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授与した学位	博　士
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学位授与の日付	令和　８年　３月２５日
学位授与の要件	医歯薬学総合研究科社会環境生命科学専攻 （学位規則第４条第１項該当）
学位論文の題目	Effects of miR-128-3p on Renal Inflammation in a Rat Periodontitis Model （ラット歯周炎モデルにおける miR-128-3p による腎臓の炎症への影響 ）
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学位論文内容の要旨

Introduction

Extracellular vesicles (EVs) are lipid-encapsulated vesicles and have the capacity to transport bioactive molecules, including DNA, RNA, lipids, and proteins, to other cells to regulate their biological functions. EVs mediate a complex intercellular communication network in sepsis, shuttling a variety of key mediators, such as microRNAs (miRNAs). MiRNAs regulate gene expression by inhibiting translation or promoting mRNA degradation. Some miRNAs have organ-specific expression and can circulate from one organ to an-other, potentially changing the expression of specific genes, including the promotion or suppression of inflammatory pathways in the kidney. One specific miRNA, miR-128-3p, has a significant role in inflammation, and contributes to sepsis-associated acute kidney injury. However, whether miR-128-3p from periodontitis can cause renal inflammation re-mains unclear. The study aim was to investigate the role of miR-128-3p from EVs associated with in periodontitis in renal inflammation and to identify the associated target genes of miR-128-3p in the rat periodontitis model.

Methods

Male Wistar rats aged 10 weeks old (Japan SLC, Inc., Hamamatsu, Japan) were used in this study. The rats were randomly divided into two groups: a control group (n = 8) and a LPS group (n = 8). The LPS group received LPS derived from *Porphyromonas gingivalis* and 10 μL injection of LPS was administered between the maxillary first and second molars on both sides of the rats every 2 days for a total of 7 days. The control group received an equivalent volume of distilled water without LPS at the same intervals. At the end of the experiment, plasma, gingival tissue, and kidney samples were collected. Hematoxylin and eosin (H&E) staining was performed to evaluate the glomerular tissue injury score. Bioinformatic analysis was conducted to identify potential target genes of miR-128-3p. The reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was performed to evaluate miR-128-3p, cytokine, chemokine and predicting gene expression. Welch's *t*-test was used

and p-values < 0.05 were considered to indicate statistical significance.

Results

Histological analysis using H&E staining revealed a higher infiltration of inflammatory cells (eosinophils and neutrophils) in the glomerular area of renal tissues in the LPS group compared with the control group. The expression of miR-128-3p was significantly higher in the gingival tissue and plasma of the LPS group compared with the control group. Gene expression of *Interleukin (IL)-1β*, *tumor necrosis factor (TNF)-α*, *C-X3-C motif chemokine ligand 1 (CX3CL1),* and C-X-C motif chemokine ligand 7 *(CXCL7)* was significantly higher in the kidney of LPS group. The potential target genes of *activin A receptor type I* (*Acvr1*), *ribosomal protein S6 kinase B1 (Rps6kb1)*, and *transforming growth factor beta receptor type 1 (Tgfbr1)* were significantly lower in the kidneys of the LPS group.

Discussion

In the present study, the expression levels of miR-128-3p in gingival tissue and blood plasma were significantly higher in the LPS group, suggesting that miR-128-3p may be originated from EVs derived LPS-induced periodontitis. Higher infiltration of inflammatory cells in the glomerular area of renal tissues in the LPS group were observed. The inflammatory gene *COX2* and pro-inflammatory cytokine and chemokine genes *IL-1β*, *TNF-α*, *CX3CL1*, *CXCL7*, and *CCL5* were upregulated in the kidney. In addition, target genes (*Rps6kb1*, *Tgfbr1*, and *Acvr1*) of miR-128-3p were significantly downregulated in the LPS group compared with the control group. TGF-β signaling pathway was associated with three mRNAs (*Rps6kb1*, *Tgfbr1*, and *Acvr1*) as a potential target gene of miR-128-3p, which may play a role in promoting inflammation in the kidney. In conclusion, EV-derived miR-128-3p from LPS-induced periodontitis may cause renal inflammation and be mediated by the *Rps6kb1*, *Tgfbr1*, and *Acvr1* genes through the TGF-βsignaling pathway. Our findings could be used for further study in the context of renal inflammation linked to periodontitis.

論文審査結果の要旨

Introduction: Periodontitis is an inflammatory disease affecting the tissues that support the teeth. In this condition, microRNAs (miRNAs) in periodontal tissue can be transferred to other organs. This study aimed to examine the effects of extracellular vesicle (EV)-derived miR-128-3p, known to be involved in various inflammatory responses, on renal inflammation in a rat model of periodontitis.

Methods: Ten-week-old male Wistar rats were divided into two groups: a control group (n=8) and a lipopolysaccharide (LPS) group (n=8). The control group received an equivalent volume of distilled water without LPS. The LPS group was subjected to gingival infections with LPS (*Porphyromonas gingivalis*) for 7 days. A 10 μL injection of LPS (10 μg/μL) was administered between the maxillary first and second molars on both sides of each rat every 2 days. At the end of the experiment, plasma, gingival tissue, and kidney samples were collected. Hematoxylin and eosin staining was performed to assess the glomerular tissue injury score. Bioinformatic analysis was conducted to identify potential target genes of miR-128-3p. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was used to evaluate miR-128-3p, inflammatory cytokines, chemokines, and predicted genes. The control and LPS groups were compared using Welch's *t*-test. *P*-values less than 0.05 were considered statistically significant.

Results: The kidney glomerular tissue injury score was significantly higher in the LPS group compared to the control group, although no functional abnormalities were found. miR-128-3p expression was also significantly higher in the gingival tissue and plasma of the LPS group. The mRNA levels of interleukin -1β, tumor necrosis factor, C-X3-C motif chemokine ligand 1, and C-X-C motif chemokine ligand 7 were elevated in the kidneys of the LPS group. Conversely, the potential target genes of activin A receptor type I (*Acvr1*), ribosomal protein S6 kinase B1 (*Rps6kb1*), and transforming growth factor beta receptor type 1 (*Tgfbr1*) were significantly decreased in the kidneys of the LPS group.

Conclusions: This study found that EVs-derived miR-128-3p in LPS-induced periodontitis may cause kidney inflammation, potentially mediated by *Rps6kb1*, *Tgfbr1*, and *Acvr1* through the TGF-β signaling pathway. The new finding suggests that miR-128-3p is a potential target for investigating the link between periodontitis and kidney inflammation.

This dissertation has already been published in "Dentistry Journal" (Basel) and is recognized internationally.Therefore, the defense committee hereby approves this article as a doctoral dissertation in Dentistry.