Comprehensive Analysis of the Microbiome of the Horny Plug, and Interaction of *Malassezia* Species and *Propionibacterium acnes* in Keratinocytes

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Introduction

Both *Malassezia* and *Propionibacterium acnes* are found in the horny plug and the overgrowth of these microorganisms can result in inflammation. A better understanding of the microbiome of the horny plug would thus provide insights into the pathology of *Malassezia* and *P. acnes*. Although *P. acnes* is known to induce the production of proinflammatory cytokines by keratinocytes *in vitro*, the mechanisms underlying the regulation of *P. acnes*-induced inflammatory responses are unclear.

In this study, the microbiome of the horny plug was analyzed comprehensively and the effect of *Malassezia* species and *P. acnes* on cytokine production by keratinocytes (NHEK cells) investigated.

Materials and Methods

1) Sample collection

One hundred horny plug samples were collected from 10 healthy Japanese individuals (6 males and 4 females).

2) Microbiota analysis

3) Quantitative analysis

The DNA of *Malassezia* species and *P. acnes* was quantified using genus specific primers and TaqMan probes.

4) Enzyme-Linked immunosorbent assay

Malassezia species and *P. acnes* were cultured in the presence or absence of a keratinocyte cell line (NHEK), and the production of interleukin (IL)-6, IL-1α, IL-8 and tumor necrosis factor (TNF)-α by the keratinocytes was examined.

Distribution of fungal and bacteria microbiota in plug

Fungal microbiota

Bacterial microbiota

PCoA analysis

Localization of fungal and bacteria in plug

Quantitative real-time PCR analysis

Malassezia was located on the outside of the plug, and *P. acnes* throughout the plug.

Malassezia increase *P. acnes*-induced inflammatory Response of keratinocyte

Conclusion

- ◆ *M. restricta* predominated in the plug fungal microbiota, followed by *M. globosa*.
- ◆ *P. acnes* predominated in the plug bacterial microbiota, followed by *Bifidobacterium* and *Staphylococcus*.
- ◆ *Malassezia* was mainly located on the outside of the plug, and *P. acnes* throughout the plug.
- ◆ *Malassezia* increase *P. acnes* induced IL-6, IL-8 and IL-1α production by keratinocytes.