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学位授与の要件	医歯薬学総合研究科機能再生・再建科学専攻 （学位規則第４条第１項該当）
学位論文の題目	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）
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学位論文内容の要旨

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.

論文審査結果の要旨

Introduction

Porphyromonas gingivalis (*P. gingivalis*), a keystone pathogen in periodontal disease, is known to evade immune responses through its unique proteinase, gingipain. PD-L1 undergoes alternative splicing (AS), producing multiple isoforms, including PD-L1 with an IgV-like domain (PD-L1IgV+), which is essential for binding PD-1 and suppressing T cell activity. However, it remains unclear whether *P. gingivalis* influences PD-L1 isoform switching to promote immune evasion. In this study, we investigated whether gingipains affect AS in macrophages after *P. gingivalis* infection, focusing on the role of AS in immune evasion by analyzing selected immune-related candidate genes.

Methods

THP-1-derived macrophages were infected with *P. gingivalis* ATCC33277 (wild-type) or a gingipain-deficient mutant strain (*ΔKDP*). Separately, macrophages were treated with recombinant gingipains (RgpA, RgpB, and Kgp). RNA was extracted from *P. gingivalis*-infected macrophages and used for RNA-seq and qPCR. Proteins extracted from *P. gingivalis*-infected macrophages were used for Western blot analysis. Isoform-specific splicing events were analyzed using isoformSwitchAnalyzeR. Structural prediction of PD-L1 isoforms in complex with PD-1 was performed using AlphaFold 3, and binding affinity was evaluated through chain_pair_iptm scores and hydrogen bond prediction.

Results

Infection with wild-type *P. gingivalis* increased total PD-L1 expression and shifted isoform usage toward PD-L1^{IgV+}, while decreasing PD-L1$^{IgV^-}$. These changes were not observed in *ΔKDP*-infected cells. RgpB and Kgp individually enhanced PD-L1^{IgV+} expression. The inclusion of exon 3, which encodes the IgV-like domain, seems to be gingipain-dependent. AlphaFold 3 modeling showed that PD-L1^{IgV+} forms more hydrogen bonds and has a higher predicted affinity for PD-1 compared to PD-L1$^{IgV^-}$.

Conclusion

This study demonstrated that gingipains regulated PD-L1 alternative splicing in macrophages, specifically enhancing the expression of the immunosuppressive PD-L1^{IgV+} isoform. These results reveal a new RNA-level immune evasion mechanism used by *P. gingivalis* and suggest promising therapeutic options that target isoform-specific immune checkpoint regulation during infection.

This study shows that gingipains promote the immunosuppressive PD-L1^{IgV+} isoform through alternative splicing in *P. gingivalis*-infected macrophages, supported by transcriptomic, biochemical, and structural evidence. The article has been published in Scientific Reports and has received academic recognition. Therefore, the defense committee has accepted this article as a doctoral dissertation in Dentistry.