ABSTRACT

We analyzed bacterial modulation of miRNAs in bone-marrow-derived macrophages (BMMs) induced by infection with either wild type Porphyromonas gingivalis (Pg) or mutant Pg (∆FimA), through a microarray analysis. TNF-α and IL-10 concentrations in Pg infected BMMs transfected with selected miRNAs were also assessed. The inhibition of mmu-miR-2137 increased the secretion of anti-inflammatory IL-10, while mmu-miR-155-5p decreased TNF-α. In vivo: injecting these miRNAs with Pg in mice reduced the size of the lesion significantly.

INTRODUCTION

Periodontitis (PD) is a common chronic inflammatory disease inducing tooth-supporting tissue destruction (1) related to the dysbiosis of periodontal microbiota, associated with Pg. Macrophages are directly in contact with infectious agents or their byproducts (2). miRNAs are involved in many physiological and pathological processes (3). The expression and role of miRNAs in inflammatory and infectious diseases are not fully understood yet.

AIM OF THE STUDY

Finding miRNAs that could be a potential therapeutic target and analyzing their biological effects. Particularly, the ones that affect the immune host-response in relation to Pg infection.

METHODS

Wild type and mutant Pg was utilized to infect BMMs. In the animal module only wild type tested. (7) In calvarial bone-resorption mouse module, effect of two miRNA was confirmed after Pg infection. (6) ELISA technique were used to assess the level of cytokines in BMMs infected with Pg. (6) Marine bone marrow drive macrophages were collected for the study. L929 (M-CSF) stimulate hematopoetic stem cell into macrophages. (4) miRNA expression monitored using microarray analysis. (5)
Identification of miRNA differentially expressed in macrophages exposed to Porphyromonas gingivalis infection

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RESULTS

CONCLUSIONS

Our results indicate miRNA-155 and miRNA-2137-inhibitor could contribute to the pathogenicity of Pg in PD. This is supported by the decrease in TNF-α secretion when BMMs were transfected with this miRNA in vitro, and the reduction of inflammation in vivo. Inhibition of miRNA-2137 in vitro increased IL-10 secretion. More importantly, the inhibition of miRNA2137 lead to significantly reduced inflammation in vivo.

Further research in this field may potentially lead to the identification of new therapeutics miRNA aiming to reduce the manipulation of invasive bacteria on host-immune, which in turn reduce bacterial survival.

REFERENCES

Figure 1: Heatmap of differentially expressed miRNA of all of the conditions tested. Red and blue indicate $z$-scores of $\geq 2$ or $\leq -2$, respectively, and white indicates a $z$-score of 0 (row-wise mean). $p$ and $q \leq 0.001$. 
**Figure 2: TNF-α.** Dosage of TNF-α in supernatants of transfected BMMs infected with *Pg* at 24h. *p*<0.05. All *in vitro* experiments were performed in triplicate.

**Figure 3: IL-10.** Dosage of IL-10 in supernatants of BMMs infected with *Pg* at 24h. *p*<0.05. All *in vitro* experiments were performed in transfected triplicate.
Figure 4: Size of Lesions. Size of calvarial lesions 7 d after *P. g.* (5x10^8 CFU) infection and the injection of the mmu-miR-155-5p mimic and mmu-miR-2137 inhibitor. *p<0.05, n=4.*
Figure 5: Histological Sections. Representative samples for skin & underlying calvarial bone at the middle of the lesion from each of the following groups: *Pg* (A-C); *Pg* + combination group (D-F); *Pg* + anti-miRNA-2137 (G-I); and *Pg* + miR 155 (L-N). TRAP staining for bone (bottom row, 200x) and H&E staining for the skin (Top row, 100x; middle row, 200x). Arrows indicate TRAP stained multi-nucleated osteoclasts attached to the bone and bone resorption lacunae.