

Biological Effects of Sprouty2 Inhibition in Periodontal Ligament Cells

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Introduction

Periodontitis is initiated by specific bacterial insult, resulting in the destruction of tooth-supporting periodontal tissues, and mainly develops in adults [1,2]. The periodontal tissues are composed of four major tissues: the periodontal ligament (PDL) tissue, cementum, gingiva, and alveolar bone. In particular, the PDL plays a pivotal role in anchoring functional teeth to the alveolar bone, repairing the wounded periodontium, and maintaining periodontal tissue homeostasis [3,4]. Functional proliferation and migration of PDL cells are essential for appropriate wound healing and successful periodontal tissue regeneration. Recombinant basic fibroblast growth factor (bFGF) has been shown to induce significant periodontal tissue regeneration *in vivo*, accompanied by regeneration of the PDL tissue and alveolar bone [5,6]. In addition, bFGF stimulates the proliferation and migration of PDL cells, and plays a crucial role in periodontal tissue regeneration [7].

Sprouty (Spry) proteins were identified as inhibitors of the FGF signaling pathway during tracheal branching in *Drosophila* [8]. Spry also regulates the epidermal growth factor (EGF) signaling pathway during *Drosophila* organogenesis [9]. In mammals, four human Spry homologs (Spry1–4) have been identified [8], and Spry2 specifically suppresses activation of the extracellular signal-regulated kinase (ERK) pathway in response to various growth factors such as FGF [10,11]. Moreover, Spry2 interacts with growth factor receptor-bound protein 2 (Grb2), an adaptor protein responsible for communication between receptor tyrosine kinase (RTK) activation and the Ras/Raf1/ERK pathway in response to growth factor stimulation [12,13]. The interaction between Spry2 and Grb2 disturbs the association between RTK and Grb2 and inhibits RTK-induced signal transduction [10,14]. In addition, both hypoxia and nickel exposure increased the level of H3K9me2 at the Spry2 promoter by inhibiting histone demethylase JMJD1A [15]. Since the main histone methyltransferases that required for the establishment of H3K9me2 are G9a/GLP complex [16], therefore it is very likely that G9a/GLP complex is recruited to the Spry2 promoter. Furthermore, as G9a/GLP complex physically interacts with *de novo* DNA methyltransferases [17] and is also involved in the maintenance of DNA methylation in mammals [18], Spry2 gene could be irreversibly down-regulated by gain of promoter DNA methylation. This epigenetic mechanism might also explain the down-regulation of Spry2 in non-small cell lung cancer [19].

Spry proteins have a short region of sequence similarity in their amino termini that contains a conserved tyrosine residue. In

mammalian Spry2, this tyrosine residue is located at amino acid position 55 (Y55), and many of the inhibitory functions of Spry2 are dependent on this residue [20,21]. In a dominant-negative mutant of Spry2 (Y55A-Spry2), the tyrosine at position 55, which is crucial for Spry2 function, is substituted with alanine [22]. In a previous study, we showed that transfection of Y55A-Spry2 promoted RTK signaling by inducing ERK activation in osteoblasts, thereby increasing osteoblastic cell proliferation and osteoblastogenesis after co-stimulation of bFGF and EGF. By contrast, the Spry2 mutation showed decreased ERK activation and cell proliferation in gingival epithelial cells. Therefore, we concluded that the topical administration of Spry2 inhibitors, bFGF, and EGF may effectively promote the growth of alveolar bone, maintain the wound space, and interfere with the healing wound area from gingival epithelial down growth, thereby creating favourable conditions for periodontal tissue regeneration [23]. However, it is fundamental to determine the effects of Spry2 on the regulation of PDL cell functions for establishing an effective therapeutic strategy to promote new periodontal tissue regeneration.

In our recent study entitled “Sprouty2 inhibition promotes proliferation and migration of periodontal ligament cells”, we demonstrated that Spry2 depletion by bFGF and EGF stimulation markedly enhanced PDL cell proliferation and migration [24]. Spry2 knockdown increased bFGF and EGF-induced ERK activation and proliferation in PDL cells, and increased cell migration in a scratch wound-healing assay and Boyden chamber migration assay. In addition, we showed that Spry2 inhibition promoted lamellipodia formation via AKT/phosphoinositide 3-kinase (PI3K) and Rho family GTPases, specifically via Rac1 activation in PDL cells, thereby activating cell migration upon stimulation with bFGF and EGF (Figure 1). Moreover, Spry2 inhibition induced a highly inhibitory effect on osteoblastic differentiation by abolishing the mRNA transcription of osteogenesis-related genes, such as collagen type I, alkaline phosphatase, bone sialoprotein and runt-related transcription factor-2. These results suggested that Spry2-downregulated PDL cells could be clinically applied to facilitate cell proliferation and migration along the root surface of the tooth, while interfering with the differentiation of PDL cells into osteoblastic cells, thereby maintaining the periodontal ligament space and avoiding “dental ankylosis”, which is characterized by the direct fusion of the tooth roots to the bone. Therefore, determining whether Spry2 suppression by bFGF and EGF stimulation is linked to wound healing of the PDL is an important issue that should be further investigated *in vivo*.

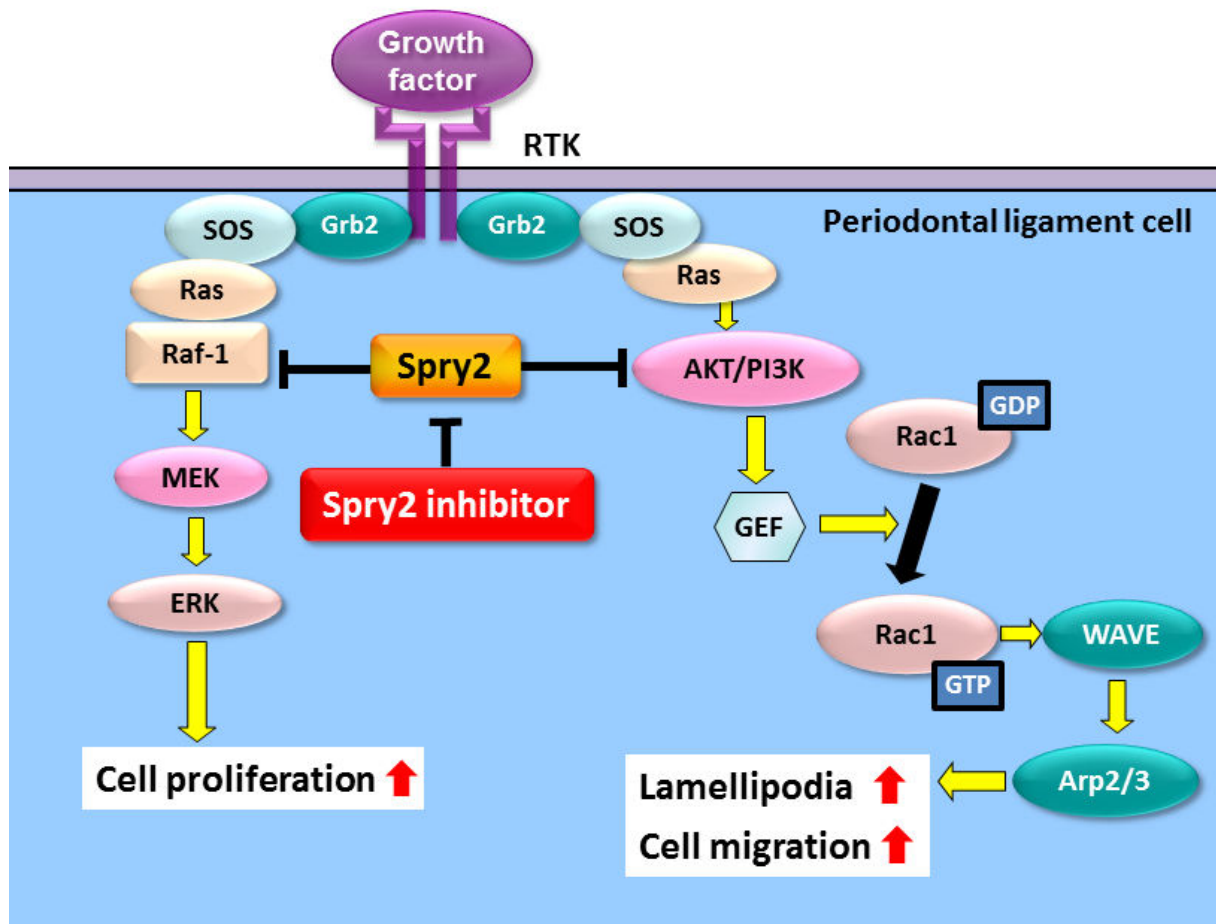
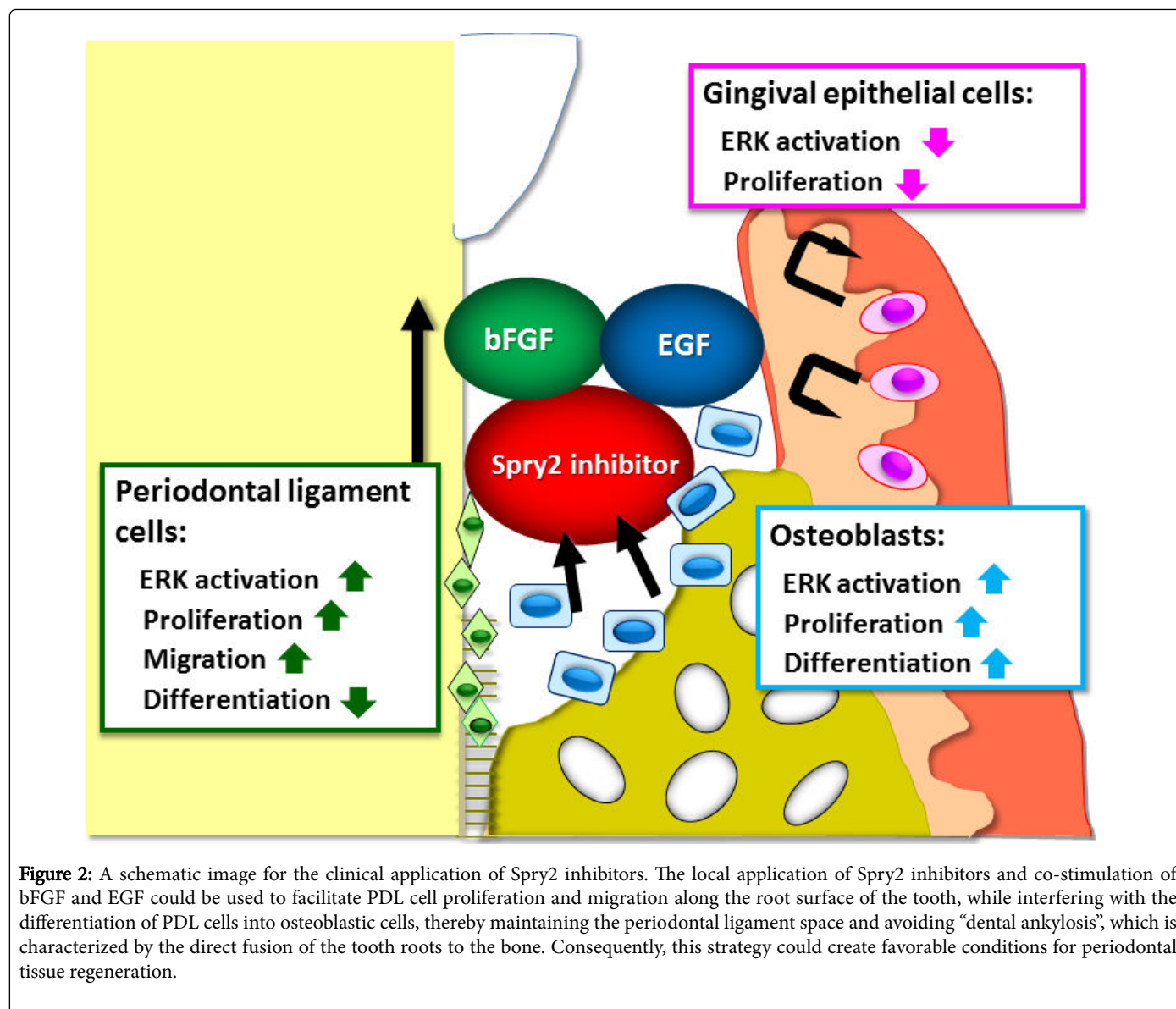


Figure 1: Proposed model of the involvement of Spry2 and a Spry2 inhibitor in growth factor signaling pathway for cell proliferation and migration in PDL cells. Upon growth factors binding to their receptors and activating the signal pathways, Spry2 becomes activated and interacts with various components of the MAPK signaling pathway. A Spry2 inhibitor suppresses endogenous Spry2, thereby promoting the growth factor-induced Raf/MEK/ERK pathway and proliferation in PDL cells. In addition, it enhances lamellipodia formation via AKT/PI3K and Rho family GTPases, specifically via Rac1 activation in PDL cells, thereby activating cell migration.

Furthermore, we recently showed that Spry2 depletion by interferon- γ and *Porphyromonas gingivalis* lipopolysaccharide stimulation leads to a shift toward M2 alternative activated macrophage polarization, and plays crucial roles in the resolution of inflammation by producing anti-inflammatory cytokines such as interleukin-10 in macrophages [25]. We also found that Spry2 inhibition increased the efferocytosis of apoptotic cells by enhancing Rac1 activation and promoted the production of growth factors such as bFGF and EGF [25]. In conclusion, our studies show that Spry2

downregulation in combination with bFGF and EGF stimulation could enhance PDL cell proliferation and migration without inducing osteoblastogenesis, further supporting a favorable environment for ideal periodontal regeneration (Figure 2). Collectively, these results indicate that the local application of Spry2 inhibitors and co-stimulation of bFGF and EGF to bony defects may create a suitable environment for periodontal wound healing by orchestrating PDL cell proliferation and migration, thereby contributing to the prevention of tooth ankylosis.



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