

RESEARCH HIGHLIGHT

Spry2 is a novel therapeutic target for periodontal tissue regeneration through fibroblast growth factor receptor signaling and epidermal growth factor signaling

Terukazu Sanui¹, Takao Fukuda¹, Urara Tanaka¹, Kyosuke Toyoda¹, Takaharu Taketomi², Fusanori Nishimura¹

¹Department of Periodontology, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Fukuoka 812-8582, Japan

²Dental and Oral Medical Center, Kurume University School of Medicine, Kurume, Fukuoka 830-0011, Japan

Correspondence: Terukazu Sanui

E-mail: sanuteru@dent.kyushu-u.ac.jp

Received: February 04, 2015

Published online: March 29, 2015

Sprouty2 (Spry2) inhibits the activation of the extracellular signal-regulated kinase (ERK) pathway via receptor tyrosine kinase signaling. In a recent paper published in Journal of Cellular Biochemistry, we demonstrated that transfection of dominant-negative Spry2 mutation increased fibroblast growth factor receptor (FGFR) - and epidermal growth factor receptor (EGFR)-induced ERK activation in osteoblasts. Conversely, it diminished their activation in gingival epithelial cells. Similar to these results, the sequestration of Spry2 increased osteoblast proliferation by FGFR and EGFR stimulation, whereas it decreased gingival epithelial cell proliferation via the ubiquitination and degradation of EGFR. Moreover, reduction of Spry2 activity upregulated the expression of Runx2 and downregulated Twist, an inhibitor of Runx2 through FGFR and EGFR signaling, resulting in enhanced osteoblastogenesis in osteoblasts. Furthermore, we also found that Spry2 inhibition upregulated proliferation and migration in human periodontal ligament cell lines when they were stimulated by both fibroblast growth factor (FGF) and epidermal growth factor (EGF), and led to a shift in macrophage polarization, exerted immunosuppressive and tissue-repairing effects in macrophages. These results suggest that the topical administration of Spry2 inhibitors may efficiently resolve inflammation by periodontitis, allow periodontal ligament and alveolar bone to grow, and prevent the healing wound area from gingival epithelial downgrowth and may create a suitable environment for periodontal wound healing such as guided tissue regeneration (GTR). This approach has potential for the establishment of an effective therapeutic strategy for novel periodontal regeneration.

Keywords: Spry2; periodontal regeneration; FGFR; EGFR; osteoblasts; gingival epithelial cells

To cite this article: Terukazu Sanui, et al. Spry2 is a novel therapeutic target for periodontal tissue regeneration through fibroblast growth factor receptor signaling and epidermal growth factor signaling. Receptor Clin Invest 2015; 2: e597. doi: 10.14800/rci.597.

What is Spry2?

Genetic analysis has shown Sprouty (Spry) to be a negative regulator of fibroblast growth factor receptor (FGFR) signaling during tracheal, eye, and wing development in *Drosophila melanogaster*. Spry proteins

modulate the effects of fibroblast growth factor (FGF) signaling by interfering with the extracellular signal-regulated kinase (ERK) pathway [1-3]. Four Spry isoforms in mammals, Spry1 to Spry4, have been identified. Spry2 inhibits the activation of ERK pathway in response to various growth factors [4-7]. The human Spry2 protein

comprises 315 amino acid residues and contains a conserved tyrosine residue at the N terminus. This tyrosine residue is situated at amino acid position 55 (Y55) in mammalian Spry2, and mutation of this residue results in a dominant-negative form of Spry2 that suppresses the functions of the wild-type counterparts on ERK activation^[8]. Spry2 is directly associated with epidermal growth factor receptor (EGFR), and Y55 is phosphorylated after the stimulation of epidermal growth factor (EGF). The binding of Y55 of Spry2 to c-Cbl, which is known as an E3 ubiquitin-protein ligase, results in the suppression of c-Cbl, preventing it from association with EGFR, which results in decreased EGFR degradation and thus, maintained EGFR activation^[9, 10]. Therefore, transfection of Spry2 dominant-negative mutant enhances FGFR-induced ERK activation, whereas it reduces EGFR-induced ERK activation^[8].

Current concept for successful periodontal tissue regeneration

Periodontal disease is initiated by persistent bacterial insult, resulting in the loss of tooth-supporting periodontal tissues mainly in the adults^[11, 12]. Recently, many experimental and clinical studies have been performed to establish methods for periodontal tissue regeneration, such as guided tissue regeneration (GTR). GTR, which was introduced in the 1980s, has been widely used to regenerate lost periodontal tissues. It involves the placement of a barrier membrane to cover the bony defect during surgery so that the wound space is preserved and the ingrowth of gingival epithelia toward the healing wound area is prevented, thereby inducing periodontal tissue regeneration^[13, 14]. However, the skills and experience of the operator often affect the success of treatment by GTR method because it is technically difficult to place a barrier thin membrane beneath the gingiva.

Spry2 regulates the proliferation and differentiation of osteoblastic cells and gingival epithelial cells through FGFR and EGFR stimulation

In our recent study entitled “Mutation of Spry2 induces proliferation and differentiation of osteoblasts but inhibits proliferation of gingival epithelial cells”^[15], we demonstrated that the dominant-negative Spry2 mutation enhanced FGFR and EGFR-induced ERK phosphorylation in osteoblasts, whereas it diminished its activity in gingival epithelial cells *in vitro*. Similar to this result, the sequestration of Spry2 promoted osteoblast proliferation but inhibited gingival epithelial cell proliferation. In addition, we showed that the Y55 mutation of Spry2 enhanced FGFR signaling by interfering with interaction between endogenous

Spry2 and Grb2, and attenuated EGFR signaling by enhancing c-Cbl-mediated EGFR ubiquitination in osteoblasts and gingival epithelial cells.

In contrast, osteogenic differentiation was promoted by the dominant-negative Spry2 mutation. Enhancement of FGFR stimulation due to suppression of Spry2 increased cell proliferation and the expression of runt-related transcription factor 2 (Runx2) via the ERK signaling pathway. Runx2 is a well-known transcription activator of osteoblast differentiation, and upregulates skeletal development; it has been linked to osteoblastic differentiation via regulation of the expression of numerous osteoblast-specific genes^[16]. The effect of Runx2 is suppressed by Twist which is known as a basic helix–loop–helix transcription factor. Twist functions by directly associating with Runx2 and interfering with Runx2 function, inhibits osteoblast maturation, and maintains preosteoblastic status. Therefore, Twist is thought to be a negative regulator of osteoblastogenesis^[17]. Furthermore, Runx2 is linked to the phosphorylation and the activation of ERK, which increases osteogenesis-related gene expression in bone formation^[18]. We also found that Spry2 dominant-negative mutant osteoblasts upregulated Ras-responsive Runx2 expression by inducing FGFR activation and EGFR degradation through the inhibition of Twist.

Conversely, Spry2 depletion enhanced the EGFR degradation; consequently, the expression of EGFR reduced, thereby decreasing proliferation in gingival epithelial cells. This decrease in proliferation counteracted the proliferative increment that is induced by increased FGFR signaling because gingival epithelial cells have the more quantitative or qualitative predominance of EGFR signaling than FGFR signaling.

Spry2 is a novel therapeutic target for treatment of periodontal disease

Collectively, our current understanding on the physiology of Spry2 showed that the tyrosine residue at amino acid position 55 mutation of Spry2 promoted proliferation in osteoblasts as well as osteoblastogenesis through the co-stimulation of FGFR and EGFR. In contrast, it reduced cell proliferation in gingival epithelial cells. Therefore, the tyrosine 55 of Spry2 has a novel therapeutic effect for treating periodontal diseases. Periodontal tissue is composed of four defined structure; the alveolar bone, gingiva, periodontal ligament, and cementum. We also noted that Spry2 RNAi upregulated cell proliferation and migration in human periodontal ligament cell lines when they were stimulated with an FGF and EGF cocktail (manuscript in preparation). Furthermore, reduction in Spry2 levels leads to

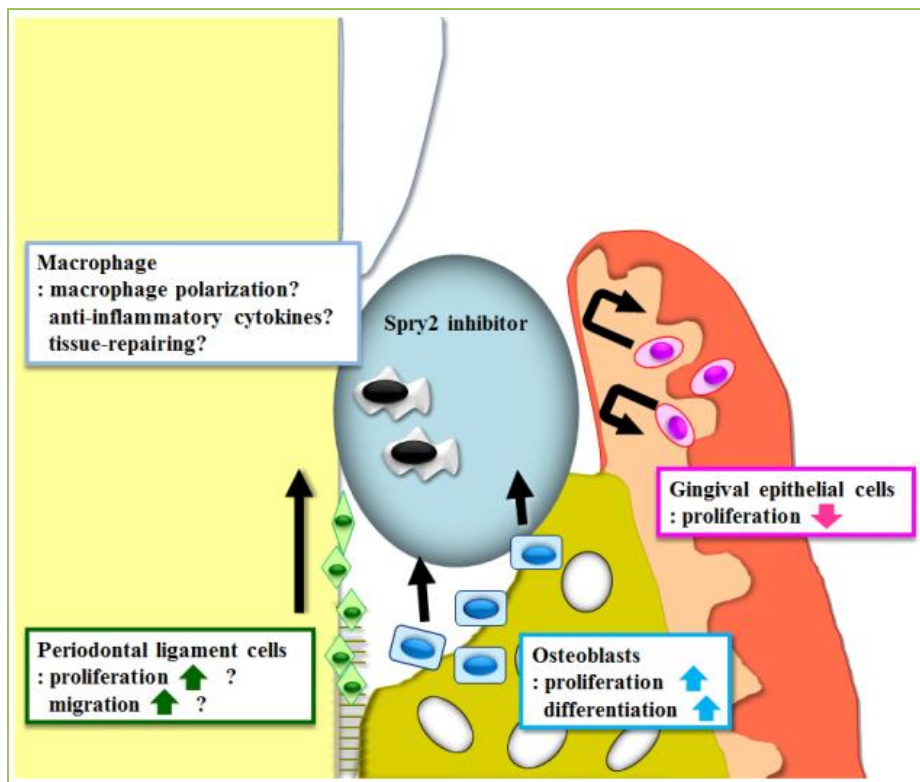


Figure 1. Possible effects of a Spry2 inhibitor on the various cell types involved in periodontal tissue regeneration. Suppression of Spry2 increases osteoblast proliferation and differentiation by FGFR and EGFR stimulation, whereas it decreases gingival epithelial cell proliferation via the ubiquitination and degradation of EGFR. In addition, it may also upregulate proliferation and migration in periodontal ligament cells and lead to a shift in macrophage polarization, exerted immunosuppressive and tissue-repairing effects in macrophages. These results suggest that the topical administration of Spry2 inhibitors may efficiently resolve inflammation by periodontitis and allow periodontal ligament and alveolar bone to grow and prevent the healing wound area from gingival epithelial downgrowth, and may create a suitable environment for periodontal wound healing such as conventional GTR.

a shift in macrophage polarization, exerts immunosuppressive and tissue-repairing effects, and plays important roles in the resolution of injurious inflammation by producing anti-inflammatory mediators in macrophages (unpublished observation).

These *in vitro* findings provide a molecular basis and an effective therapeutic strategy for novel periodontal regeneration. That is, the topical administration of Spry2 inhibitors may efficiently resolve inflammation by periodontitis and allow periodontal ligament and alveolar bone to grow and prevent the healing wound area from gingival epithelial downgrowth, and may create a suitable environment for periodontal wound healing such as conventional GTR (Fig. 1). This approach has potential for the establishment of an effective therapeutic strategy for novel periodontal regeneration.

Conflict of interests

The authors declare no potential conflict of interests.

Acknowledgements

This work was supported by Grants-in-Aid for Young Scientists B (22792092 and 24792334) from the Japan Society for the Promotion of Science, Takeda Science Foundation, and Kyushu University Interdisciplinary Programs in Education and Projects in Research Development.

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