Biochemical and Biophysical Research Communications 424 (2012) 182–188

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Effects of intermittent versus continuous parathyroid hormone administration on condylar chondrocyte proliferation and differentiation

Qi Liu [a,1], Qilong Wan [a,1], Rongtao Yang [a], Haihua Zhou [a], Zubing Li [a,b,*]

[a] The State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) & Key Laboratory of Oral Biomedicine Ministry of Education, School & Hospital of Stomatology, Wuhan University, 237# Luoyu Road, Wuhan 430079, China
[b] Department of Oral and Maxillofacial Surgery, School and Hospital of Stomatology, Wuhan University, 237# Luoyu Road, Wuhan 430079, China

ARTICLE INFO

Article history:
Received 16 June 2012
Available online 27 June 2012

Keywords:
Parathyroid hormone (PTH)
Proliferation
Differentiation
Chondylar chondrocyte
Intermittent PTH
Continuous PTH

ABSTRACT

Endochondral ossification is a complex process involving chondrogenesis and osteogenesis regulated by many hormones and growth factors. Parathyroid hormone (PTH), one of the key hormones regulating bone metabolism, promotes osteoblast differentiation and osteogenesis by intermittent administration, whereas continuous PTH administration inhibits bone formation. However, the effects of PTH on chondrocyte proliferation and differentiation are still unclear. In this study, intermittent PTH administration presented enhanced effects on condylar chondrocyte differentiation and bone formation, as demonstrated by increased mineral nodule formation and alkaline phosphatase (ALP) activity, up-regulated runt-related transcription factor 2 (RUNX2), ALP, collagen type X (COL10a1), collagen type I (COL1a1), osteocalcin (OCN), bone sialoprotein (BSP), bone morphogenetic protein 2 (BMP2) and osterix (OSX) mRNA and/or protein expression. On the contrary, continuous PTH administration promoted condylar chondrocyte proliferation and suppressed its differentiation, as demonstrated by up-regulated collagen type II (COL2a1) mRNA expression, reduced mineral nodule formation and down-regulated expression of the mRNAs and/or proteins mentioned above. Our data suggest that PTH can regulate condylar chondrocyte proliferation and differentiation, depending on the type of PTH administration. These results provide new insight into the effects of PTH on condylar chondrocytes and new evidence for using local PTH administration to cure mandibular asymmetry.

1. Introduction

Condylar cartilage is a special structure in the mandible. It consists of layers of chondrocytes at the top of the condyle. Although condylar cartilage persists throughout post-natal life similar to other articular cartilages, it possesses some unique characteristics. Normal articular cartilage is permanent tissue and cannot adapt to the changes and stimulations after adolescence because chondrocytes in normal articular cartilages no longer participate in endochondral ossification [1]. However, condylar cartilage can grow and remodel to adapt to positional changes or mechanical stimulations by regenerating chondrogenesis and the subsequent endochondral ossification [2].

Endochondral ossification is a complex process involving chondrocyte proliferation, differentiation and osteogenesis, which is regulated by many factors such as parathyroid hormone (PTH), parathyroid hormone-related peptide (PTHrP), bone morphogenetic proteins (BMPs), insulin-like growth factors, Indian hedgehog and the Wnt family. PTH, an 84 amino acid peptide, is one of the key factors in controlling bone remodeling. The function of PTH is performed by its cleavage products, which contain at least 31 of the N-terminal amino acids; PTH 1-34 (teriparatide) is the only bone anabolic agent approved by the US Food and Drug Administration (FDA) for osteoporosis treatment [3]. Elevated levels of PTH can increase bone turnover, by exerting either an anabolic or catabolic effect on the skeleton depending on the dosage and exposure duration [4,5]. When PTH is administered intermittently, the anabolic effect is shown as increased bone mass, density, strength, and improved bone fracture prognosis. Continuous delivery of PTH, in contrast, usually causes bone resorption. However, the molecular mechanism of the above contradictory effects is still not fully understood.

As an analog of PTH, PTHrP binds on the same receptor with PTH [6]. Effects of PTHrP on chondrocyte proliferation and differentiation have widely been studied, demonstrating that PTHrP is one of the important factors regulating the pace of chondrocyte differentiation and endochondral ossification [7–9]. Chondrocyte proliferation is promoted when PTHrP is overexpressed, while

* Corresponding author at: Department of Oral and Maxillofacial Surgery, School and Hospital of Stomatology, Wuhan University, 237# Luoyu Road, Wuhan 430079, China. Fax: +86 027 87873260.
E-mail address: lizubing0827@163.com (Z. Li).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.06.106

endochondral ossification is enhanced when PTHrP is down-regulated or when the PTH/PTHrP receptor is blocked. However, PTHrP function in these studies is gained or lost at the genetic level, which leads to the continuous up- or down-regulation of PTHrP. It has recently been reported that PTH regulates bone metabolism by enhancing proliferation and differentiation and by inhibiting apoptosis of osteoblasts *in vivo* and *in vitro* [6,10–12]. In addition, intermittent PTH administration can stimulate mandibular bone formation in rats [13]. However, (1) whether PTH administration affects chondrogenesis during the process of regulating bone metabolism and (2) what is the effect of PTH on chondrocytes, especially on condylar chondrocytes, remain to be elucidated.

In this study, primary condylar chondrocytes isolated from newborn rats were cultured in an osteogenic medium that provided an endochondral ossification environment [14]. PTH peptide fragment 1-34 (PTH 1-34) was administered intermittently or continuously to the cultured condylar chondrocytes to test our previous hypothesis [15]. Chondrocyte proliferation-, differentiation- and endochondral ossification-related markers were analyzed to study the effects of the different methods of PTH administration on condylar chondrocytes during osteogenic culture.

2. Materials and methods

2.1. Isolation and culture of condylar chondrocytes

All animal protocols were approved by the Animal Care and Use Committee of the School of Stomatology, Wuhan University. Condylar chondrocytes were isolated from newborn female Sprague–Dawley (S–D) rats by using the enzymatic digestion method as previously described [14]. Briefly, condylar cartilage tissue was carefully dissected under a stereomicroscope from the bone-cartilage junction, after the condyles had been harvested from euthanatized newborn female S–D rats. Then, these condylar cartilage tissue samples were digested with 0.2% type II collagenase (Gibico) for 4–6 h at 37 °C. The suspension cells were seeded in plastic flasks or 6-well plates at a density of 5×10^3 cells/cm^2 in DMEM containing 10% FBS and 100 μg/mL penicillin/streptomycin and cultured at 37 °C in 5% CO_2. The culture medium was changed every two days. The adherent cells were then verified as chondrocytes by collagen type II immunohistochemical staining (data not shown).

2.2. PTH administration

According to the PTH administration methods, condylar chondrocytes were divided into three groups: the control group (without PTH administration), the intermittent PTH administration (I-PTH) group and the continuous PTH administration (C-PTH) group (Fig. 1A). When the primary cultured condylar chondrocytes were approximately 80% confluent, the culture medium was changed to the osteogenic culture medium, which is DMEM containing 10% FBS, 5 mM β-glycerophosphate (Sigma), 50 μg/mL ascorbic acid (Sigma), 100 nM dexamethasone (Sigma) and 100 μg/mL penicillin/streptomycin. Thereafter (Day 0), the chondrocytes were stimulated for the first 6 h with (I-PTH group and C-PTH group) or without (Control group) 10 nM PTH 1-34 (Sigma). After 6 h of stimulation, the culture medium in the I-PTH group was changed to osteogenic culture medium without PTH. The PTH administration cycle was repeated every 48 h as shown in Fig. 1A.

2.3. Mineral nodule detection

Alizarin red S staining was used to detect the mineral nodule formation in the three groups on day 6 and day 14. Condylar chondrocytes cultured in 6-well plates were fixed with 95% alcohol for 30 min after being washed twice with PBS. These cells were then incubated in 1% alizarin red S staining solution (Sigma) at 37 °C for 30 min. After removing the staining solution, the excess dye was rinsed off with distilled water.

2.4. Alkaline phosphatase (ALP) activity assay

After being treated with PTH 1-34 for 6 or 14 days, the condylar chondrocytes in each group were collected and lysed in M-PER® Mammalian Protein Extraction Reagent (Thermo Scientific) according to the manufacturer's instructions. The protein concentrations were then measured with the Pierce® BCA Protein Assay Kit (Thermo Scientific). ALP activity was analyzed with the same volume (30 μL) of lysate in each sample by measuring the color changes of 4-p-nitrophenylphosphate (Sigma) in the spectrophotometer at 405 nm. The enzyme activity was normalized based on the protein concentration of each sample. The specific activity of ALP was calculated as units/μg protein.

2.5. Western blot analysis

The protein samples from different groups containing 40 μg total proteins were used for the Western blot analysis. The samples were separated by 10–15% SDS–PAGE. These proteins were then transferred to polyvinylidene difluoride (PVDF, Millipore) membranes. After blocking with 5% non-fat milk, the PVDF membranes were incubated with primary antibodies in a TBS buffer overnight at 4 °C. These primary antibodies were against runt-related transcription factor 2 (RUNX2), bone sialoprotein (BSP) and BMP2 (Santa Cruz). On the following day, PVDF membranes were incubated with appropriate secondary antibodies for 1 h at room temperature. After the membranes had been soaked in an enhanced chemiluminescence reagent (Thermo Scientific) for 5 min, the blots were visualized using X-ray films. All the blots were quantified by densitometry using Quantity One® software (Bio-Rad), and the relative protein expression levels were normalized to β-actin.

2.6. Real-time PCR analysis

The total RNA in each group was extracted using the TRIzol reagent (Invitrogen), and total RNA was extracted following the manufacturer's recommended protocol. One microgram of RNA was subjected to reverse transcription using the ReverTra Ace-α-First strand cDNA Kit (TOYOBO) to synthesize cDNA. The obtained cDNA was then amplified via real-time PCR using an ABI 7500 Real-time PCR System (Applied Biosystems) and SYBR® Green Realtime PCR Master Mix (TOYOBO). The primers used for real-time PCR are listed in Table 1, in which glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene. Quantification of the relative expression levels of these target genes was achieved by normalizing to GAPDH using the ΔΔCt method.

2.7. Statistical analysis

All the above experiments were repeated at least three times independently. All the data was presented as mean ± SD. Statistical significance among the groups was assessed with one-way ANOVA using SPSS software 16.0. The level of significance was $P < 0.05$.

3. Results

3.1. Effects of different PTH administration methods on mineral nodule formation in condylar chondrocytes during osteogenic culture

To investigate the effects of intermittent or continuous PTH administration on mineral nodule formation in condylar chondro-

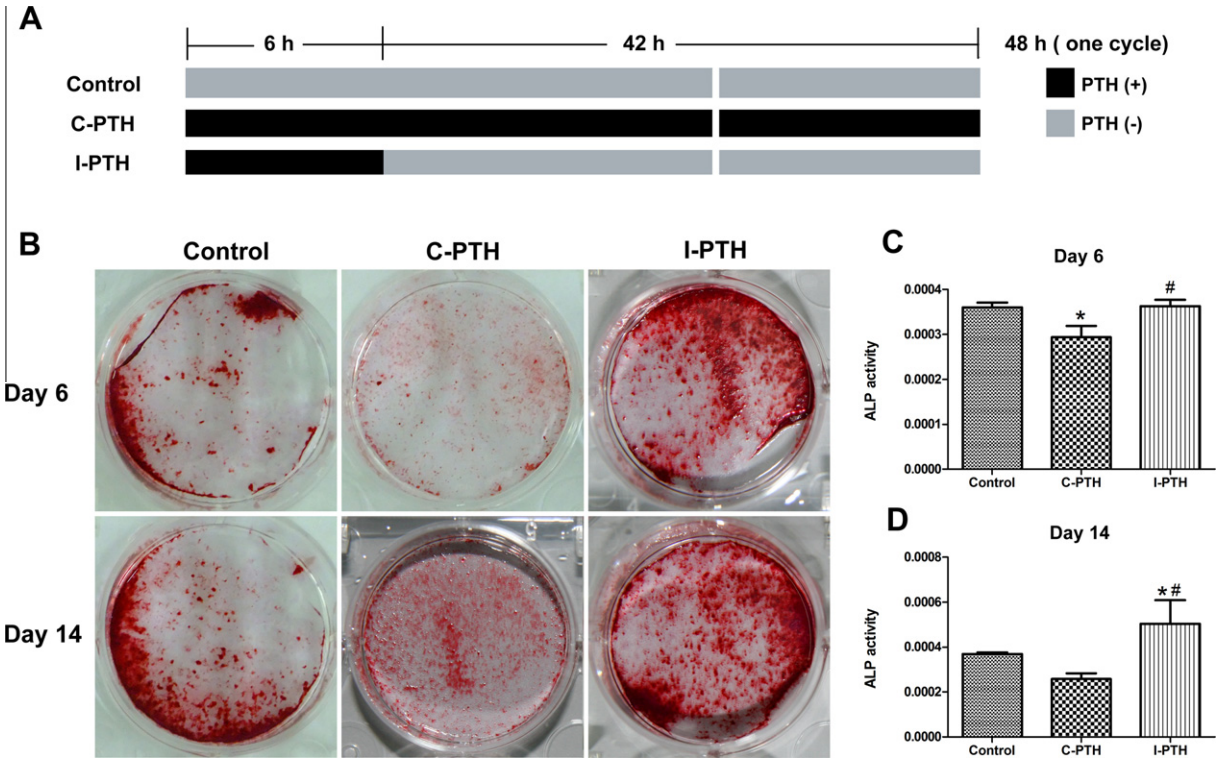

Fig. 1. Diagram of the PTH administration methods (A) and effects of the different PTH administration methods on mineral nodule formation (B) and ALP activity (C and D) in condylar chondrocyte cultured in osteogenic medium on day 6 and day 14. $^{*}P < 0.05$ when PTH administration groups were compared to the control group, $^{\#}P < 0.05$ when the I-PTH group was compared to the C-PTH group.

Table 1
Primers used for real-time PCR.

Gene	Sense primers (5′–3′)	Anti-sense primers (5′–3′)
GAPDH	AGACAGCCGCATCTTCTTGT	TGATGGCAACAATGTCCACT
RUNX2	GCCGGGAATGATGAGAACTA	GGACCGTCCACTGTCACTTT
ALP	GACATGCAGTATGAGTTGAAT	TCAATCCTGCCTCCTTCCACTA
COL2a1	GCGAGAGAGGACGGACTG	CGAGAACCTTGAGCACCTTC
COL10a1	TCCTGTCAAGCTCATCCTAAT	CGGAGTGCACTTTAGAAAGC
COL1a1	ATCACCAGACGCAGAAGTCA	CCATAGGACATCTGGGAAGC
OCN	ATCACCAGACGCAGAAGTCA	GAGCTCACACACCTCCCTGT
BMP2	ATCACGAAGAAGCCATCGAG	TGTTCCCGAAAAATCTGGAG
OSX	CACTGGCTCCTGGTTCTCTC	GGACTGGAGCCATAGTGAGC

cytes during osteogenic culture, alizarin red S staining was performed on day 6 and day 14. The mineral nodules were stained red as shown in Fig. 1B. More mineral nodules were formed in the I-PTH group than in the C-PTH and control groups. On the other hand, fewer mineral nodules were found in the C-PTH group than in the control group (Fig. 1B). These results confirmed our previous hypothesis that different PTH administration methods might demonstrate opposing PTH functions during condylar endochondral ossification [15].

3.2. Effects of different PTH administration methods on ALP activity and endochondral ossification-related protein expression in condylar chondrocytes

Because different PTH administration methods had different abilities to induce mineral nodule formation in condylar chondrocytes during osteogenic culture, we investigated whether different PTH administration also altered ALP activity and endochondral ossification-related protein expression among the three groups. On day 6, ALP activity in the I-PTH and control groups was significantly higher than that of the C-PTH group, while no significant difference was observed between the I-PTH and control groups (Fig. 1C). ALP activity in the I-PTH group was the highest among the three groups on day 14 (Fig. 1D).

Expression profiles of the chondrocyte differentiation- and bone formation-related proteins, RUNX2, BSP and BMP2, are illustrated in Fig. 2. The expression level of RUNX2 was remarkably higher in the I-PTH group than that in the C-PTH and control groups. In addition, the RUNX2 expression level in the C-PTH group was significantly lower than that in the control group. The expression levels of BSP and BMP2 in the I-PTH group were highest among the three groups. Furthermore, BSP expression level in the C-PTH group was lower, while BMP2 expression level was higher than in the control group.

3.3. Effects of different PTH administration methods on chondrocyte proliferation-, differentiation- and bone formation-related gene mRNA expression in condylar chondrocytes

To analyze the effects of the different PTH administration methods on condylar chondrocyte proliferation and differentiation, the mRNA expression levels of the chondrocyte proliferation marker collagen type II (COL2a1) and the differentiation marker collagen type X (COL10a1) were detected by real-time PCR. The findings indicated that COL2a1 mRNA expression in the I-PTH and control groups was significantly down-regulated compared to that in the C-PTH group on day 2 and day 6, whereas COL10a1 mRNA expression in the C-PTH group was obviously down-regulated compared to the I-PTH and control groups (Fig. 3A and B). As typical bone formation and chondrocyte terminal differentiation regulators, mRNA levels of RUNX2 and ALP were significantly up-regulated in the I-PTH group compared to the other two groups on both day 2 and

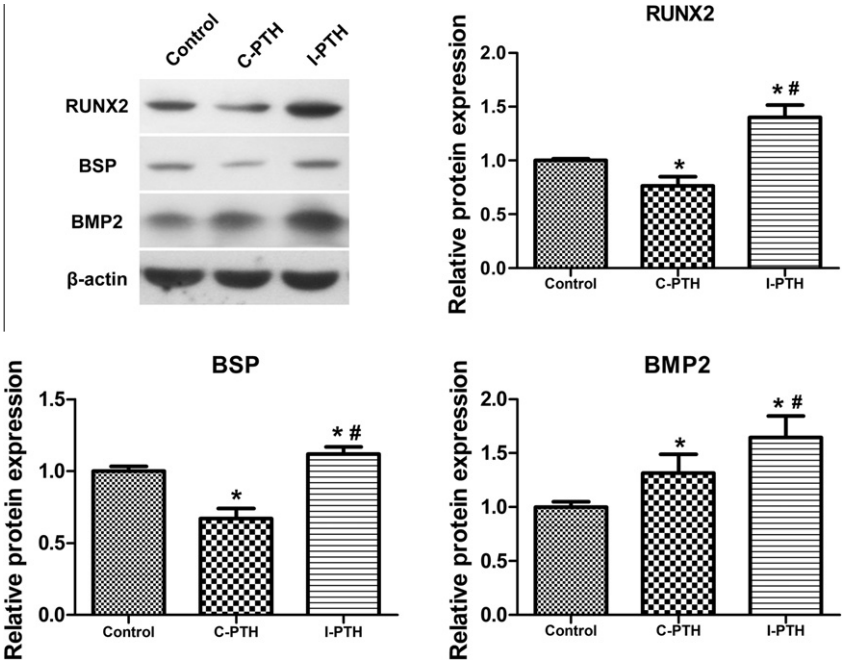

Fig. 2. Effects of different PTH administration methods on the expression of chondrocyte differentiation- and endochondral ossification-related proteins in condylar chondrocytes on day 14. $^{*}P < 0.05$ when PTH administration groups compared to the control group, $^{\#}P < 0.05$ when the I-PTH group compared to the C-PTH group.

day 6. Meanwhile, mRNA levels of RUNX2 and ALP in the C-PTH group were the lowest among the three groups (Fig. 3C and D).

To further explore the effects and mechanisms of the different PTH administration methods on condylar chondrocyte differentia-

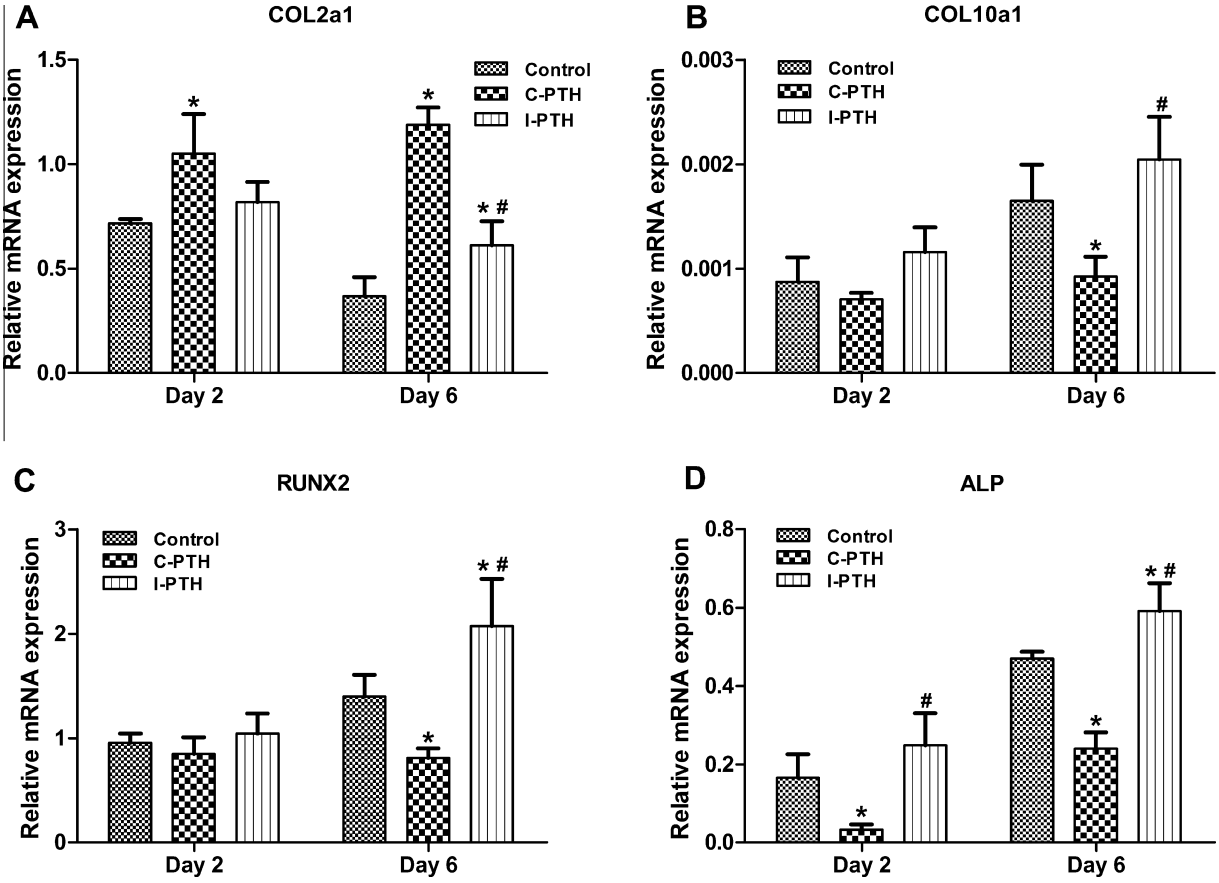

Fig. 3. Effects of different PTH administration methods on the mRNA levels of chondrocyte-specific markers and differentiation markers on day 2 and day 6. $^{*}P < 0.05$ when PTH administration groups were compared to the control group, $^{\#}P < 0.05$ when the I-PTH group was compared to the C-PTH group.

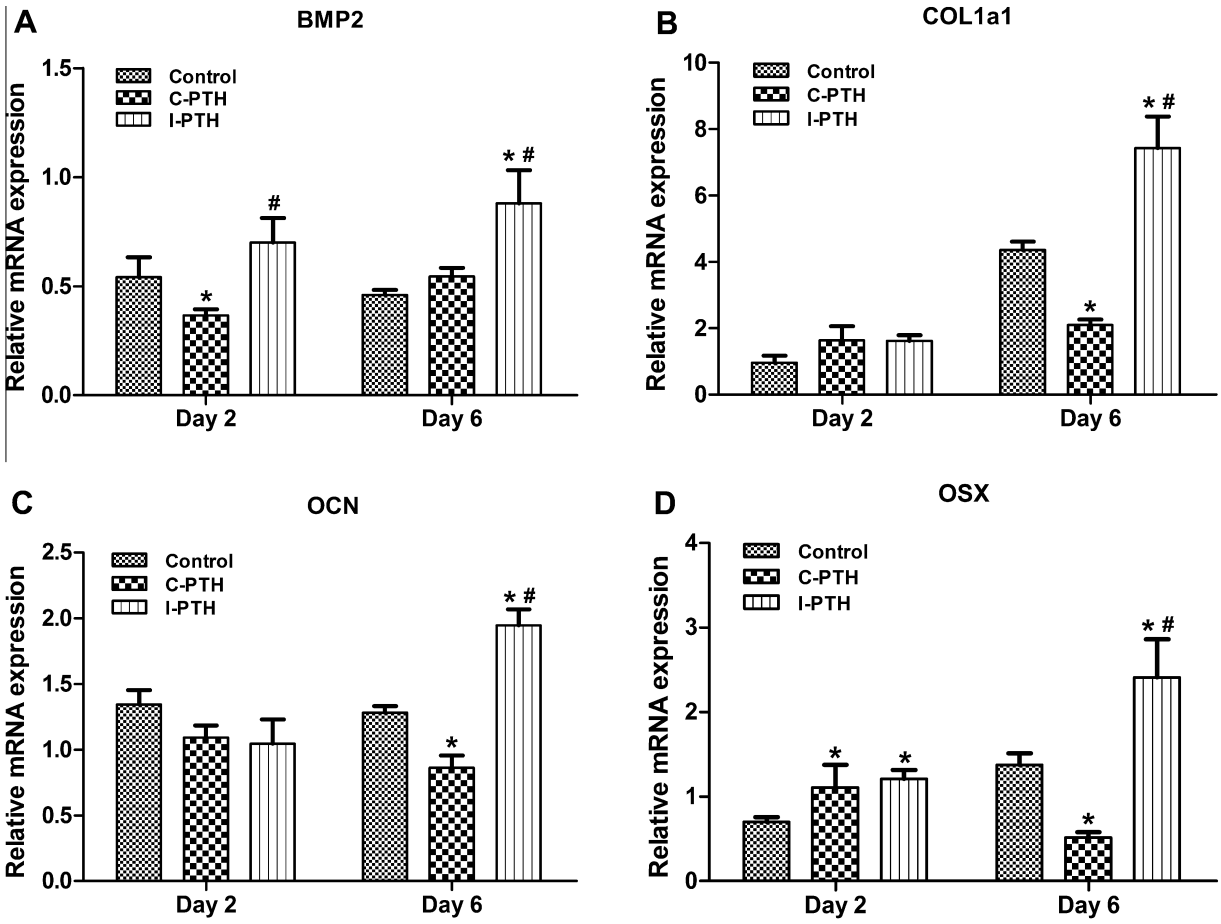

Fig. 4. Effects of different PTH administration methods on the mRNA levels of chondrocyte late stage differentiation- and ossification-related markers on day 2 and day 6. $^{*}P < 0.05$ when PTH administration groups were compared to the control group, $^{\#}P < 0.05$ when the I-PTH group was compared to the C-PTH group.

tion and ossification, other chondrocyte differentiation and ossification markers, including BMP2, COL1a, OCN and OSX mRNA expression were measured. The mRNA levels of these genes in the I-PTH group were significantly up-regulated compared to the C-PTH and control groups after 6 days of PTH administration. On the contrary, the expression levels of these mRNA in C-PTH were down-regulated compared to the I-PTH and control groups (Fig. 4).

4. Discussion

Condylar cartilage plays an important role in the development and remodeling processes of the mandible, in which endochondral ossification is the main way that bone tissue is formed during the post-natal period. The effects of different PTH administration methods on osteogenic cells have widely been studied, and many reports on the roles of PTHrP or PTH/PTHrP receptor on chondrocyte proliferation and differentiation have been published [6–9]. However, there have been few reports on the effects of intermittent or continuous PTH administration on chondrocytes. Previous studies have suggested that using 10 nM PTH 1-34 in the first 6-h period (intermittent administration) or throughout the period of each 48 h (continuous administration) should be the ideal methods for studying the influence of PTH on osteoblastic differentiation and bone formation [10,16]. Furthermore, the chondrocyte is another important cell type in endochondral ossification besides the osteoblast. Therefore, for the first time in this study, we treated the primary cultured condylar chondrocytes with intermittent or continuous PTH 1-34 administration to analyze the effects of PTH on condylar chondrocyte proliferation, differentiation and endochondral ossification *in vitro*.

Mineral nodule formation is one of the signs of bone formation in the cell culture system *in vitro*. In our study, mineral nodules were detected in the three groups, which proved that osteogenic culture medium could provide an environment for ossification in condylar chondrocytes cultured *in vitro*. The results of mineral nodule formation indicated that PTH affected the ossification of condylar chondrocytes in osteogenic conditions, depending on the manner of administration. Previous studies have reported that overexpression of PTHrP or the PTH/PTHrP receptor in chondrocytes enhances their proliferation and delays differentiation [6–9,17]. These effects of PTHrP or PTH/PTHrP receptor overexpression on chondrocytes are similar to the characteristics of the C-PTH group in our study. Therefore, it can be inferred that the lowest frequency mineral nodule formation in the C-PTH group is due to a higher proportion of chondrocytes staying in the proliferation stage than the differentiation stage.

During the process of chondrocyte differentiation and ossification, many factors are synthesized by the hypertrophic chondrocyte. ALP is one of the most important factors synthesized by the hypertrophic chondrocyte at the late stage of differentiation and the following ossification process. Under the effect of ALP, endochondral ossification starts from mineralized cartilage matrix [2]. In ALP knockout mice, chondrocytes in growth cartilage fail to undergo terminal hypertrophy because the effect of promoting mineralization is blocked [18]. In our study, ALP activity and ALP

mRNA expression in the I-PTH and C-PTH groups were opposite. This means that different PTH administration method differently regulates condylar chondrocyte differentiation.

Chondrocyte proliferation and differentiation are also regulated by BMPs. It is reported that BMP2 can stimulate bone formation by enhancing both chondrocyte proliferation and endochondral ossification [19,20]. BMP2 knockout mice exhibit severe disorganization of chondrocytes in the growth plate and severe bone defects [21]. In our study, both BMP2 mRNA and protein expression levels in the C-PTH group were higher than the control group, which could be explained by the continuous PTH administration promoting condylar chondrocyte proliferation. This explanation is supported by the data on mRNA expression of the chondrocyte-specific markers COL2a1 and COL10a1 in our study. COL2a1 is highly expressed in proliferating chondrocytes, while COL10a1 is especially expressed in differentiated chondrocytes [22]. In our study, COL2a1 mRNA expression was up-regulated in the C-PTH group, and COL10a1 expression was higher in the I-PTH than the C-PTH group. Moreover, previous reports have noted that continuous PTH enhances chondrocyte proliferation while suppressing the late stage maturation [23,24]. We can propose that intermittent PTH administration stimulates condylar chondrocyte differentiation, while continuous PTH administration enhances condylar chondrocyte proliferation and inhibits its differentiation.

RUNX2 is involved in endochondral ossification as a well-known osteo-/chondrogenic differentiation and bone formation regulator [18]. It has been reported that chondrocyte maturation is delayed and bone formation is inhibited when the RUNX2 gene is knocked-out or blocked by RNAi technology, implying that RUNX2 is essential for endochondral ossification [8,25]. However, the impact of PTH or PTHrP on RUNX2 expression in chondrocytes is not well illustrated. It is reported that PTHrP suppresses RUNX2 mRNA expression in chick sternal chondrocytes in a continuous PTHrP treatment model [9]. Nonetheless, in our study, RUNX2 expression in condylar chondrocytes was up-regulated in the I-PTH group, both at the transcription level and the translation level, while down-regulated in the C-PTH group. These results suggest that PTH regulates condylar chondrocyte differentiation and bone formation, but depending on the pattern of administration.

In this study, BSP, COL1a, OCN and OSX, which are expressed in chondrocytes at the late stage of differentiation and the duration of endochondral ossification, were studied to further verify the effects of PTH on chondrocyte differentiation and endochondral ossification. Previous studies have reported that PTH treatment can stimulate these genes expression to enhance bone formation in osteogenic cell lines [10,11,26]. In our study, the results of these markers at the mRNA and/or protein synthesis levels further proved our inferences, which is different PTH administration with different effects on condylar chondrocyte differentiation and endochondral ossification.

Another potential explanation for the discrepancies observed between our study and other previous studies could be ascribed to the different culture conditions. Cell culture conditions can influence the effects of PTH as shown in Yang et al.'s report, in which they found that the same PTH administration method could lead to different results in two different culture media [16]. The previous studies investigating the effects of PTH or PTHrP on chondrocytes were rarely performed in an osteogenic culture medium, which could provide an environment to induce endochondral ossification demonstrated by both the previous study and our results [14]. In our study, condylar chondrocytes were cultured in osteogenic medium when treated by PTH. Apparently, distinctive results were observed between the two different PTH administration groups, thus demonstrating our hypothesis [15].

In conclusion, the present study elaborates the effects of intermittent or continuous PTH administration on condylar chondrocyte proliferation and differentiation in an osteogenic condition *in vitro*. Intermittent PTH administration promoted condylar chondrocyte differentiation and endochondral ossification, whereas continuous PTH administration enhanced condylar chondrocyte proliferation and suppressed simultaneously condylar chondrocyte differentiation. This study provides an insight into the effects of PTH administration on chondrocytes and supports the local administration of PTH 1-34 for the treatment of mandibular asymmetry.

Acknowledgment

This study was supported by Grant from the National Natural Science Foundation of China (No. 30672341). We thank Dr. Ongodia David for his help on the language revision.

References

[1] Y. Sakamoto, Y. Takano, Morphological influence of ascorbic acid deficiency on endochondral ossification in osteogenic disorder Shionogi rat, Anat. Rec. 268 (2002) 93–104.
[2] G. Shen, M.A. Darendeliler, The adaptive remodeling of condylar cartilage–a transition from chondrogenesis to osteogenesis, J. Dent. Res. 84 (2005) 691–699.
[3] L. Qin, L.J. Raggatt, N.C. Partridge, Parathyroid hormone: a double-edged sword for bone metabolism, Trends Endocrinol. Metab. 15 (2004) 60–65.
[4] C.A. Frolik, E.C. Black, R.L. Cain, J.H. Satterwhite, P.L. Brown-Augsburger, M. Sato, J.M. Hock, Anabolic and catabolic bone effects of human parathyroid hormone (1-34) are predicted by duration of hormone exposure, Bone 33 (2003) 372–379.
[5] K.E. Poole, J. Reeve, Parathyroid hormone–a bone anabolic and catabolic agent, Curr. Opin. Pharmacol. 5 (2005) 612–617.
[6] N.S. Datta, A.B. Abou-Samra, PTH and PTHrP signaling in osteoblasts, Cell Signaling 21 (2009) 1245–1254.
[7] A.B. Rabie, G.H. Tang, H. Xiong, U. Hagg, PTHrP regulates chondrocyte maturation in condylar cartilage, J. Dent. Res. 82 (2003) 627–631.
[8] J. Guo, U.I. Chung, D.H. Yang, G. Karsenty, F.R. Bringhurst, H.M. Kronenberg, PTH/PTHrP receptor delays chondrocyte hypertrophy via both Runx2-dependent and -independent pathways, Dev. Biol. 292 (2006) 116–128.
[9] T.F. Li, Y.F. Dong, A.M. Ionescu, R.N. Rosier, M.J. Zuscik, E.M. Schwarz, R.J. O'Keefe, H. Drissi, Parathyroid hormone-related peptide (PTHrP) inhibits Runx2 expression through the PKA signaling pathway, Exp. Cell Res. 299 (2004) 128–136.
[10] T. Ishizuya, S. Yokose, M. Hori, T. Noda, T. Suda, S. Yoshiki, A. Yamaguchi, Parathyroid hormone exerts disparate effects on osteoblast differentiation depending on exposure time in rat osteoblastic cells, J. Clin. Invest. 99 (1997) 2961–2970.
[11] B.L. Wang, C.L. Dai, J.X. Quan, Z.F. Zhu, F. Zheng, H.X. Zhang, S.Y. Guo, G. Guo, J.Y. Zhang, M.C. Qiu, Parathyroid hormone regulates osterix and Runx2 mRNA expression predominantly through protein kinase A signaling in osteoblast-like cells, J. Endocrinol. Invest. 29 (2006) 101–108.
[12] D. Jiang, R.T. Franceschi, H. Boules, G. Xiao, Parathyroid hormone induction of the osteocalcin gene. Requirement for an osteoblast-specific element 1 sequence in the promoter and involvement of multiple-signaling pathways, J. Biol. Chem. 279 (2004) 5329–5337.
[13] S.C. Miller, J. Hunziker, M. Mecham, T.J. Wronski, Intermittent parathyroid hormone administration stimulates bone formation in the mandibles of aged ovariectomized rats, J. Dent. Res. 76 (1997) 1471–1476.
[14] R. Garimella, X. Bi, N. Camacho, J.B. Sipe, H.C. Anderson, Primary culture of rat growth plate chondrocytes: an in vitro model of growth plate histotype, matrix vesicle biogenesis and mineralization, Bone 34 (2004) 961–970.
[15] Q. Wan, Z.B. Li, Intra-articular injection of parathyroid hormone in the temporomandibular joint as a novel therapy for mandibular asymmetry, Med. Hypotheses 74 (2010) 685–687.
[16] C. Yang, H. Frei, H.M. Burt, F. Rossi, Effects of continuous and pulsatile PTH treatments on rat bone marrow stromal cells, Biochem. Biophys. Res. Commun. 380 (2009) 791–796.
[17] M. Amling, L. Neff, S. Tanaka, D. Inoue, K. Kuida, E. Weir, W.M. Philbrick, A.E. Broadus, R. Baron, Bcl-2 lies downstream of parathyroid hormone-related peptide in a signaling pathway that regulates chondrocyte maturation during skeletal development, J. Cell Biol. 136 (1997) 205–213.
[18] E.J. Mackie, Y.A. Ahmed, L. Tatarczuch, K.S. Chen, M. Mirams, Endochondral ossification: how cartilage is converted into bone in the developing skeleton, Int. J. Biochem. Cell. Biol. 40 (2008) 46–62.
[19] W.S. Toh, Z. Yang, H. Liu, B.C. Heng, E.H. Lee, T. Cao, Effects of culture conditions and bone morphogenetic protein 2 on extent of chondrogenesis from human embryonic stem cells, Stem cells 25 (2007) 950–960.
[20] M. Wuelling, A. Vortkamp, Chondrocyte proliferation and differentiation, Endocr. Dev. 21 (2011) 1–11.

[21] B. Shu, M. Zhang, R. Xie, M. Wang, H. Jin, W. Hou, D. Tang, S.E. Harris, Y. Mishina, R.J. O'Keefe, M.J. Hilton, Y. Wang, D. Chen, BMP2, but not BMP4, is crucial for chondrocyte proliferation and maturation during endochondral bone development, J. Cell Sci. 124 (2011) 3428–3440.
[22] S. Provot, E. Schipani, Molecular mechanisms of endochondral bone development, Biochem. Biophys. Res. Commun. 328 (2005) 658–665.
[23] B. Zerega, S. Cermelli, P. Bianco, R. Cancedda, F.D. Cancedda, Parathyroid hormone [PTH(1-34)] and parathyroid hormone-related protein [PTHrP(1-34)] promote reversion of hypertrophic chondrocytes to a prehypertrophic proliferating phenotype and prevent terminal differentiation of osteoblast-like cells, J. Bone Miner. Res. 14 (1999) 1281–1289.
[24] A. Hollnagel, M. Ahrens, G. Gross, Parathyroid hormone enhances early and suppresses late stages of osteogenic and chondrogenic development in a BMP-dependent mesenchymal differentiation system (C3H10T1/2), J. Bone Miner. Res. 12 (1997) 1993–2004.
[25] H. Enomoto, M. Enomoto-Iwamoto, M. Iwamoto, S. Nomura, M. Himeno, Y. Kitamura, T. Kishimoto, T. Komori, Cbfa1 is a positive regulatory factor in chondrocyte maturation, J. Biol. Chem. 275 (2000) 8695–8702.
[26] B. Demiralp, H.L. Chen, A.J. Koh, E.T. Keller, L.K. McCauley, Anabolic actions of parathyroid hormone during bone growth are dependent on c-fos, Endocrinology 143 (2002) 4038–4047.