Tissue and Cell 48 (2016) 334–339

Contents lists available at ScienceDirect

Tissue and Cell

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

The parathyroid hormone-related protein is secreted during the osteogenic differentiation of human dental follicle cells and inhibits the alkaline phosphatase activity and the expression of DLX3

C. Klingelhöffer, A. Reck, T. Ettl, C. Morsczeck*

Department of Cranio- and Maxillofacial Surgery, Hospital of the University of Regensburg, Regensburg, Germany

ARTICLE INFO

Article history:
Received 18 March 2016
Received in revised form 24 May 2016
Accepted 28 May 2016
Available online 1 June 2016

Keywords:
Parathyroid hormone-related protein
Dental follicle cells
Periodontal development
Osteogenic differentiation

ABSTRACT

The dental follicle is involved in tooth eruption and it expresses a great amount of the parathyroid hormone-related protein (PTHrP). PTHrP as an extracellular protein is required for a multitude of different regulations of enchondral bone development and differentiation of bone precursor cells and of the development of craniofacial tissues. The dental follicle contains also precursor cells (DFCs) of the periodontium. Isolated DFCs differentiate into periodontal ligament cells, alveolar osteoblast and cementoblasts. However, the role of PTHrP during the human periodontal development remains elusive. Our study evaluated the influence of PTHrP on the osteogenic differentiation of DFCs under in vitro conditions for the first time. The PTHrP protein was highly secreted after 4 days of the induction of the osteogenic differentiation of DFCs with dexamethasone (2160.5 pg/ml ± 345.7 SD. in osteogenic differentiation medium vs. 315.7 pg/ml ± 156.2 SD. in standard cell culture medium; Student's t Test: $p < 0.05$ ($n = 3$)). We showed that the supplementation of the osteogenic differentiation medium with PTHrP inhibited the alkaline phosphatase activity and the expression of the transcription factor DLX3, but the depletion of PTHrP did not support the differentiation of DFCs. Previous studies have shown that Indian Hedgehog (IHH) induces PTHrP and that PTHrP, in turn, inhibits IHH via a negative feedback loop. We showed that SUFU (Suppressor Of Fused Homolog) was not regulated during the osteogenic differentiation in DFCs. So, neither the hedgehog signaling pathway induced PTHrP nor PTHrP suppressed the hedgehog signaling pathway during the osteogenic differentiation in DFCs. In conclusion, our results suggest that PTHrP regulates independently of the hedgehog signaling pathway the osteogenic differentiated in DFCs.

1. Introduction

Multiple signaling pathways are involved in the development of bone (Kronenberg, 2006). Especially, the influence of the parathyroid hormone related peptide (PTHrP) synthesis is essential for enchondral and calvarial ossification (Kronenberg, 2006; Pan et al., 2013). PTHrP affects directly the osteogenic differentiation of perichondrial cells into osteoblast (Kronenberg, 2006; Pan et al., 2013). Moreover, PTHrP keeps chondrocytes proliferating and delays the differentiation into hypertrophic chondrocytes and it represses the expression of hedgehog proteins via a negative feed back loop (Vortkamp, 2000). In addition, PTHrP interferes not only within the osteogenic differentiation, but it is also responsible for numerous cell interactions and is secreted ubiquitously in benign and malignant cells (Cao et al., 2013; Fiaschi-Taesch and Stewart, 2003; Otsuki et al., 2001; Philbrick et al., 1996). Interestingly, PTHrP regulates cell cycle in tissue cells in two different manners (Kronenberg, 2006; Miao et al., 2008). PTHrP inhibits cell cycle progression and induces senescence via its surface receptor, but it also shuttles into the nucleus and keeps the cell cycle progression after the inhibition of P27 (Miao et al., 2008; Sun et al., 2016). A recent study showed that recombinant hPTHrP (1–84) enhances renal calcium reabsorption and stimulates bone formation in vivo and the osteogenic differentiation in bone marrow cells (Wang et al., 2014). Interestingly, the mandibular and dental development was hampered in PTHrP (1–84) knock-in mice (Sun et al., 2016).

PTHrP is also required for tooth eruption in mice by regulating osteoclasts and subsequently for the alveolar bone resorption (Philbrick et al., 1998; Rakian et al., 2013). Here, cells in the dental follicle, which surrounds the developing tooth, secretes PTHrP and trigger the formation of osteoclasts in the dental crypt (Nakchbandi

* Corresponding author at: Department of Cranio– and Maxillofacial Surgery, University Hospital Regensburg, Franz–Josef–Strauss–Allee 11, 93053 Regensburg, Germany.
E-mail address: christian.morsczeck@ukr.de (C. Morsczeck).

http://dx.doi.org/10.1016/j.tice.2016.05.007
0040-8166/

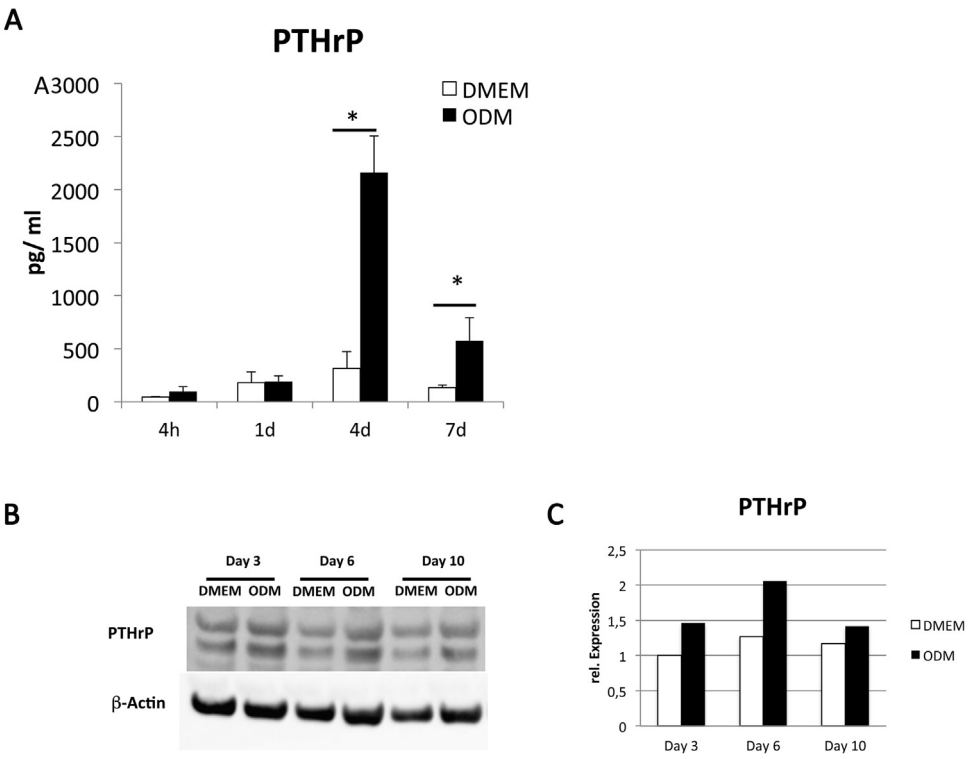

Fig. 1. (A) Estimation of the extracellular PTHrP concentration in cell culture medium by ELISA after the induction of the osteogenic differentiation. Columns represents means of three biological replicates S.D., *: Student's tTest $p < 0.05$. (B) Western Blot analysis for the evaluation of the intracellular PTHrP concentration at days 3, 6 and 10 after the osteogenic differentiation. A Western-Blot analysis with a specific antibody for β-Actin was used for loading control. (C) Diagram shows relative pixel intensities of protein bands of the PTHrP protein. The PTHrP protein band intensities were normalized to the pixel intensity of the housekeeping protein β-Actin.

et al., 2000). Interestingly, the dental follicle is also involved in the development of the periodontium of the dental root. Therefore, dental follicle cells (DFCs) differentiate into three different cell types: periodontal ligament cells, alveolar osteoblasts and cementoblasts (Diekwisch, 2001; Morsczeck et al., 2005; Wise et al., 1992). In previous studies we could show that DFCs differentiate into alveolar osteoblast-like and cementoblast-like cells under in vitro conditions and that these cells can be used for detailed studies about molecular processes and signaling transduction during the osteogenic differentiation (Morsczeck and Schmalz, 2010; Saugspier et al., 2010). Even though PTHrP is differentially expressed in DFCs during the osteogenic differentiation (Felthaus et al., 2014; Viale-Bouroncle et al., 2012), the influence of PTHrP on the osteogenic differentiation of DFCs remains elusive. The aim of this study was to examine the role of PTHrP during the osteogenic differentiation of DFCs. We showed that the protein secretion of PTHrP was up-regulated after the induction of the osteogenic differentiation and that PTHrP inhibited the osteogenic differentiation of DFCs.

2. Materials and methods

2.1. Cell culture

Human dental follicle cells (DFCs) were purchased from ALL Cells. The standard culture medium was DMEM (Dulbecco's Modified Eagle Medium; Sigma-Aldrich, Munich, Germany) supplemented with 10% fetal bovine serum, FBS (Sigma-Aldrich) and 100 μg/mL Penicillin/Streptomycin. DFCs at passage 6 were used for experiments.

2.2. Osteogenic differentiation

DFCs were cultivated until sub-confluence (>80%) in standard cell culture medium before the osteogenic differentiation was stimulated with 100 nM dexamethasone (Sigma.Aldrich) in a DMEM (Sigma-Aldrich) based cell culture medium comprising 10% fetal bovine serum (Sigma-Aldrich), 100 μmol/l ascorbic acid 2-phosphate, 10 mmol/l KH_2PO_4, HEPES (20 mmol/l) and 100 μg/mL Penicillin/Streptomycin. Differentiation of DFCs was determined by quantification of Alkaline Phosphatase (ALP) activity (see below) and Alizarin Red staining, which was previously described (Saugspier et al., 2010).

2.3. Transfection experiments

The siRNAs were purchased from Qiagen (Hilden, Germany) targeting human PTHrP mRNA (Gene Bank No. NM_153000). A non-specific siRNA (ALLStar) was also purchased from Qiagen. DFCs were seeded at a cell density of $7 \times 10^3/cm^2$ and cultivated until sub-confluence in standard cell culture medium. The cells were transfected with 12 μM of the respective siRNAs using Hiperfecta reagent (Qiagen) according to the manufacturer's instructions. After 48 h, qRT-PCR determined the expression of PTHrP in DFCs.

2.4. Measurement of alkaline phosphatase (ALP) activity

The osteogenic differentiation potential was evaluated after 7 days of cultivation in differentiation medium with dexamethasone or standard culture medium (s. above). DFCs were washed with PBS buffer, disrupted by 1.5 M alkaline buffer (Sigma-Aldrich) and mixed with 100 mM *p*-nitrophenylphosphate (Sigma-Aldrich). After incubation at 37 °C for 60 min, the reaction was stopped by adding 300 μl of 0.3 M NaOH and the liberated *p*-nitrophenol was