Streptococcus gordonii antagonizes *Porphyromonas gordonii*-induced OLFM4 in epithelial cells

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Introduction

Porphyromonas gingivalis (Pg) is an oral obligate anaerobe that is considered a keystone pathogen in periodontal disease. Additionally, Pg has been strongly associated with oral squamous cell carcinoma (OSCC), which is the most common oral malignancy with roughly a 50% survival rate after 5 years. Pg has been shown to inhibit apoptosis, accelerate cell cycle progression, and induce epithelial-to-mesenchymal transition through ZEB1/ZEB2 in gingival epithelial cells. Interestingly, the oral commensal *Streptococcus gordonii* (Sg) can inhibit ZEB2 upregulation by Pg, thereby preventing EMT, as well as prevent the enhanced cell cycle progression and inhibited apoptosis induced by Pg. OLFM4 is strongly associated with gastric cancer, and is primarily known for its role in inhibiting apoptosis. OLFM4 also requires Notch signaling for regulation. Here we show that Pg upregulates OLFM4 by activating Notch signaling, and Sg blocks this upregulation in gingival epithelial cells.

Objective

To investigate OLFM4 regulation by Pg in gingival epithelial cells

Methods

Telomerase immortalized gingival keratinocytes (TIGKs) were grown to 80% confluence. Cells were challenged with bacteria for 3h, media was changed and RNA was harvested at 24h. RNA was reverse transcribed, then qPCR was performed and fold change calculated using ΔΔCT. siRNA transfection was performed 48h before bacterial challenge, and scrambled siRNA was used as a control. All qPCR data was normalized to GAPDH.

Results

a)

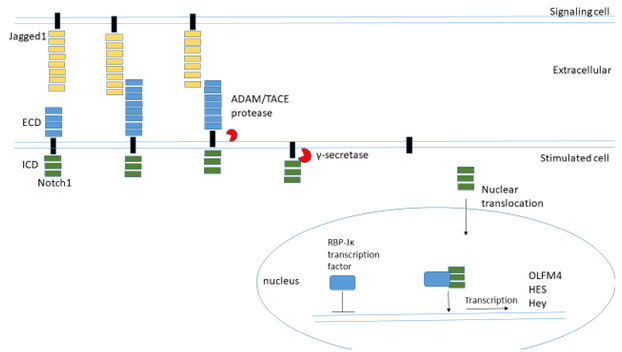
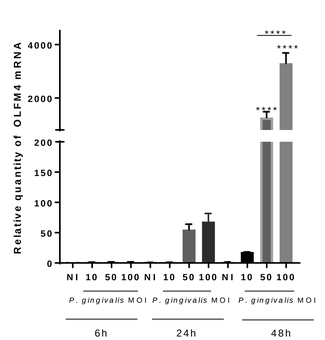


Figure 1: a) Schematic of Notch Signaling

a)



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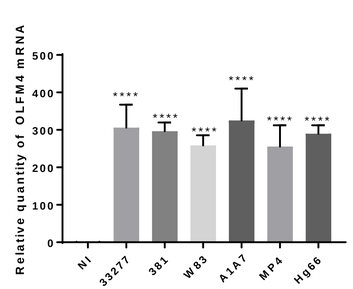
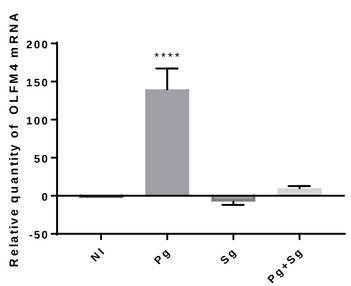


Figure 2: a) TIGK cells were grown to 80% confluence, then challenged with *P. gingivalis* ATCC33277 at indicated MOIs and times. RNA was then harvested, reverse transcribed, and qPCR used to determine relative quantities of OLFM4 mRNA. B) TIGK cells were challenged with multiple laboratory strains and a low passage clinical isolate *P. gingivalis* strain as described in a. One-way ANOVA was used to determine statistical significance.

a)



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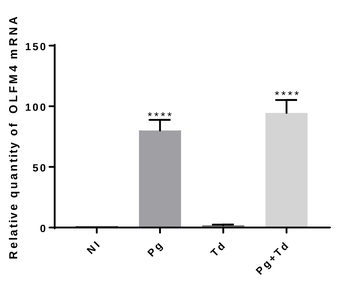

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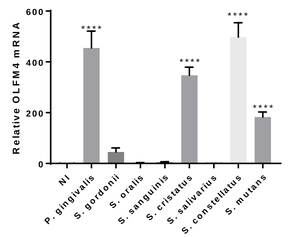
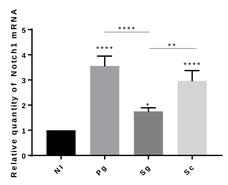


Figure 3: a) TIGK cells were grown to 80% confluence, then challenged with Pg and/or Sg. RNA was then harvested, reverse transcribed, and qPCR used to determine relative quantities of OLFM4 mRNA. b) TIGK cells were challenged with Pg and/or *T. denticola* as described in a. c) TIGK cells were challenged with Pg, or co-challenged with different streptococci as described in a. One-way ANOVA was used to determine statistical significance.

Figure 4: a-b) TIGK cells were challenged as previously described with Pg, or co-challenged with Sg or Sc. RNA was then harvested, reverse transcribed, and qPCR used to determine relative quantities of Notch1 and Jagged1 mRNA. c) Cells were transfected with siRNA targeting Jagged1 or a scrambled siRNA 48h before infection with Pg. RNA was then harvested, reverse transcribed, and relative quantities of OLFM4 determined by qPCR. d) TIGK cells were transfected with siRNA targeting Notch1 and/or Notch3 as previously described, then challenged with Pg for 24h. RNA was then harvested, reverse transcribed, and relative quantities of OLFM4 determined by qPCR.

a)



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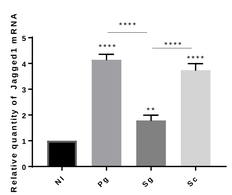

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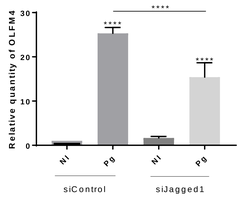

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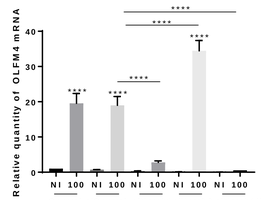

Conclusions

Our lab has established Sg as a homeostatic commensal, and here we show an additional antagonism of Pg induced oncogenes. Interestingly, this antagonism is specific to certain commensal streptococci. Understanding this relationship, and the mechanism by which Pg activates and Sg inactivates oncogenic pathways will be valuable for understanding OSCC initiation and progression.

Acknowledgments

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