論　文　内　容　要　旨

The involvement of TGF-β/smad2 signaling pathway in *Aggregatibacter actinomycetemcomitans*-induced apoptosis on human gingival epithelial cells

(TGF-β/smad2 シグナル伝達経路と *Aggregatibacter actinomycetemcomitans* が誘導するヒト歯肉上皮細胞におけるアポトーシスとの関連性)

主指導教員：栗原　英見教授
（応用生命科学部門歯周病態学）
副指導教員：谷本　幸太郎教授
（応用生命科学部門歯科矯正学）
副指導教員：柴　秀樹教授
（応用生命科学部門健康増進学）

吉本　哲也
（医歯薬学総合研究科　創生医科学専攻）

論　文　内　容　要　旨

論文題目

The involvement of TGF-β/smad2 signaling pathway in Aggregatibacter actinomycetemcomitans-induced apoptosis on human gingival epithelial cells
(TGF-β/smad2 シグナル伝達経路と Aggregatibacter actinomycetemcomitans が誘導するヒト歯肉上皮細胞におけるアポトーシスとの関連性)

学位申請者　吉本　哲也

Periodontitis is the most prevalent infectious disease caused by periodontopathic bacteria. The junctional epithelium is located at a strategically important interface at the base of the gingival sulcus. Since junctional epithelium is the first line of defense against bacteria, its disruption by bacterial infection is involved in the initiation and progression of periodontitis. Therefore, revealing how bacteria induce the disruption of junctional epithelium can elucidate to the pathogenesis of periodontitis. It is well known that junctional epithelium is sustained by the vigorous proliferation of progenitor cells and rapid apoptosis of the superficial cells, with gingival epithelial cells self-renewing within as little as 3 days. Apoptosis of superficial cells at junctional epithelium is a highly conserved and regulated process for eliminating aged, damaged, or infected cells from tissues, thus serving as an intrinsic mechanism against bacterial infection. Accordingly, the disruption of this apoptosis mechanism contributes to the destruction of junctional epithelium. Previous studies reported that smad2 signaling pathway plays an important role in apoptosis of superficial gastric epithelial cells. From these findings, we hypothesized that periodontopathic bacteria facilitate apoptosis by activating smad2 signaling pathway in gingival epithelial cells, lead to the hypofunction such as barrier function, and initiate to periodontits. In the present study, we aimed to reveal whether periodontopathic bacteria facilitate smad2 phosphorylation, which result in induce apoptosis in human gingival epithelial cells

We used primary human gingival epithelial cells (HGECs) or the human gingival epithelial cell line, OBA9 cells (gifted by Prof. Murakami, Osaka university). As a stimulant, we used *Porphyromonas gingivalis*W83 (*Pg*W83), *Aggregatibacter actinomycetemcomitans* (*Aa*) strain Y4, *Aa* strain IDH781, *Fusobacterium nucleatum* (*Fn*), *Prevotela intermedia* (*Pi*), and *Escheria coli* (*E.coli*) (ATCC, Manassas, VA), which were fixed with 1% formalin and recombinant human TGF-β1 (R&D systems, Minneapolis, MN, USA). We performed Western blotting to analyze protein levels and TUNEL staining to detect apoptosis positive cells. To

determine whether these bacteria facilitate the phosphorylation of smad2 in human gingival epithelial cells, we exposed HGECs and OBA9 cells to these bacteria at 10^7 cells per well (MOI: 10^2), respectively. While *Aa* Y4, *Aa* IDH781, *Fn* and *E.coli* stimulation apparently facilitate smad2 phosphorylation in human gingival epithelial cells, *Pg*W83 and *Pi* did not. To reveal the mechanism on the phosphorylation of smad2 in human gingival epithelial cells, we employed OBA9 cells and treatment with *Aa*Y4 in the following experiments. To examine the relationship between bacteria-facilitated smad2 phosphorylation and apoptosis in human gingival epithelial cells, we performed siRNA transfection for smad2. Knockdown by siRNA for smad2, which reduced smad2 protein levels, attenuated the activation of cleaved-caspsae3 expression and suppressed apoptosis in OBA9 cells.

From these results, we addressed the involvement of TGF-β type I receptor (TGF-βRI) in *Aa*Y4-activated apoptosis cascade on OBA9 cells. *Aa*Y4 induced phosphorylation of the serine residue of TGF-βRI in OBA9 cells. The siRNA transfection for TGF-βRI, which reduced both TGF-βRI mRNA and protein levels, markedly attenuated the phosphorylation of smad2 and the activation of cleaved caspase-3 expression, and repressed apoptosis in OBA9 cells treated with *Aa*Y4. Furthermore, we investigated the mechanism how *Aa*Y4 activate TGF-βRI/smad2 cascade in OBA9 cells. Recombinant Omp29 (1.0 μg/ml) were used to stimulate OBA9 cells. Similar to *Aa*Y4, Omp29 induced apoptosis via TGF-βRI/smad2 signaling pathway in OBA9 cells. Here, we focused on the involvement of TGF-β1 in Omp29-activated TGF-βRI/smad2 cascade in OBA9 cells. To test whether OBA9 cells treated with Omp29 released TGF-β1, we checked TGF-β1 in the medium by using ELISA. The amount of active TGF-β1 in medium was increased in OBA9 cells treated with Omp29. Furthermore, the pretreatment with the neutralization antibody of TGF-β1 or TGF-β type II receptor decreased the Omp29-facilitated smad2 phosphorylation in OBA9 cells. On the other hands, Omp29 did not affect the mRNA level of TGF-β1 in OBA9 cells. These findings implied that Omp29 activate precursor TGF-β1, and activated TGF-β1 induces apoptosis via TGF-βR/smad2 signaling pathway in human gingival epithelial cells.

In summary, TGF-β/smad2 signaling pathway is involved in *Aggregatibacter actinomycetemcomitans*-induced apoptosis on human gingival epithelial cells. The present study provided a novel insight into bacterial infection system and introduced novel molecular targets in the search for a therapeutic chemical compound that can protect human gingival epithelial cells from bacteria-induced apoptosis. These results may lead to the development of novel preventive or therapeutic interventions against periodontitis.