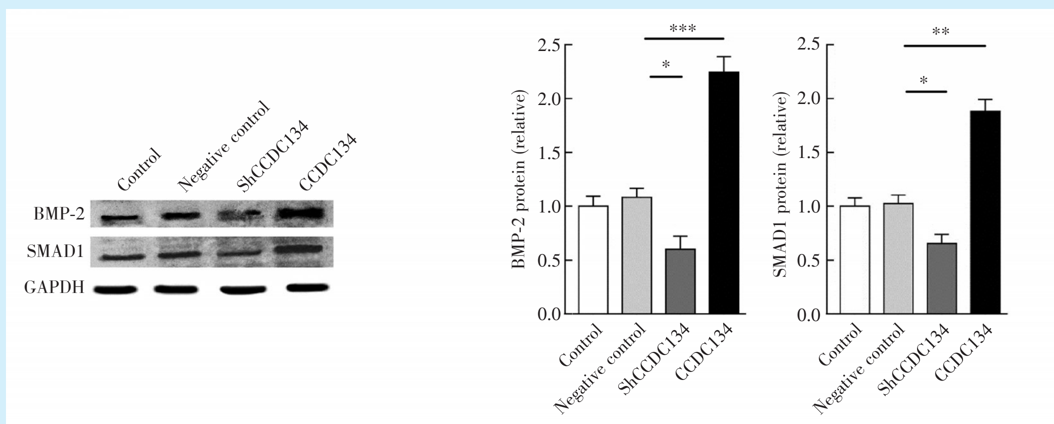
Figure 4　Effect of CCDC134 on osteogenic differentiation of human dental pulp stem cells

图4　CCDC134对人牙髓干细胞成骨分化功能的影响

分子机制对于精准调控种子细胞功能，促进骨组织修复与再生具有重要意义。

CCDC134是新发现的一种高度保守的分子，在胚胎发育过程中参与心脏、脑、肝脏等多种重要组织器官的代谢，其表达缺失可导致这些重要脏器的发育和功能障碍[11]。CCDC134的高度保守性保证了其可以从动物实验延伸至人源性样本的研究。研究表明，过表达CCDC134可以显著改善小鼠关节炎的症状[12]。不仅如此，CCDC134表达缺失可导致多种成骨相关基因的表达异常，进而导致严重的骨发育不全[2]。此外，CCDC134基因突变可造成患Ehlers-Danlos综合征母亲所怀胎儿的骨折，甚至是致死性骨折[3]。以上研究均提示CCDC134在骨发育与代谢中具有关键作用。

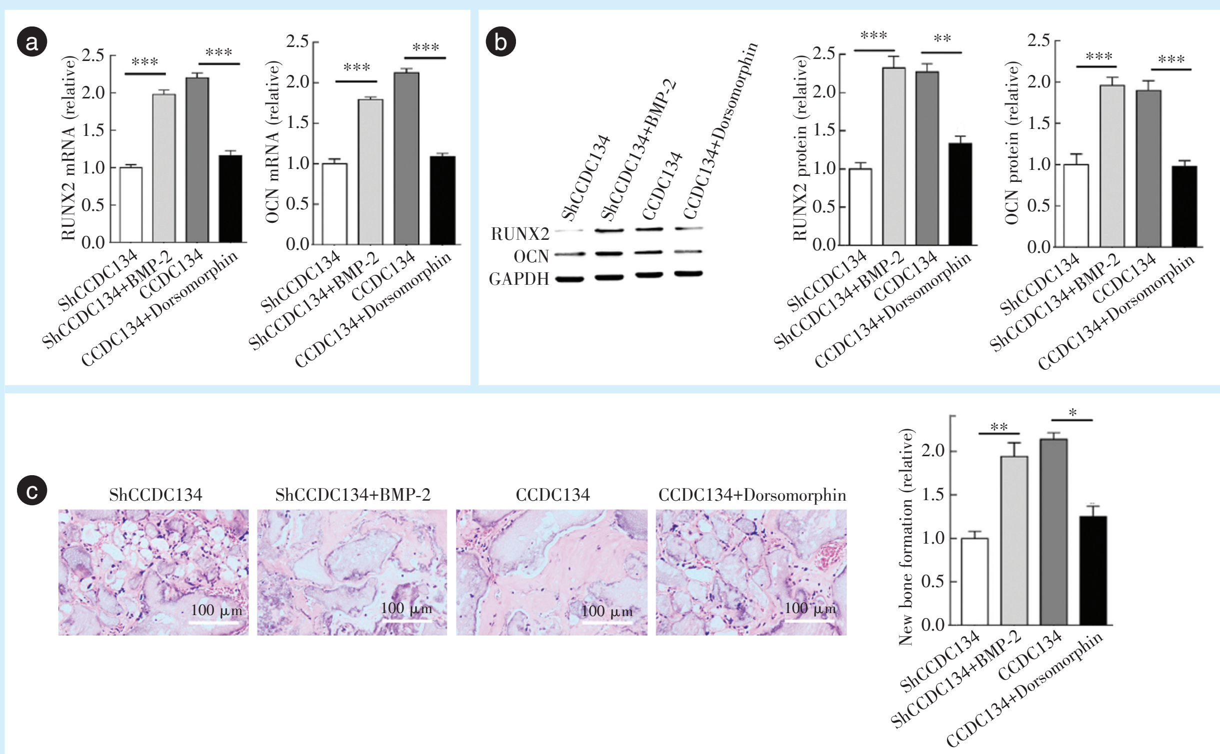
本实验首先对进行了hDPSCs鉴定，显示其间充质干细胞表面标志物高表达，造血干细胞表面标志物低表达，同时具有自我更新和多向分化潜能，以确保后续实验的可靠性。其次，发现hDPSCs




Control group: the hDPSCs were not infected with any lentivirus; negative control group: the hDPSCs were infected with blank plasmid lentivirus; shCCDC134 group: the hDPSCs were infected with CCDC134 low expression lentivirus; CCDC134 group: the hDPSCs were infected with CCDC134 overexpression lentivirus; hDPSCs: human dental pulp stem cells; BMP-2: bone morphogenetic protein-2; SMAD1: mothers against decapentaplegic homolog 1; $n$=4, *$P$ < 0.05, **$P$ < 0.01, ***$P$ < 0.001

Figure 5　Expression level of BMP-2/SMAD1 signal pathway protein in human dental pulp stem cells after lentivirus infection

图5　慢病毒感染人牙髓干细胞后BMP-2/SMAD1信号通路蛋白表达水平




a: mRNA expressions of RUNX2 and OCN; b: protein expressions of RUNX2 and OCN; c: subcutaneous new bone formation; shCCDC134 group: the hDPSCs were infected with CCDC134 low expression lentivirus; shCCDC134 + BMP - 2 group: the hDPSCs were infected with CCDC134 low expression lentivirus and added BMP-2 signal activator; CCDC134 group: the hDPSCs were infected with CCDC134 overexpression lentivirus; CCDC134+Dorsomorphin group: the hDPSCs were infected with CCDC134 overexpressing lentivirus and added inhibitor of BMP-2 signaling pathway (Dorsomorphin); hDPSCs: human dental pulp stem cells; RUNX2: Runt-related transcription factor 2; OCN: osteocalcin; BMP-2: bone morphogenetic protein-2; $n$ = 4, *$P$ < 0.05, **$P$ < 0.01, ***$P$ < 0.001

Figure 6　CCDC134 regulates the osteogenic differentiation of human dental pulp stem cells through BMP-2 signaling pathway

图6　CCDC134通过BMP-2信号通路调控人牙髓干细胞的成骨分化能力

成骨过程中的CCDC134表达增高，提示其在成骨中起重要作用；并通过慢病毒调控hDPSCs中CCDC134的水平，发现过表达CCDC134可以显著增强hDPSCs的成骨分化能力，为精准调控hDPSCs在骨组织工程中的作用，提供了新的靶点。

研究显示，CCDC134可调控细胞外调节蛋白激酶（extracellular regulated protein kinases，ERK）、c-Jun氨基末端激酶（c-Jun N-terminal kinase，JNK）以及丝裂原活化蛋白激酶（mitogen-activated protein kinase，MAPK）信号通路在其他骨组织细胞中发挥作用[13-14]。例如，CCDC134突变可以引发ERK1/2磷酸化，抑制成骨相关分子骨桥蛋白（osteopontin，OPN）、Ⅰ型胶原α链（collagen type I alpha 1 chain，COL1A1）的表达，进而导致成骨细胞分化异常[2]。也有研究报道CCDC134不直接调控MAPK信号通路[13]，这可能与两项研究选用的疾病模型和细胞种类不同有关。除以上信号通路外，BMP-2/SMAD1信号通路也是参与细胞骨代谢的重要信号通路，可以促进成骨细胞[15-16]、间充质干细胞[17-20]的成骨分化，其与CCDC134的相关研究尚未见报道。本实验结果发现，过表达CCDC134可上调BMP-2/SMAD1信号的表达，而抑制CCDC134则下调该信号的表达，提示BMP-2/SMAD1是CCDC134的下游信号通路。此外，通过BMP-2信号的激活和抑制，可以有效逆转CCDC134低表达慢病毒或过表达慢病毒对hDPSCs的作用，从而明确了CCDC134可通过BMP-2/SMAD1信号通路调控hDPSCs的成骨分化。

辅助性T细胞1（helper T cell 1，Th1）和辅助性T细胞17（helper T cell 17，Th17）是两类重要的免疫细胞，在骨代谢的负向调控中起重要作用，二者的活化直接或间接影响成骨和破骨过程[21-22]。研究显示，CCDC134可以抑制Th1和Th17细胞的功能，并能有效控制骨关节炎的发展[12]，这也提示CCDC134调控骨代谢的另一潜在机制。此外，CCDC134还能通过与转录激活因子hADA2a（human alteration/deficiency in activation 2a）相互作用，抑制其诱导的细胞凋亡和细胞周期抑制，发挥保护细胞的作用[23]。另有研究发现，CCDC134通过调控Wnt信号通路，在神经系统发育及运动神经的协调中发挥关键作用，而hDPSCs为神经嵴来源的细胞，与神经系统具有同源性，可能具有类似的信号调控途径[24]。

本实验明确了CCDC134在hDPSCs成骨分化中的作用及相关分子机制，进一步完善了CCDC134调控成骨分化的信号网络，为多方位调控hDPSCs的成骨分化功能，促进其在颌面部骨缺损修复与再生中的应用提供实验依据。

**【Author contribution】** Xu WT designed the study, performed the experiments and wrote the article. Dong WR performed the experiments. Zhu WY performed the experiments. All authors read and approved the final manuscript as submitted.

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(编辑 周春华,邵龙泉)

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