Biochimica et Biophysica Acta xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Canonical Wnt signaling differently modulates osteogenic differentiation of mesenchymal stem cells derived from bone marrow and from periodontal ligament under inflammatory conditions

Wenjia Liu [a,b,1], Anna Konermann [c,1], Tao Guo [d,1], Andreas Jäger [c], Liqiang Zhang [a], Yan Jin [a,b,*]

[a] Research and Development Center for Tissue Engineering, The Fourth Military Medical University, Xi'an, Shaanxi, People's Republic of China
[b] Department of Oral Histology and Pathology, School of Stomatology, The Fourth Military Medical University, Xi'an, Shaanxi, People's Republic of China
[c] Department of Orthodontics, Medical Faculty, University of Bonn, Bonn, Germany
[d] Department of Orthodontics, School of Stomatology, The Fourth Military Medical University, Xi'an, Shaanxi, People's Republic of China

ARTICLE INFO

Article history:
Received 20 April 2013
Received in revised form 14 September 2013
Accepted 3 November 2013
Available online xxxx

Keywords:
Bone marrow derived mesenchymal stem cell
Canonical Wnt signaling
Inflammation
Osteogenesis
Periodontal ligament stem cell

ABSTRACT

Background: Cellular plasticity and complex functional requirements of the periodontal ligament (PDL) assume a local stem cell (SC) niche to maintain tissue homeostasis and repair. Here, pathological alterations caused by inflammatory insults might impact the regenerative capacities of these cells. As bone homeostasis is fundamentally controlled by Wnt-mediated signals, it was the aim of this study to characterize the SC-like capacities of cells derived from PDL and to investigate their involvement in bone pathophysiology especially regarding the canonical Wnt pathway.

Methods: PDLSCs were investigated for their SC characteristics via analysis of cell surface marker expression, colony forming unit efficiency, proliferation, osteogenic differentiation and adipogenic differentiation, and compared to bone marrow derived mesenchymal SCs (BMMSCs). To determine the impact of both inflammation and the canonical Wnt pathway on osteogenic differentiation, cells were challenged with TNF-α, maintained with or without Wnt3a or DKK-1 under osteogenic induction conditions and investigated for p-IκBα, p-NF-κB, p-Akt, β-catenin, p-GSK-3β, ALP and Runx2.

Results: PDLSCs exhibit weaker adipogenic and osteogenic differentiation capacities compared to BMMSCs. TNF-α inhibited osteogenic differentiation of PDLSCs more than BMMSCs mainly through regulating canonical Wnt pathway. Blocking the canonical Wnt pathway by DKK-1 reconstituted osteogenic differentiation of PDLSCs under inflammatory conditions, whereas activation by Wnt3a increased osteogenic differentiation of BMMSCs.

Conclusions: Our results suggest a diverse regulation of the inhibitory effect of TNF-α in BMMSCs and PDLSCs via canonical Wnt pathway modulation.

General significance: These findings provide novel insights on PDL cells with SC-like capacities and their involvement in bone pathophysiology under the impact of the canonical Wnt pathway.

1. Introduction

The periodontal ligament (PDL) is a complex soft connective tissue located at the interface of the alveolar bone and cementum, which maintains tooth attachment, nutrition and homeostasis as well as structural repair of damaged tissues [1]. Embedding diverse cell types [2], PDL cells represent the predominant cellular component of the PDL and are characterized by phenotypic heterogeneity as much as the capability to transform into neighboring cell types like osteoblasts or cementoblasts [3]. This cellular plasticity, attended by the fact that the PDL exhibits one of the upmost turnover rates in the body assumes the existence of a local stem cell (SC) niche for the maintenance of tissue homeostasis and repair [2,4]. However, the complex structural composition comprising both hard and soft tissues and the diverseness of the elements composing the PDL aggravate the detection of potential SC-like elements responsible for regeneration. Considering the osteoblast-like properties and the mesenchymal origin of PDL cells [5], a possible interconnection with the characteristics of bone marrow derived mesenchymal SCs (BMMSCs) can be assumed. Furthermore, MSC markers like STRO-1 and CD146 have already been identified on a subpopulation of PDL cells that potentially disclose them as periodontal ligament stem

Abbreviations: BMMSCs, bone marrow derived mesenchymal stem cells; PDLSCs, periodontal ligament mesenchymal stem cells; PCR, polymerase chain reaction; Runx2, runt-related transcription factor 2; ALP, alkaline phosphatase; PPARγ, peroxisome proliferator-activated receptor-γ; LPL, lipoprotein lipase; p-IκBα, phosphonated inhibitor of nuclear factor kappa B; p-NF-κB, phosphonated nuclear factor kappa B; p-Akt, v-Akt murine thymoma viral oncogene; p-GSK-3β, phosphonated glycogen synthase kinase 3 beta

* Corresponding author at: Research and Development Center for Tissue Engineering, The Fourth Military Medical University, 145 West Changle Road, Xi'an, Shaanxi 710032, People's Republic of China. Tel.: +86 29 84776147; fax: +86 29 83218039.
E-mail address: yanjin@fmmu.edu.cn (Y. Jin).
[1] These authors contributed equally to this work.

0304-4165/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.bbagen.2013.11.003

cells (PDLSCs) [2]. BMMSCs represent one of the best described multipotent MSC type that provided the basis for the establishment of gold standard criteria characterizing SCs, namely colony-forming unit (CFU) capacity, persistent self-renewal reflected by a high proliferation potency and developmental plasticity in terms of differentiation into multiple cell lineages [6]. However, the features of PDLSCs may differ from BMMSCs, as the mesenchymal origin of dental structures is designated by its developmental interdependency with the neural crest and therefore denoted as ectomesenchyme [6].

One of the central questions in PDL pathophysiology is the identification of the factors determining its turnover quality and regenerative efficiency, particularly regarding its involvement in pathological alterations of the alveolar bone caused by inflammatory insults. Investigations revealed that PDL cells are required for bone remodeling and regeneration, as they are involved in both the regulation of osteogenic differentiation and osteoclastic differentiation [7]. Furthermore, inflammatory processes in periodontal tissues seem to be modulated by resident PDL cells [8,9], correlating with data on MSC that have been shown to feature immunomodulatory capacities as well [10]. Consequently, a subpopulation of PDL cells with SC-like capacities might play a particular role in the regulation of bone pathophysiology.

Bone homeostasis is known to be fundamentally controlled by Wnt-mediated signals that comprise two main molecular pathways, namely the β-catenin-dependent canonical and the β-catenin-independent noncanonical Wnt pathway [11]. The canonical Wnt/β-catenin pathway enhances bone formation and entails osteogenic lineage differentiation of progenitor cell as well as PDL cells [7], thus capacitating these cells to implement hard tissue repair. Furthermore, Wnt/β-catenin signals suppress bone resorption by stimulating OPG expression but simultaneously suppressing RANKL expression in osteoblasts, and by inhibition of osteoclastogenesis [11]. The canonical Wnt/β-catenin pathway is mainly driven by Wnt1 and Wnt3a and leads to activation of β-catenin and in turn inhibition of GSK-3β kinase [12]. This cascade can be blocked by antagonists like Dickkopf-1 (DKK-1) through binding to one of the two Wnt receptor complexes, low-density lipoprotein receptor-related protein 5/6 (LRP5/6) [11]. β-catenin signals are not only required by osteoclast precursors for the process of osteoclastogenesis, but also inhibit their differentiation upon prolonged stimulation [13].

Bennett et al. reported that canonical Wnt pathway promotes the osteogenesis of murine BMMSCs and osteoprogenitor cells through up-regulation osteoblast-related genes [14,15]. However, our group observed that canonical Wnt signaling promoted osteogenesis of PDLSCs in full culture medium while inhibited it in osteogenic differentiation medium [16]. In MSCs, both an osteoinhibitory and osteoinductive impact of Wnt/β-catenin signals could be observed depending on the environmental conditions [17]. Knowing that periodontitis has a significantly higher prevalence than osteomyelitis, and that the regenerative capacities of periodontal tissues are significantly constrained under inflammatory conditions [18–21], inflammation may differently affect osteogenic commitment of PDLSCs and BMMSCs. However, the role of the canonical Wnt pathway in regulating osteogenic differentiation of BMMSCs and PDLSCs in an inflammatory microenvironment remains to be elucidated.

In the present study, PDLSCs were found to feature weaker adipogenic and osteogenic lineage commitment than BMMSCs, but exhibited to be more sensitive to the inhibitory effect of TNF-α on osteogenic differentiation. Blockage of the canonical Wnt pathway by DKK-1 reconstituted the process of osteogenic differentiation of PDLSCs under inflammatory conditions, whereas activation by Wnt3a increased osteogenic differentiation of BMMSCs, indicating the dual impact of the canonical Wnt pathway in regulating osteogenesis. Taken together, our findings provided novel insights in the SC-like capacities of PDLSCs and investigated their involvement in bone pathophysiology. Furthermore, our results suggest a diverse regulation of the inhibitory effect of TNF-α in PDLSCs and BMMSCs via modulation of the canonical Wnt pathway.

2. Material and methods

2.1. Cell culture and identification of stem cells

Primary cultures of human PDL cells (n = 6, aged from 16 to 18 years) were explanted from the middle third of the root surface of the teeth extracted for orthodontic reasons at the Dental Clinic of the Fourth Military Medical University, Xi'an, China. Adolescent donors were examined on defined variables for clinically healthy periodontal tissues with the absence of bleeding on probing, probing depth <4 mm and loss of attachment level <3 mm. Written informed consent was provided by all participants and the study was approved by the hospital's ethics committee. The PDL explants were enzymatically digested with type 1 collagenase (0.66 mg/ml; Sigma, St Louis, MO, USA) for 20 min. For the generation of single-cell suspensions, the tissues were filtrated through a 70-μm cell strainer (Millipore, Billerica, MA, USA), washed and incubated in a-MEM (Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% FBS, 0.292 mg/ml glutamine (Invitrogen, Carlsbad, CA, USA), 100 U/ml penicillin, and 100 mg/ml streptomycin (Gibco BRL) at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

The explants were maintained in 6-well culture dishes (Costar, Cambridge, MA, USA) for 2 weeks until subconfluence was reached. To obtain homogeneous populations of PDLSCs, single-cell-derived colony cultures were generated by the limiting dilution technique [20,22]. Multiple colony-derived PDLSCs were used from passages 2 to 4.

To investigate the MSC phenotype, 5×10^5 PDLSCs were incubated with PE or FITC conjugated monoclonal antibodies for human CD14, CD31, CD90, CD105 (eBioscience, San Diego, CA, USA), CD146 and Stro-1 (R&D Systems, Inc., Minneapolis, MN, USA), or isotype-matched control IgGs. Cells were subjected to flow cytometric analysis using a Beckman Coulter Epics XL (Beckman Coulter, Fullerton, CA, USA).

Human BMMSCs (n = 6, aged from 16 to 25) were used as control cell line and cultured as previously described [23].

2.2. Colony forming unit (CFU) assay

To assess the CFU efficiency of PDLSCs and BMMSCs, day 14 cultures of single-cell suspensions (2×10^3 cells) were seeded in 10-cm-diameter culture dishes (Corning, Lowell, MA, USA). The newly formed colonies were visualized with 0.1% toluidine blue following 4% paraformaldehyde fixation. Aggregates of 50 or more cells were scored as colonies under the microscope (Leica Microsystems, Heerbrugg, Switzerland). CFU efficiency was determined by the number of colonies relative to the total number of seeded cells in each plate. Experiments were performed in triplicate.

2.3. Proliferation assays

For proliferation analyses of PDLSCs and BMMSCs, 5×10^3 cells/well were cultured in 96-well plates. The 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried out for 8 days according to the manufacturer's protocol (Sigma). Absorbance was determined at 490 nm with a microplate reader (Bio-Tek Instruments, Winooski, VT, USA). Besides, cell proliferation was analyzed by an ehynedeoxyuridine (EdU) assay. 3×10^3 cells/well were seeded in 24-well culture plates overnight and cell proliferation was subsequently assessed by analysis of EdU incorporation into DNA with an EdU staining kit (RiboBio) according to the manufacturer's instruction. Furthermore, cell cycle analysis was performed on PDLSCs and BMMSCs. Single cell suspensions of both cells were harvested and fixed in ice-cold 75% ethanol 4 °C for 24–48 h, washed twice with PBS, stained with 100 mg/ml propidium iodide at 4 °C for 30 min and subjected to cell cycle analysis using an Elite ESP flow cytometer (Beckman Coulter, Fullerton, CA, USA). All experiments were performed in triplicate.

Please cite this article as: W. Liu, et al., Canonical Wnt signaling differently modulates osteogenic differentiation of mesenchymal stem cells derived from bone marrow and from..., Biochim. Biophys. Acta (2013), http://dx.doi.org/10.1016/j.bbagen.2013.11.003