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·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
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CCDC134 regulates the osteogenic differentiation of human dental pulp stem cells

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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

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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

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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5×10^4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1×10^5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1×10^5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)分别干预后的

CCDC134下调及上调hDPSCs以1 × 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

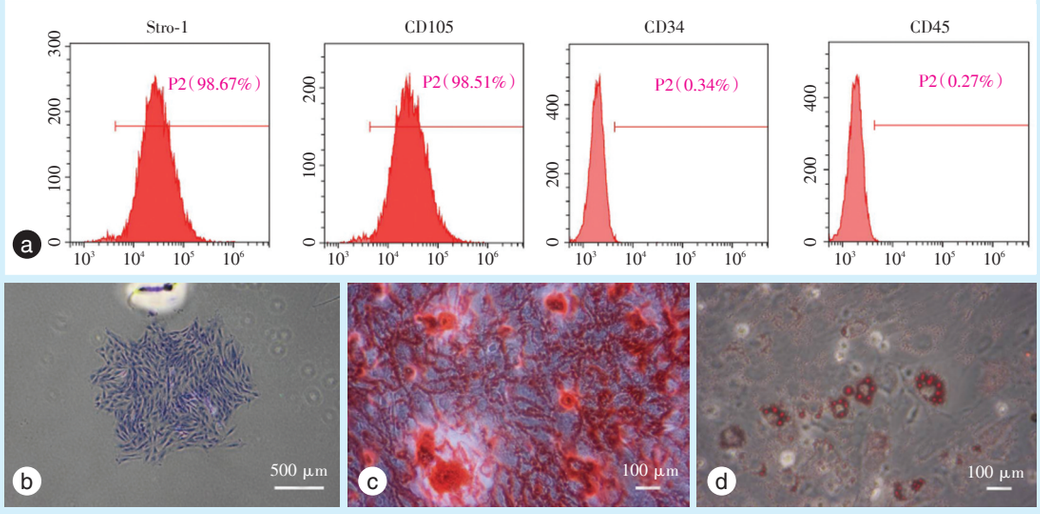
hDPSCs高表达间充质来源的表面标志物Stro-1（98.67%）和CD105（98.51%），低表达造血系来源的表面标志物CD34（0.34%）和CD45（0.27%）。hDPSCs可形成细胞克隆，在成骨或成脂诱导条件下，可产生矿化结节或脂肪滴。以上结果说明hDPSCs分离培养成功，可进行后续实验。见图1。

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

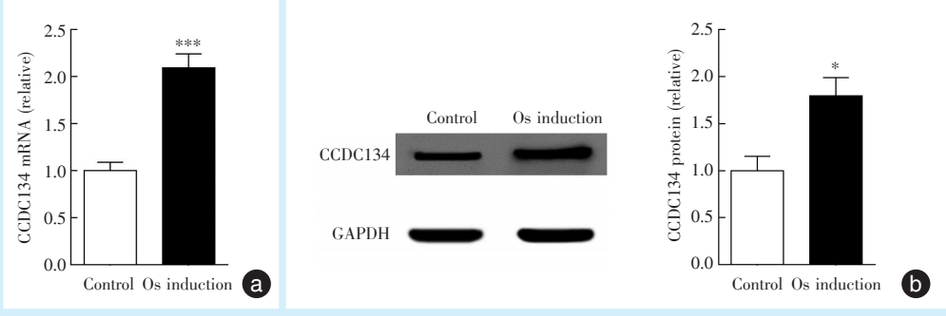
与空白对照组相比，阴性对照组的CCDC134的



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

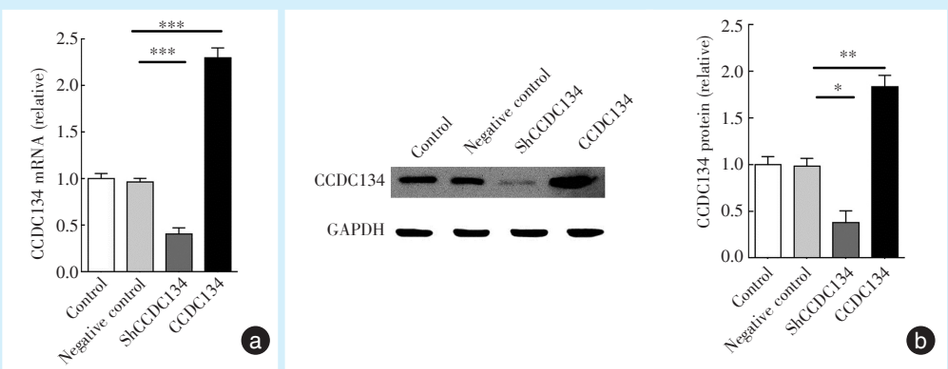


a: CCDC134 mRNA expression; b: CCDC134 protein expression; $n = 4$; *$P < 0.05$; ***$P < 0.001$

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。

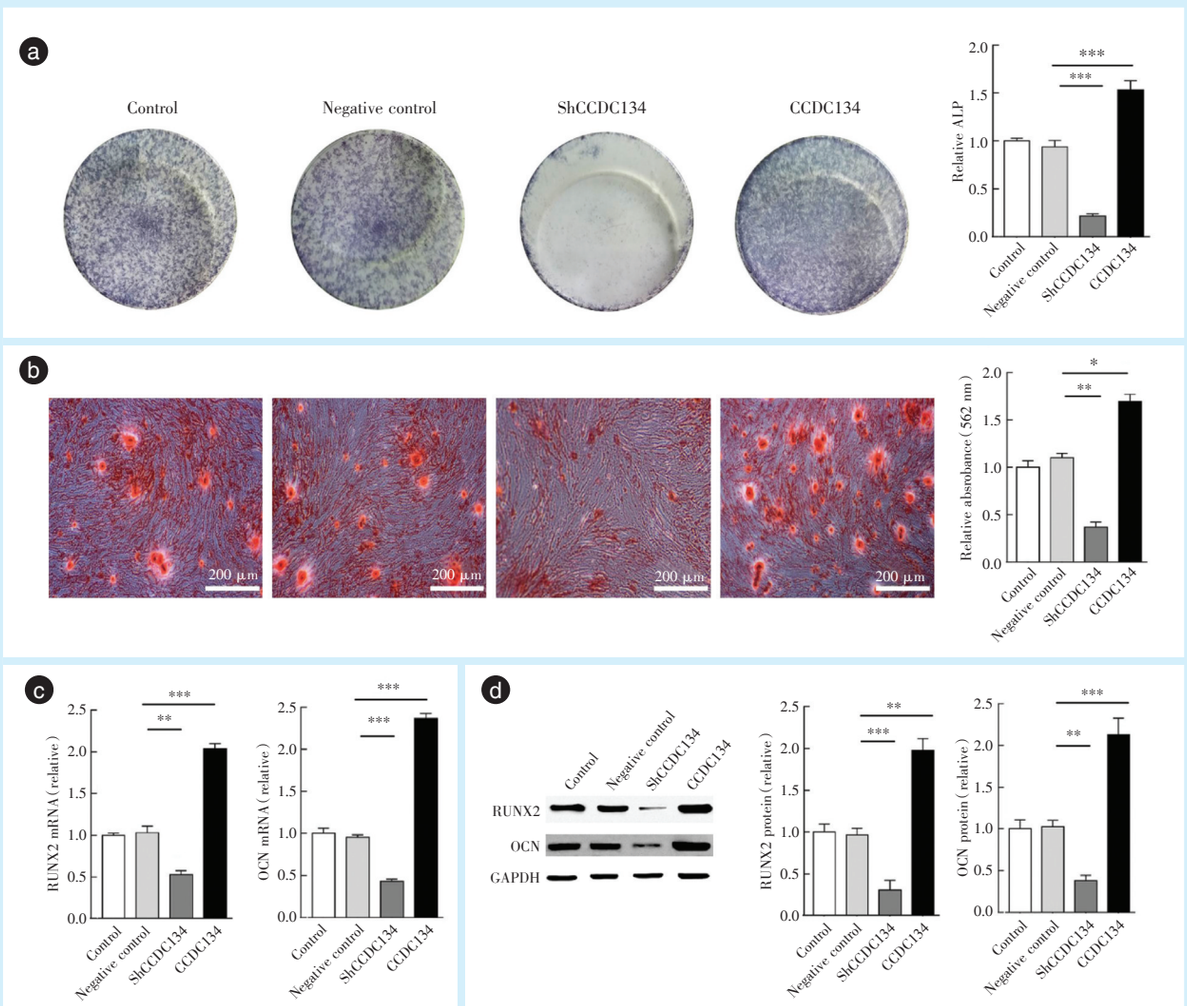
2.5　CCDC134通过BMP-2/SMAD1信号通路调控hDPSCs成骨分化功能

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; $n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

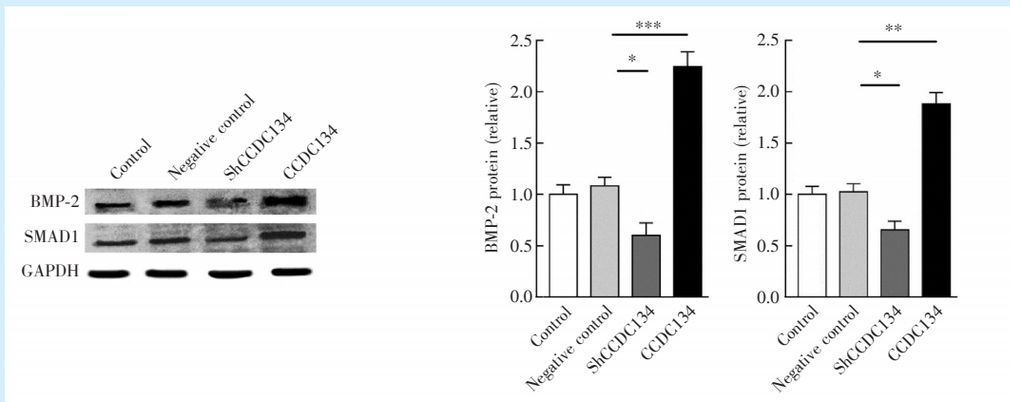
Figure 4　Effect of CCDC134 on osteogenic differentiation of human dental pulp stem cells

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。

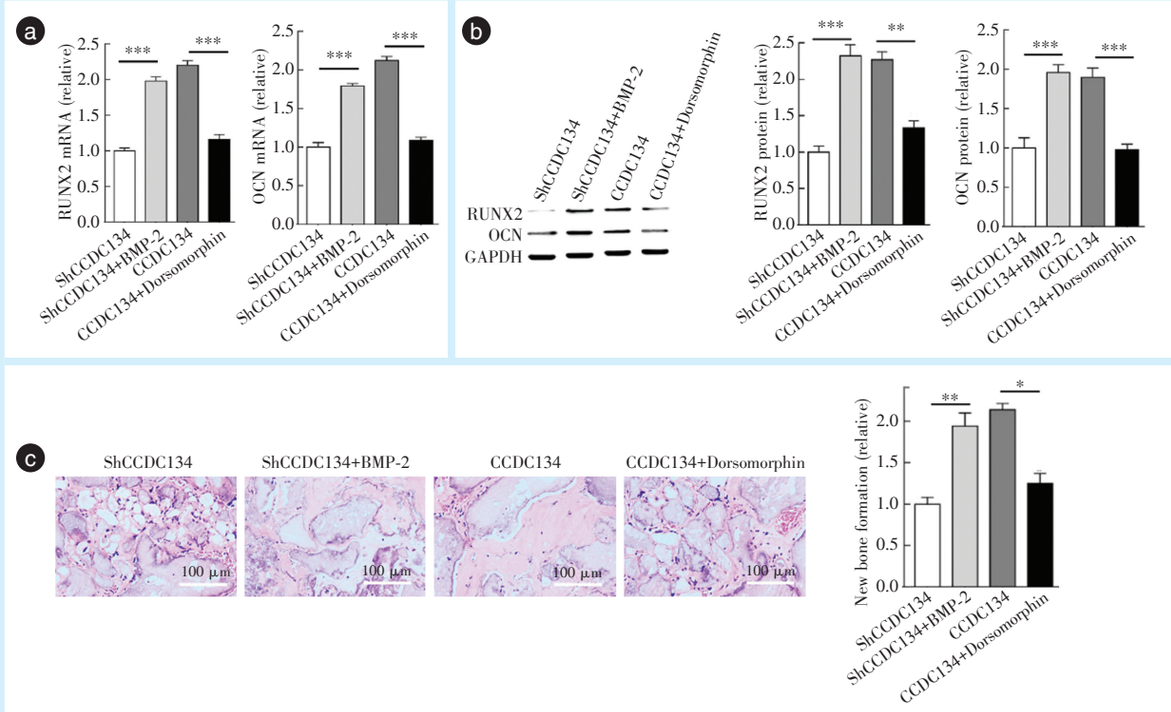
本实验首先对进行了hDPSCs鉴定，显示其间充质干细胞表面标志物高表达，造血干细胞表面标志物低表达，同时具有自我更新和多向分化潜能，以确保后续实验的可靠性。其次，发现hDPSCs



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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