

The expressions of tooth eruption relevant genes are different in incisors and molars dental follicle cells in rat: an in vitro study

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Abstract

Background The incisors and molars showed different patterns of tooth eruption in rodents and the dental follicle cells play key roles in tooth eruption. Little is known about the differences in incisors and molars dental follicle cells during tooth eruption in rodents. The purpose of this study was to investigate the differences between incisor dental follicle cells and molar dental follicle cells during tooth eruption in rat.

Methods Incisor dental follicle cells and molar dental follicle cells were obtained as previously described. Immunofluorescence was used to identify the cells. Gene expression was measured by real-time qPCR and western blot.

Results Compared with molar dental follicle cells, the incisor dental follicle cells showed higher expression of OPG, BMP-2 and BMP-3. The molar dental follicle cells showed higher expression of MCP-1 and RANKL.

Conclusions The expression patterns of genes related to tooth eruption were different in incisors and molars dental follicle cells in rat.

Background

Many diseases can cause abnormal tooth eruption such as delayed tooth eruption (DTE)^[1] and cleidocranial dysplasia (CCD)^[2]. Understanding the mechanism of tooth eruption is very important for solving the problem of abnormal tooth eruption. Tooth eruption is a complex process, in which there are not only the histological changes of alveolar bone and tooth germ, but also cytologic changes of dental follicle cells, osteoblasts, osteoclasts and other cells, and a variety of cytokines are involved^[3]. The dental follicle cells play an important role in the process of tooth eruption^[4]. Two obvious requirements were needed for tooth eruption, formation of eruption pathway and tooth moving through it^[5]. Osteoclasts formation and subsequent resorption of alveolar bone are necessary for the formation of tooth eruption pathway^[6]. During tooth eruption, monocytes are recruited into the dental follicle and differentiate into osteoclasts. Recruitment of monocytes is regulated by monocyte chemoattractant protein-1 (MCP-1) and colony-stimulating factor-1 (CSF-1) secreted by dental follicle cells^[7, 8]. Epidermal growth factor (EGF), transforming growth factor-beta 1 (TGF-β1) and interleukin-1 alpha (IL-1α) can enhance the expression of the MCP-1 in dental follicle cells.^[8] In addition, the receptor activator of nuclear factor-kappa B ligand (RANKL) and osteoprotegerin (OPG) are also expressed in dental follicle cells^[9]. RANKL and OPG were key regulators of osteoclasts formation. The expression of RANKL was increased while OPG was inhibited during tooth eruption^[10]. Wnt(Wingless and INT - 1)/β-catenin, RUNX2(runt-related transcription factor 2)-MiR31-SATB2(sensor array test-bed 2), JNK (Jun N-terminal kinase) and other signaling pathways are involved in alveolar bone resorption^[11–13].

In the process of tooth eruption, the tooth dental follicle cells not only regulate bone resorption, but also regulate bone formation. The basal part of dental follicle showed bone formation^[14]. Bone morphogenetic protein-2 (BMP-2) and bone morphogenetic protein-3 (BMP-3) can promote osteogenic differentiation of dental follicle cells^[15]. BMP-2 can induce osteogenic differentiation of tooth dental follicle through NOTCH hedgehog signaling pathways^[16, 17]. In order to coordinate the opposite biological processes of tooth eruption, namely osteoclastogenesis and osteogenesis, the expression of CSF-1, MCP-1, RANKL, OPG, BMP-2 and BMP-3 in dental follicle cells may be different in space-time.

The incisors and molars of rodent exhibit two different development patterns. The incisors can erupt throughout their life, but the molars will not erupt initiatively after the root have developed^[18]. Therefore, the incisor dental follicle and molar dental follicle may play different roles in tooth eruption in rats. Exploring the difference of IF and MF cells during eruption can provide a theoretical basis for the occurrence of abnormal tooth eruption.

Results

Cell culture and identification

Purified IF and MF cells of rats were obtained after twice differential trypsin digestion. IF and MF cells were characterized by a typical fibroblasts-like morphology of stellate or spindle shape (Fig. 1 A, B). Both IF and MF cells were positive for the mesenchymal cell maker vimentin, but negative for the epithelial cell marker CK14 (Fig. 1 C-F). These results indicated that the methods to isolate IF and MF cells are effective with a high purification.

IF and MF cells exhibited approximate proliferation ability

CCK-8 analysis showed that there was no significant difference in proliferation rates between IF and MF cells. Both IF and MF cells proliferation rates reached the peak at the 6th day and the numbers of IF and MF cells started decreasing. (Fig. 2).

IF and MF cells produced different amount of the minerals.

Alkaline phosphatase staining showed different amount of the minerals between the IF and MF cells (Fig. 3). Numerous minerals were formed in IF group and connected together while minerals were relatively few in the MF group. IF cells presented higher mineral formation than MF cells.

IF and MF cells showed differential expression patterns of eruption related genes.

There was a significantly different genes expression profile between IF and MF cells. Compared with MF cells, IF cells showed higher expression of CSF-1, but lower expression of MCP-1, which were monocytes recruitment relevant genes. IF showed higher expression of OPG, BMP-2, BMP-3 which were

osteogenesis relevant genes, while MF showed higher expression of RANKL which was osteoclastogenesis (Fig. 4). Western blotting showed that compared with MF cells, IF cells showed higher expression of BMP-2, BMP-3 and OPG, but lower expression of CSF-1, MCP-1 and RANKL. (Fig. 5).

Discussion

The dental follicle cells play key roles in the process of tooth eruption^[4]. The incisors and molars showed two different patterns of eruption in rodents that have attracted many researches in which the dental follicle may play a crucial role. Therefore, exploring the difference in the expression of genes related to tooth eruption in incisors and molars dental follicle in rats can further clarify the mechanism of tooth eruption and provide a possible theoretical basis for the occurrence of abnormal tooth eruption.

A variety of signaling molecules and transcription factors have been showed to be expressed in the dental follicle and participate in tooth eruption such as CSF-1, MCP-1, RANKL, OPG, BMP-1 and BMP3^[15, 19]. However, the differences in their expression of incisors and molars dental follicle cells in rodents have not been studied. Since incisors and molars in rats showed different patterns of eruption, the expression of tooth eruption related genes in IF and MF cells will not be same. Therefore, a hypothesis was proposed that the expression patterns of genes related to tooth eruption were different in incisors and molars dental follicle cells in rat.

MCP-1 and CSF-1 are effective chemokine of monocytes. Study has shown that MCP-1 gene is expressed in dental follicle. On the third day after the birth of rat, the expression of MCP-1 achieved the peak, which was consistent with the gathering of monocytes. The dental follicle recruits a large number of monocytes, which then differentiation into osteoclasts, is the key to tooth eruption^[12]. Injection of CSF-1 can increase the numbers of monocytes and osteoclasts in the dental follicle of normal mice, thus accelerating tooth eruption^[20]. Our results showed that the MF cells expressed higher MCP-1 and CSF-1 protein which indicated that MF cells had stronger ability to recruit monocytes. MF cells also expressed higher RANKL which enhanced bone resorption than IF cells. Together, these results revealed that MF cells had stronger osteoclastogenic ability. During tooth eruption, the dental follicle also express genes related to osteogenesis such as OPG, BMP-2, BMP-3. Our results showed that IF cells expressed higher OPG, BMP-2 and BMP-3. The results indicated that the IF cells had stronger osteogenic ability.

In this study, we used the third passages of IF and MF cells of rats. The results showed that the IF cells expressed higher OPG, BMP-2, and BMP-3 than MF cells, while MF cells expressed higher MCP-1, RANKL. The results indicate that the IF cells have stronger osteogenic ability than MF cells while MF cells have stronger osteoclastogenic ability. However, what causes this difference needs further studies.

Conclusion

In summary, the study demonstrates that the expressions of genes related to tooth eruption are different in IF and MF cells. The IF cells showed stronger osteogenic ability than MF cells, while MF cells showed

stronger osteoclastogenic ability. This may be the reason why incisors and molars have different eruption patterns in rat. The incisors in rodents can erupt throughout life may relate to the continuous expression of osteogenic relevant genes. But the specific mechanism needs further study.

Methods

All the animal experimental procedures employed in this study were approved by the Ethics Committee of West China School of Stomatology, Sichuan University, China. All the animals used in the experiments were bought from CHENGDU DOSSY EXPERIMENTAL ANIMALS CO., LTD.

Cell isolation, purification and identification

Incisor dental follicle cells (IF) and molar dental follicle cells (MF) were isolated from incisor and molar germs of post-natal (PN) 7-day Sprague-Dawley (SD) rats referring to a modified method as previously described^[21]. Briefly, 5 post-natal 7-day old SD rats were euthanized by cervical dislocation under overdose anesthesia (chloral hydrate, 10%, 0.5ml per rat). The mandibles were dissected and then the incisor and first molar germs were isolated with the aid of a stereomicroscope. The Incisor dental follicle was isolated from the incisor germ while the molar dental follicle was isolated from the molar germ. Then minced the tissues into pieces, and digested with a mixture of 625 U/mL type I collagenase (Sigma-Aldrich, USA) and 2.4U/mL Dispase II (Sigma-Aldrich, USA) at 37 °C for 0.5h. Using α -MEM supplemented 10% fetal bovine serum and 1% penicillin/streptomycin solution to terminate digestion. After centrifugation at 1500 rpm for 5minutes, IF and MF cells were re-suspended and cultured with α -MEM supplemented with 10 FBS (Gibco, USA), 1% penicillin/streptomycin solution (P/S; Solarbio, China). Cells were incubated at 37 °C in a humidified atmosphere with 5% CO₂. The medium was changed every 2 days. After the cells grow and fuse by 70%, a differential digestion method using trypsin/EDTA (Hyclone, USA) was performed to purify the targeting cells^[22]. Purified cells were further identified by immunofluorescence staining. IF and MF cells were assessed for positive immunofluorescent staining with rat anti-vimentin antibody and negative staining with anti-CK-14 antibody. Choose the third passages of IF and MF for subsequent experiments.

Cell proliferation analysis

CCK-8 analysis (CCK-8, Dojindo, Japan) was used to quantitatively evaluated cell proliferation. IF and MF cells were seeded in a 96-well plate with an initial density of 6×10^3 /well and cultured with α -MEM supplemented with 10 FBS, 1% P/S. Replaced 120uL original medium and added 12uL CCK-8 per well, then incubated the plate at 37°. After 2h, taken 100ul of above solution from each well and added to one well of a new 96-well plate. At least three parallel replicates were prepared. Using a spectrophotometer (Thermo VARIOSKAN FLASH, Thermo, USA) to determine the optical density (OD).

Mineralization ability assay

Alkaline phosphatase staining was used to assess the mineral formation of IF and MF cells. IF and MF cells were seeded in a 6-well plate with an initial density of 4×10^4 /well and cultured in osteoblast inducing conditional media (α -MEM containing 10^{-8} mol/L Dexamethasone, 0.2mmol/L Vitamin C and 10mmol/L Disodium beta-Glycerophosphate Tetrahydrate) for 7 days. Cells were washed 3 times in PBS and fixed in 4% paraformaldehyde for 15min and then incubated in BCIP/NBT solution (Beyotime, China) at room temperature in dark for 20 minutes. After being washed 3 times in distilled water, cells were photographed by a camera (vivo NEX A, China).

Quantitative real-time PCR analysis

Quantitative real-time PCR/qRT-PCR analysis was used to detect the CSF-1, MCP-1, RANKL, OPG, BMP-2 and BMP-3 expression in IF and MF cells. After 7 days culture, cells were harvested and total RNA was isolated using Trizol reagent (Ambion, USA). cDNA synthesis was performed with SYBR[®] Premix Ex Taq II (Perfect Real Time Kit; TaKaRa, Dalian). Experiments were performed in triplicates according to the manufacturer's instructions. Sequences of the gene-specific primers synthesized by TaKaRa are listed in Table 1. Normalized gene expression values for each sample were calculated as the ratio of expression of mRNA for the target genes to the expression of mRNA for GAPDH.

Western blotting

IF and MF cells were harvested and lysed in lysis buffer containing protease inhibitor and phosphatase inhibitor (Beyotime, China). Protein concentration was tested using a BCA kit (Beyotime, China). After being blocked in 5% skim milk (Beyotime, China) for 60 min at room temperature, membranes were incubated with primary antibodies against MCP-1 (df7577, Affinity, USA), CSF-1 (ab233387, Abcam, UK), BMP2 (af5163, Affinity, USA), BMP3 (af5153, Affinity, USA), RANKL (df7006, Affinity, USA), OPG (df6824, Affinity, USA) and β -actin (ab8227, Abcam, UK) separately at 4° overnight. After washing, the membranes were incubated with HRP-Goat anti Rabbit (ab6721, Abcam, UK). The immune-reactive bands were detected by chemiluminescence (Thermo-Fisher Scientific) with the ChemiDocTM Touch Imaging System (Biorad).

Statistical analysis

Data are presented as the mean \pm SD from at least three independent experiments. Student t test was used to perform the statistical comparison. GraphPad Prism 7.00 software was used to perform statistical analysis and a $p \leq 0.05$ was considered statistical significant.

Abbreviations

IF: Incisor dental follicle

MF: Molar dental follicle

DTE: Delayed tooth eruption

CCD: Cleidocranial dysplasia

MCP-1: Monocyte chemoattractant protein-1

CSF-1: Colony-stimulating factor-1

EGF: Epidermal growth factor

TGF- β 1: Transforming growth factor-beta 1

IL-1 α : Interleukin-1 alpha

RANKL: The receptor activator of nuclear factor-kappa B ligand

OPG: Osteoprotegerin

Wnt: Wingless and INT-1

RUNX2: Runt-related transcription factor-2

SATB2: sensor array test-bed 2

JNK: Jun N-terminal kinase

BMP-2: Bone morphogenetic protein-2

BMP-3: Bone morphogenetic protein-3

PN: Post-natal

SD: Sprague-Dawley

OD: optical density

Declarations

Ethics approval and consent to participate

Animal studies were approved by State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University and complied with the guidelines for the use of animals in research.

Consent for publication

Not applicable

Availability of data materials

All datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

There are no conflicts of interest to declare.

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Author's contributions

M. H. and D. B. conceived the project, designed and supervised the experiments. M. H. and X. D. performed the experiments; M. H., P. W., Z. X., J. W., X. W. and J. C. analyzed the data AND wrote the manuscript together. All authors reviewed the draft manuscript and approved the final version of the manuscript.

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Table

Gene	Primers (5'-3')	Fragment(bp)
CSF-1	F agcaaggaagcgaacgaaccag	141
	R tcacgaggagacagaccagcag	
MCP-1	F cagcccagaaaccagccaactc	88
	R caacaggcccagaagcgtgac	
BMP2	F gccacgacggtaaaggacatcc	146
	R ggccacgatccagtcattccac	
BMP3	F agccttcagactcagcctcctg	116
	R cgcctcgccttcttcagtgtc	
RANKL	F ggaagggttcgtggctcgatgtg	82
	R gtcggcagcattgatggtgagg	
OPG	F actgtgtcccttgccctgac	131
	R ctcggttggtgggtgcggttg	

Table 1. Primer sequences of target genes.

Figures

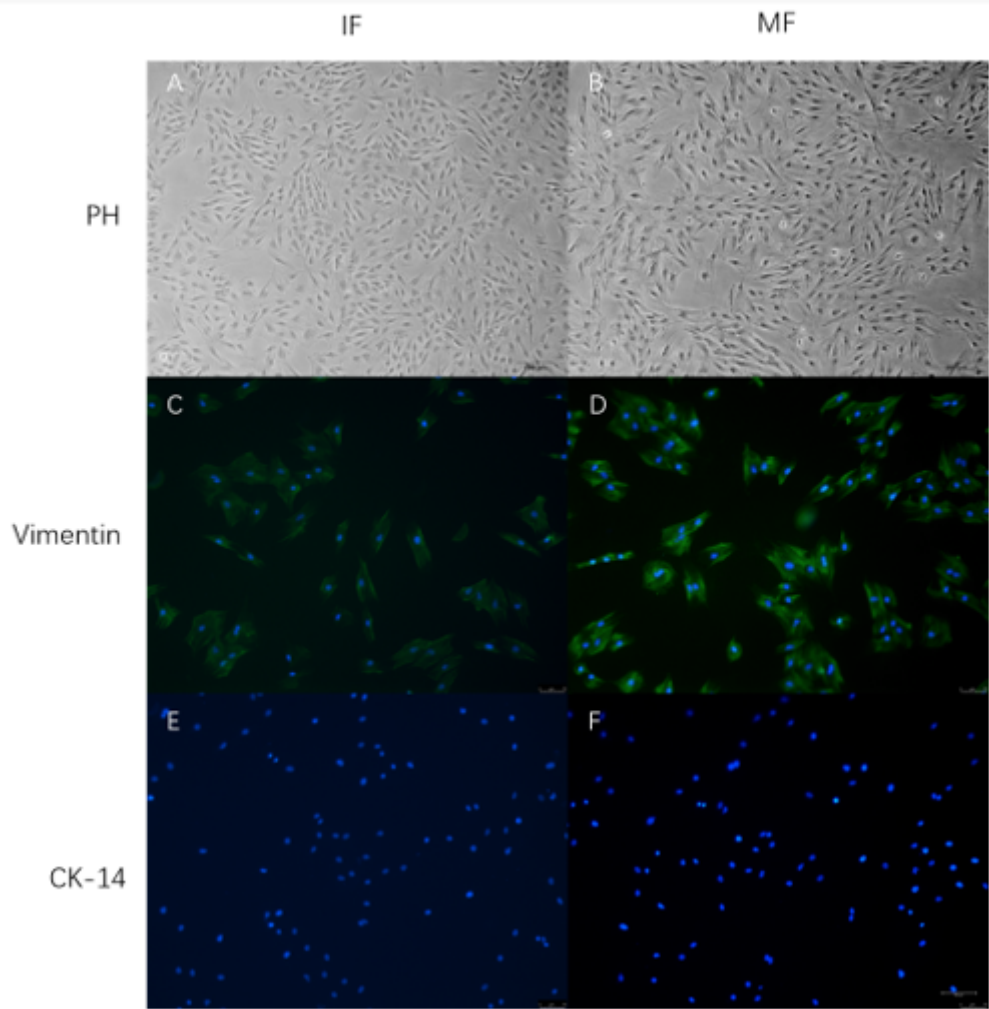


Figure 1

Purified IF and MF cells observed under phasecontrast microscope (PH) and identified by immunoinfluence staining. IF and MF cells were positive for vimentin but negative for CK-14.

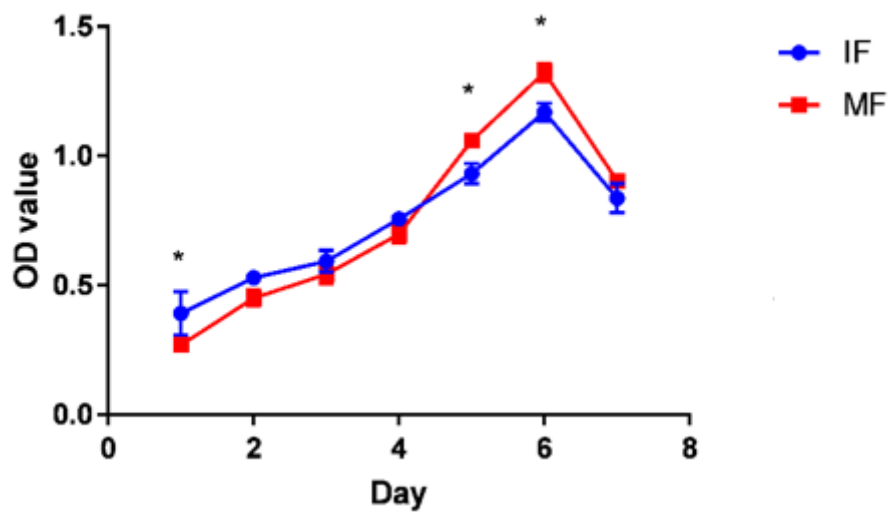


Figure 2

CCK-8 analysis of IF and MF cells. There was no significance between groups. (* $p \leq 0.05$)

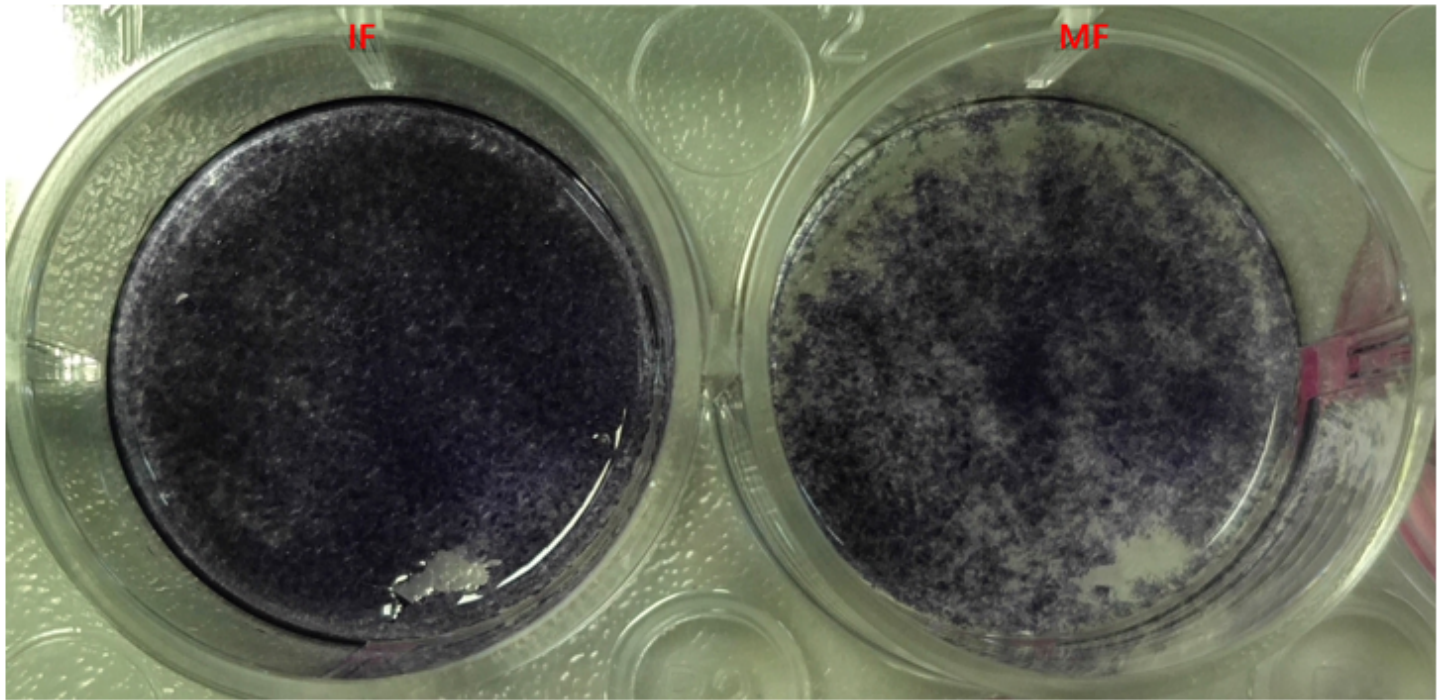


Figure 3

Alkaline phosphatase staining of IF and MF cells. IF cells presented higher mineral formation than MF cells.

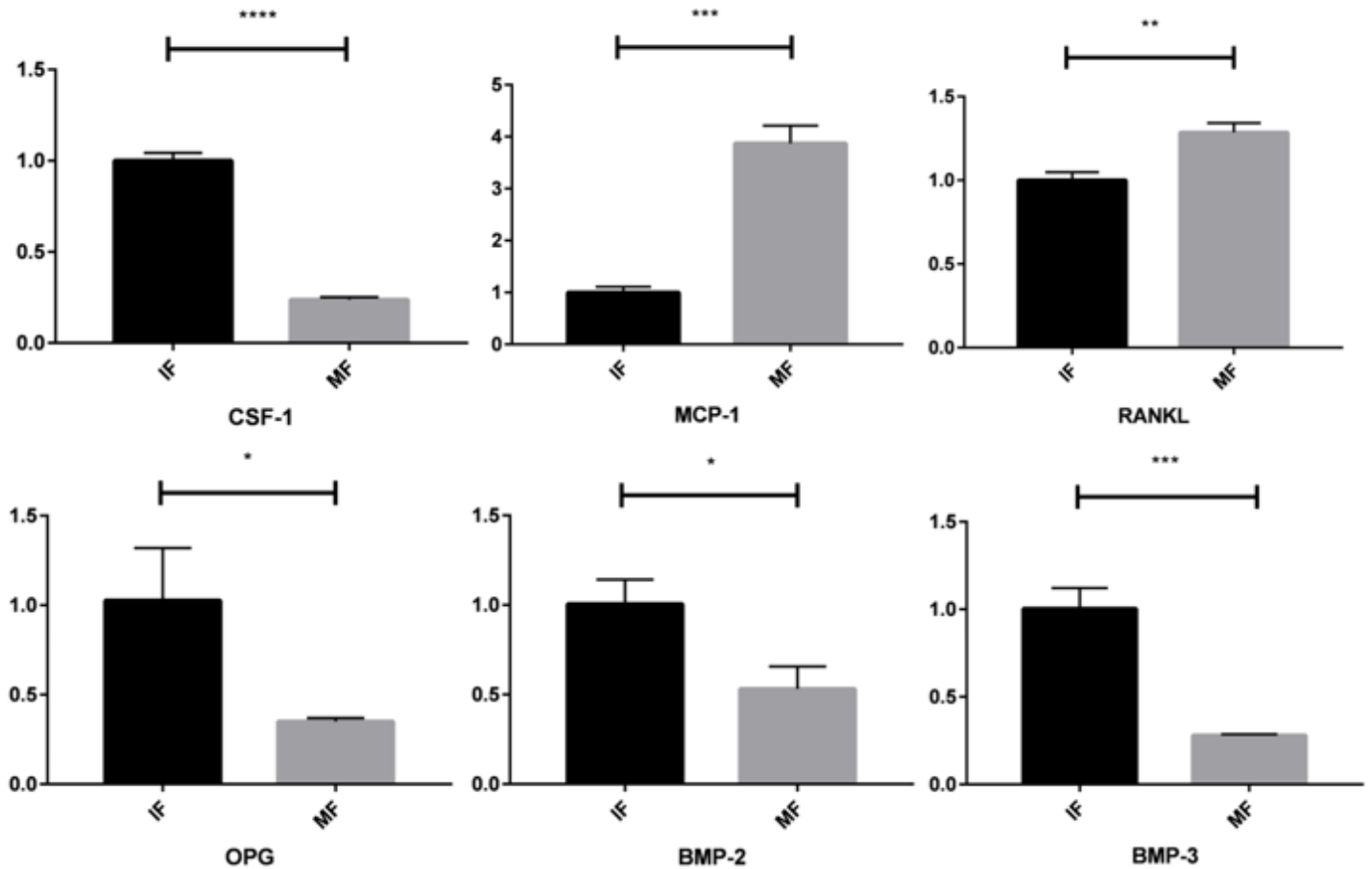


Figure 4

Differential expression patterns of tooth eruption related genes between IF and MF cells. Compared with MF cells, the IF cells showed significantly higher expression of CSF-1, BMP-2, BMP-3 OPG and lower expression of MCP-1, RANKL. (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$)

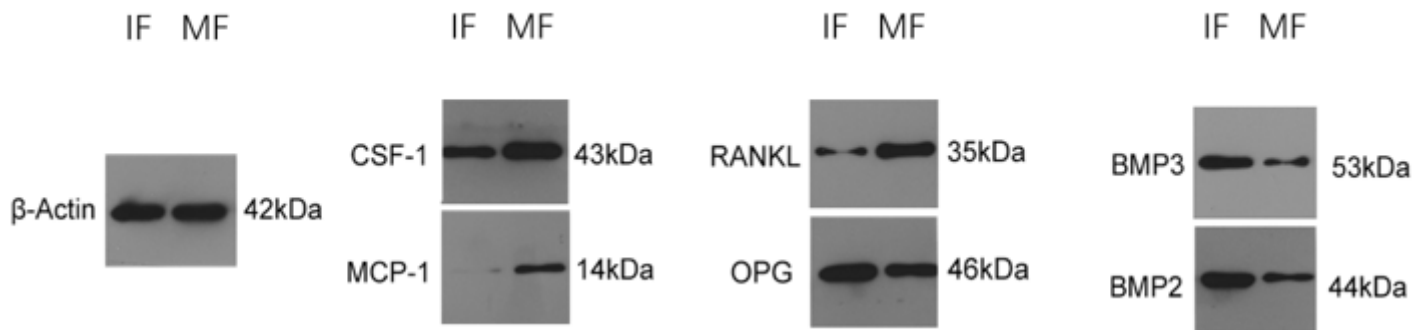


Figure 5

Differential expression patterns of tooth eruption related proteins between IF and MF cells. Compared with MF cells, IF cells showed higher expression of OPG, BMP-2, BMP-3 and lower expression of CSF-1, MCP-1, RANKL.

Supplementary Files

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