指導教授氏名	指導役割
（自署）	Supervisor and research guidance
（自署）	
（自署）	

学位論文要旨

岡山大学大学院医歯薬学総合研究科

教育研究分野 口腔形態学	身分　大学院生	氏名　ZHENG YILIN

論文題名　Gingipain regulates isoform switches of PD-L1 in macrophages infected with *Porphyromonas gingivalis*

（Gingipain は *Porphyromonas gingivalis* 感染マクロファージにおけるPD-L1のアイソフォームスイッチを制御する）

論文内容の要旨（2000字程度)

Introduction

This study investigates whether *Porphyromonas gingivalis* (*P. gingivalis*), a major periodontal pathogen, utilizes its unique proteases, gingipains, to regulate alternative splicing (AS) of host genes as a mechanism of immune evasion. Previous research has established the role of *P. gingivalis* in upregulating immune checkpoint proteins like PD-L1, which suppress T cell responses. However, it remained unclear whether *P. gingivalis* also alters PD-L1 splicing to enhance its immune suppressive effects. PD-L1 exists in multiple isoforms due to AS, notably a full-length isoform containing the Immunoglobulin Variable (IgV)-like domain (PD-L1^{IgV+}) critical for PD-1 binding, and a shorter isoform lacking this domain (PD-L1^{IgV-}). This study explores whether gingipain facilitates selective expression of PD-L1^{IgV+} in macrophages.

Methods

Wild-type *P. gingivalis* (ATCC33277) and its gingipain-deficient mutant strain (*ΔKDP*) were cultured anaerobically. THP-1 cells were differentiated into macrophages using PMA and then infected with *P. gingivalis* or *ΔKDP* (MOI = 100). Separately, recombinant gingipains (RgpA, RgpB, and Kgp) were applied to macrophages to assess individual enzyme effects. After infection or treatment, RNA and protein were extracted. Total RNA was processed for library construction using the NEBNext Ultra II RNA Library Prep Kit and sequenced using Illumina platforms. RNA-seq data were analyzed using STAR, StringTie, and isoformSwitchAnalyzeR to assess alternative splicing. Differential exon usage (DEU) was examined via DEXSeq, and differentially expressed genes (DEGs) were identified by DESeq2. Gene Ontology analysis was performed with clusterProfiler. RT-qPCR and RT-PCR were used to quantify total and isoform-specific PD-L1 transcripts. Protein expression was evaluated by SDS-PAGE

and Western blot. Structural modeling of PD-L1 isoforms in complex with PD-1 was performed using AlphaFold 3, and binding affinities were estimated via chain_pair_iptmperformed using AlphaFold 3, and binding affinities were estimated via chain_pair_iptm scores and hydrogen bond predictions (PDBePISA).

Results

RNA-seq analysis of THP-1-derived macrophages infected with wild-type *P. gingivalis* (*Pg*-inf) or its gingipain-deficient mutant strain (*ΔKDP*-inf) revealed significant changes in the AS landscape. Specifically, *P. gingivalis* infection enhanced total PD-L1 expression and significantly shifted isoform usage toward PD-L1^{IgV+}, while suppressing PD-L1$^{IgV–}$. These effects were absent in the *ΔKDP*-inf group, implicating gingipains in the regulation of PD-L1 splicing. Western blot and RT-qPCR confirmed that only *Pg*-inf cells—unlike *ΔKDP*-inf or uninfected cells—exhibited robust PD-L1 upregulation. Treatment with recombinant gingipains (RgpA, RgpB, Kgp) validated that RgpB and Kgp independently increased PD-L1^{IgV+} expression. AlphaFold 3 structural modeling revealed that PD-L1^{IgV+} has a significantly higher predicted binding affinity for PD-1, forming more hydrogen bonds compared to PD-L1$^{IgV–}$. Thus, *P. gingivalis* upregulates a functionally superior PD-L1 isoform via gingipain-mediated AS modulation.

Discussion

This study is the first to demonstrate that gingipains modulate AS of PD-L1 in macrophages, preferentially producing the immunosuppressive PD-L1^{IgV+} isoform. The findings provide molecular evidence that *P. gingivalis* evades immune surveillance not only by increasing PD-L1 expression but also by manipulating isoform selection to enhance PD-1 interaction. The modulation of exon inclusion (specifically exon 3 encoding the IgV-like domain) appears to be a gingipain-dependent event. Bioinformatic classification of AS changes revealed that some were promoted or inhibited specifically by gingipains, affecting protein domain composition and coding potential. This highlights a novel role of gingipains in host RNA processing. The study also noted a dramatic downregulation of RNA-binding protein RBPMS in *Pg*-inf macrophages, which might be involved in exon regulation, although the precise link with gingipains remains to be confirmed.

Conclusion

The study concludes that gingipains from *P. gingivalis* drive immune evasion by selectively promoting the PD-L1^{IgV+} isoform through alternative splicing regulation. This isoform has stronger PD-1 binding capacity and thus more effectively suppresses T cell activation. These findings provide new insights into the immune-modulatory strategy of *P. gingivalis*, with potential implications for targeting PD-L1 isoform regulation in infection-associated immune dysregulation.