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The expression of periostin in dental pulp cells

Robert M. Wiesen [a,1], Miguel Padial-Molina [b,c,2], Sarah L. Volk [b,2], Neville McDonald [d,3], Daniel Chiego Jr. [d,4], Tatiana Botero [d,5], Hector F. Rios [b,*]

[a] Resident of Endodontics, University of Michigan School of Dentistry, Ann Arbor, MI, USA
[b] Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, Ann Arbor, MI, USA
[c] Department of Oral Surgery and Implant Dentistry, School of Dentistry, University of Granada, Granada, Spain
[d] Department of Cariology, Restorative Sciences, and Endodontics, University of Michigan School of Dentistry, Ann Arbor, MI, USA

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ABSTRACT

Background and objective: Dental pulp repair is a common process triggered by microbial and mechanical challenges. Matricellular modulators, such as periostin, are key for extracellular matrix stability and tissue healing. In the scope of the dental pulp, periostin expression has been reported during development and active dentinogenesis. However, the specific dental pulp cell population capable of expressing periostin in response to known regulators has not been clearly defined. Among the different relevant cell populations (i.e., stem cells, fibroblasts and pre-odontoblasts) potentially responsible for periostin expression in the dental pulp, this study aimed to determine which is the primary responder to periostin regulators.
Methods: Human dental pulp stem cells (DPSCs), human dental pulp fibroblasts (DPFs), and rat odontoblast-like cells (MDPC-23) were treated with different concentrations of TGF-β1 or different regimens of biomechanical stimulation to evaluate periostin expression by qRT-PCR, Western blot and ELISA. Statistical analyses were performed by Student's *t*-test and ANOVA with Fisher's LSD post hoc tests ($p \leq 0.05$).
Results: DPSC and MDPC-23 showed a statistically significant increase in periostin mRNA expression after exposure to TGF-β1 for 48 h. TGF-β1 also up-regulated periostin protein levels in DPSC. However, periostin significantly down-regulated protein expression in DPF.

* *Corresponding author at*: Department of Periodontics & Oral Medicine, School of Dentistry, University of Michigan, 1011 N. University Ave., Room 3310G, Ann Arbor, MI 48109, USA. Tel.: +1 734 763 7215.
E-mail addresses: rwiesen@umich.edu (R.M. Wiesen), mipadial@ugr.es (M. Padial-Molina), stomanic@umich.edu (S.L. Volk), somerled@umich.edu (N. McDonald), djchiego@umich.edu (D. Chiego Jr.), tbotero@umich.edu (T. Botero), hrios@umich.edu (H.F. Rios).
[1] Address: Graduate Program in Endodontics, 1011 N. University Ave, Ann Arbor, MI 48109-1078, USA.
[2] Address: Department of Periodontics & Oral Medicine, School of Dentistry, University of Michigan, 1011 N. University Ave., Room 3310G, Ann Arbor, MI 48109, USA.
[3] Address: Department of Cariology, Restorative Sciences, and Endodontics, School of Dentistry, University of Michigan, 1011 N. University Ave., Room 2307, Ann Arbor, MI 48109, USA.
[4] Address: Department of Cariology, Restorative Sciences, and Endodontics, School of Dentistry, University of Michigan, 1011 N. University Ave., Room 1360-B, Ann Arbor, MI 48109, USA.
[5] Address: Department of Cariology, Restorative Sciences, and Endodontics, School of Dentistry, University of Michigan, 1011 N. University Ave., Room 1376-D, Ann Arbor, MI 48109, USA.
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Different regimens of biomechanical stimulation showed different patterns in protein and mRNA periostin expression.

Conclusions: Expression of periostin was identified in each of the analysed dental pulp cell lines, which can be regulated by TGF-β1 and biomechanical stimulation. Overall, DPSCs are the most responsive cells to stimulation.

1. Introduction

The structure and properties of the dentine–pulp complex are intimately related through crucial cell–matrix interactions.[1] The proper regulation of these interactions determines the adaptive dentine–pulp response by orchestrating the function of important bioactive proteins such as growth factors, cytokines, chemokines, and neuropeptides.[1–7] The proteins modulating these interactions are collectively known as matricellular molecules. These molecules act in homeostasis by providing a range of signals to the constituent cell populations and modulating their phenotype. These molecules are expressed at different stages during dentinogenesis with chemo-attractive properties that may signal endogenous cells from the pulp proper into the healing zone with the expectation of generating tertiary dentine to seal the injury, allowing for soft tissue remodelling and repair, maintaining the pulp vitality, or promoting regeneration of pulp-like tissue.[8–12] The study of such interactions may be useful to broaden the understanding of pulp biology and could be applied in future regenerative therapies.

In this context, periostin, a matricellular protein, has to be considered. Periostin is a 835-amino secreted 90-kDa protein[13] heavily localized in the periodontal ligament (PDL).[14] Periostin influences collagen fibrillogenesis[15–17] and induces cell proliferation and migration of periodontal ligament fibroblasts[18] and keratinocytes,[19] among other cell populations, by the interaction with $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin receptors. Therefore, it plays a determinant role in the maintenance of tissue function, integrity and strength.[13,20–23]

Periostin has been identified in numerous tissues throughout the body including the dental pulp.[24–26] However, the dental pulp is a highly cellular, vascular and innervated environment that contains multiple cell populations including odontoblasts, fibroblasts and dental pulp stem cells, as well as immunocompetent cells.[27] There is still little information on which specific cell line(s) is responsible for periostin expression within the pulp tissue and its regulatory mechanisms. In other cell populations, such as the periodontal ligament fibroblasts, periostin is expressed in response to mechanical stimulation through the activation of extracellular latent TGF-β1 that activates TGF-β receptors to induce the SMAD and MAPK/ERK pathways.[23,24,28] These pathways activate Twist and as a result periostin is increased.[15,29] Thus, it is reasonable to think that similar processes may happen in other cell types. Therefore, the aims of this study were to determine if periostin is expressed by three different representative populations of the dental pulp complex relevant for its maintenance and healing, and to analyse the effects of TGF-β1 or biomechanical stimulation on its expression.

2. Materials and methods

2.1. Cell culture

All cell types used in the experiments were previously established and generously donated by Dr. Tatiana Botero (University of Michigan, Ann Arbor, MI, USA)[30] following the methods described by Gronthos et al. for DPSCs[31] and Stanislawski et al. for DPF.[32] Human primary cell lines were previously obtained under the University of Michigan Institutional Review Board supervision. Human dental pulp stem cells (DPSC, passage 4–7), human dental pulp fibroblasts (DPF, passage 4–7), and murine MDPC-23 odontoblast-like cells (passage 50–55) were used in this study. Cells were cultured with Dulbecco's modified Eagle's medium (DMEM) (Gibco, Life Technologies Corporation, Grand Island, NY, USA) supplemented with heat inactivated 10% foetal bovine serum (FBS, Gibco), 1% penicillin/streptomycin (100 IU/ml penicillin and 100 μg/ml streptomycin, Gibco), and 1:1000 fungizone (Gibco). Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Cells were removed from the growth surface with a trypsin solution (0.25% trypsin, 0.1% glucose, citrate-saline buffer, pH 7.8; Gibco) and subsequently used for experiments. More than three independent experiments were performed in triplicate each time.

2.2. Application of TGF-β1 supplementation

Total cell count was done and 1×10^5 viable cells were seeded in each well of a 6-well plate until they reached 75% confluency with media changes every 48 h. Experimental groups were defined as Group 1: control (DMEM; no TGF-β1 treatment); Group 2: cells treated with 10 ng/ml TGF-β1 (Invitrogen, Grand Island, NY, US) at time point 0 and after 24 h; and Group 3: cells treated with 20 ng/ml TGF-β1 at time point 0 and after 24 h. Media were changed at 24 h. Cell collection occurred at 48 h.

2.3. Application of biomechanical stimulation

Cells were cultured as previously described until a level of 75% confluence was reached. Total cell count was made to ensure 1×10^5 viable cells were seeded on flexible-bottomed BioFlex™ Culture Plates coated with type I collagen (Flexcell International Corp., Hillsborough, NC, USA) until they reached 75% confluency with media changes every 48 h.