Differentiation 88 (2014) 97–105

Contents lists available at ScienceDirect

Differentiation

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

The difference on the osteogenic differentiation between periodontal ligament stem cells and bone marrow mesenchymal stem cells under inflammatory microenviroments

Jing Zhang [a,1], Zhi-Gang Li [b,1], Ya-Meng Si [a], Bin Chen [a], Jian Meng [a,*]

[a] Department of Stomatology, The Affiliated School of Clinical Medicine of Xuzhou Medical College, Xuzhou Central Hospital, Xuzhou, China
[b] Department of Urology, The Affiliated School of Clinical Medicine of Xuzhou Medical College, Xuzhou Central Hospital, Xuzhou, China

ARTICLE INFO

Article history:
Received 29 April 2014
Received in revised form
26 September 2014
Accepted 29 October 2014
Available online 10 December 2014

Keywords:
Periodontal ligament stem cells
Bone marrow mesenchymal stem cells
Inflammation
Osteogenic differentiation

ABSTRACT

Periodontitis is a major cause of tooth loss in adults and periodontal ligament stem cells (PDLSCs) is the most favorable candidate for the reconstruction of tissues destroyed by periodontal diseases. However, pathological alterations caused by inflammatory insults might impact the regenerative capacities of these cells. Bone-marrow-derived human mesenchymal stem cells (hBMSCs) would accelerate alveolar bone regeneration by transplantation, compared to PDLSCs. Therefore, a better understanding of the osteogenic differentiation between PDLSCs and BMSCs in inflammatory microenviroments is therefore warranted. In this study, human PDLSCs were investigated for their stem cell characteristics via analysis of cell surface marker expression, colony forming unit efficiency, osteogenic differentiation and adipogenic differentiation, and compared to BMSCs. To determine the impact of both inflammation and the NF-κβ signal pathway on osteogenic differentiation, cells were challenged with TNF-α under osteogenic induction conditions and investigated for mineralization, alkaline phosphatase (ALP) activity, cell proliferation and relative genes expression. Results showed that PDLSCs exhibit weaker mineralization and ALP activity compared to BMSCs. TNF-α inhibited genes expression of osteogenic differentiation in PDLSCs, while, it stimulates gene expressions (BSP and Runx2) in BMSCs. Enhanced NF-κβ activity in PDLSCs decreases expression of Runx2 but it does not impede the osteogenic differentiation of BMSCs. Taken together, these results may suggest that the BMSCs owned the stronger immunomodulation in local microenvironment via anti-inflammatory functions, compared to PDLSCs.

1. Introduction

Periodontitis is a chronic infectious disease that leads to a progressive destruction of periodontal tissue. This disease is highly prevalent and can affect up to 90% of the worldwide population and it is also a major cause of tooth loss in adults (Pihlstrom et al., 2005; Chen et al., 2012; Park et al., 2011). Improving the regeneration of periodontal tissue has proven effective in the treatment of periodontitis.

Human periodontal ligament (PDL) contains a novel population of multipotent stem cells that have the capacity to develop into cells with diverse phenotypes and therefore provide a unique reservoir of stem cells (Mrozik et al., 2010). In addition, periodontal ligament stem cells (PDLSCs) have been shown to form an ectopic cementum/ligament-like complex when transplanted in nude mice (Seo et al., 2004). Therefore, tissue regeneration mediated by human PDLSCs has the potential for use as a practical cell-based treatment for periodontal diseases (Liu et al., 2008; Tamaki et al., 2013). However, access to the periodontal ligament requires removal of teeth and the number of recoverable PDLSCs is limited due to their rarity and the small sample size. In contrast, bone-marrow-derived human mesenchymal stem cells (hBMSCs) can be harvested in much larger numbers and relative ease of acquisition (Kassem and Abdallah, 2008; Pittenger et al., 1999; Owen and Friedenstein, 1988). These cells are able to differentiate along several committed phenotypes including osteogenic, chrondogenic, adipogenic, and neurogenic and lineages in response to stimulation by multiple environmental factors (Wu et al., 2014; Lin and Hankenson, 2011; Welter et al., 2013; Haynesworth et al., 1992). Studies revealed that periodontal ligament cells and bone marrow own many similar characterists (Kramer et al., 2004). Periodontal ligament stem cells also express

* Corresponding author. Tel.: +86 051683956489.
E-mail address: jingz77@163.com (J. Meng).
[1] Both the authors contributed equally to this work.

http://dx.doi.org/10.1016/j.diff.2014.10.001
Join the International Society for Differentiation (www.isdifferentiation.org)
0301-4681/

early mesenchymal stem cell surface markers (Menicanin et al., 2010). In addition, PDLSCs and BMSCs expressed the same surface molecules markers of bone. These results demonstrate BMSCs' potency to develop periodontal ligament characteristics and suggest that the cells may have the potential to form other periodontal tissues. This is of potential significant since tissue regeneration is often needed in areas of an inflammatory reaction.

Inflammatory processes in periodontal tissues seem to be modulated by resident PDLSCs. The features of BMSCs may differ from PDLSCs. Some researches discovered BMSCs would accelerate periodontal tissue regeneration (Chung et al., 2012; Yang et al., 2010; Kim et al., 2009). However, the differences in regulating osteogenic differentiation of BMSCs and PDLSCs in an inflammatory microenvironment remains to be elucidated.

Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine released by macrophages is known for its substantial role in periodontitis mediated bone loss (Boyce et al., 2009). Elevated level of tumor necrosis factor-α (TNF-α) was confirmed to be associated with the severity of periodontal disease (Kornman et al., 1997; Soga et al., 2003; Zhang et al., 2013) and immune response (Teles et al., 2009). The transcription factor family, nuclear factor κB (NF-κB), is considered a central culprit in the pathogenesis of osteolysis in inflammatory diseases, including periodontitis, rheumatoid arthritis, low-grade systemic inflammation, Paget's disease of bone (PDB), and other bacterial infections (Xu et al., 2009). NF-kappaB signalling pathways are strictly regulated to maintain bone homeostasis by cytokines such as RANKL, TNF-alpha and IL-1, which differentially regulate classical and/or alternative NF-kappaB pathways in osteoclastic cells. Numerous reports have demonstrated that TNF-α activates nuclear factor (NF)-kappaB, resulting in the upregulation of several genes that regulate inflammation, proliferation, and apoptosis (Moe et al., 2014).

In this study, we aimed to analyze the effects of TNF-α on the osteogenic differentiation of hPDLSCs and hBMSCs and to determine the differential expression of target genes that are related to osteogenic differentiation. In addition, we also investigated the role of NF-κβ signal pathway on the differentiation of PDLSCs and BMSCs.

2. Materials and methods

2.1. Cell culture

Human PDLSCs were isolated and cultured as previously described (Yang et al., 2009). Briefly, normal premolar teeth ($n=6$) extracted for orthodontic treatment from 3 individuals (at 18–22 years of age) were collected after obtaining writted informed consent. The study protocol was approved by the Xuzhou Medical College's Ethics Committee. Both systemic and oral diseases were absent in all subjects. Periodontal ligament tissues were gently scraped from the surface of the middle part of the root, cut into 1 mm^3 cubes and placed into six-well culture dishes. The tissues were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 0.292 mg/ml glutamine, 100 U/ml penicillin G, 100 μg/ml streptomycin. Cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 in air and cultures obtained from each donor were processed separately.

To obtain homogeneous populations of PDLSC, single cell-derived colony cultures were obtained using the limiting dilution technique as previously described (Huo et al., 2010). After 2–3 weeks original culture, the single cell-derived clones were then harvested and cells were subcultured at approximately 80–90% confluence with trypsin/EDTA.

Human BMSCs ($n=4$, aged from 18 to 25) were established from bone marrow samples with informed consent of the donors and following the guidelines of the hospital's Ethics Committee. Cells were isolated by Ficoll density gradient centrifugation, suspended in regular culture medium consisting of DMEM with 10% FBS, 0.292 mg/ml glutamine, 100 U/ml penicillin G, 100 μg/ml streptomycin. Cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 in air. After 24 h the culture supernatants were replaced by fresh medium to discard the nonadherent cells. Cells were subcultured at approximately 80–90% confluence with trypsin/EDTA.

2.2. Flow cytometry analysis

For identification of MSC phenotype, approximately 5×10^5 BMSCs and PDLSCs were washed in phosphate buffered saline (PBS) and then incubated with the following mouse anti-human monoclonal antibodies: fluorescein isothiocyanate (FITC)-conjugated CD14, CD90 (eBioscience, San Diego, CA), CD34 (Biolegend, USA), phycoerythrin (PE)-labelled CD31, CD45 (eBioscience, San Diego, CA) and CD146 (Biolegend, USA) for 30 min at 4 °C, respectively. The cell suspension was then washed twice with PBS and analyzed on a Beckman Coulter Epics XL (Beckman Coulter, Fullerton, CA).

2.3. Colony-forming assay

To assesss colony forming efficiency, 1×10^3 PDLSCs and BMSCs were seeded into 100-mm dish and maintained for 14 days, respectively. Then they were fixed with 4% formalin and stained with 1% toluidine blue. The cells were washed twice with distilled water, and the number of colonies was counted. Aggregates of over 50 cells were counted as a colony under the microscope. Experiments were performed in triplicate.

2.4. Osteogenic and adipogenic differentiation

Induction of calcification and adipogenesis were as previously reported (Platt and El-Sohemy, 2009). HBMSCs and hPDLSCs at 3rd passage were plated into 6-well culture dishes at a concentration of 1×10^5 cells/well. Cultures were allowed to reach 80% confluence before differentiation was initiated. Then normal DMEM medium was removed, and replaced with the osteogenic medium (OM, DMEM supplemented with 10% FBS, 50 μg/ml of ascorbic acid, 10 mM of sodium β-glycerophosphate, and 100 nM of dexamethasone) or the adipogenic medium (DMEM supplemented with 10% FBS, 0.5 mM of methylisobutylxanthine, 0.5 μM of hydrocortisone, 60 μM indomethacin, and 10 μg/ml insulin). Cells were maintained with the fresh differentiation medium every 3 or 4 days for 4 weeks. All experiments were performed in triplicate.

2.5. Alkaline phosphatase (ALP) activity assay

For quantitative analysis of alkaline phosphatase (ALP) activity, single-cell suspensions of PDLSCs and BMSCs at 3rd passage were seeded at a density of 3×10^3 cells/well into 96-well plates and cultured in DMEM supplemented with 10% FBS. To investigate the effect of cytokine, cells were treated from day 2 with human TNF-α (0.01–10 ng/ml) (Peprotech, USA) throughout the differentiation assay (DMEM supplemented with 2% FBS, 50 μg/ml of ascorbic acid, 10 mM of sodium β-glycerophosphate, and 100 nM of dexamethasone). Fresh cytokines added at every medium change. After 7 and 14 days in vitro culture, the ALP activity of each cell was detected with a commercially acailable assay kit (Zhongsheng Co, Beijing, China). In brief, cells were washed 3 times in PBS and incubated in Triton X-100 (2 ml/l in PBS) for overnight at 4 °C. One hundred microliters of *p*-nitrophenol phosphate substrate solutions was added to each well and the cells were incubated for 40 min at 37 °C. The addition of NaOH quenched the reaction, and