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研究課題名（和文）基底膜による皮膚細菌叢の制御機構の解明

研究課題名（英文）Modulation of skin microbiota through basememt membrane

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研究成果の概要（和文）：皮膚基底膜蛋白欠損マウスの皮膚細菌叢を解析した。生後1日目の段階では、対照群のマウスでむしろ皮膚細菌量が多く、生後4日目では皮膚基底膜蛋白欠損マウスとその対照群の皮膚細菌量が同等であることが確認された。FISH法を用いた皮膚細菌叢の可視化実験では、皮膚基底膜蛋白欠損マウスとその対照群ともに主に表皮の角層において細菌叢が確認された。陰性コントロールとして用いた、皮膚に存在しない真菌のプローブによるFISH法ではシグナルを認めなかった。今後は、長期生存する皮膚基底膜蛋白欠損マウスの皮膚や表皮水疱症患者皮膚を用いてさらに皮膚細菌叢の解析を進める必要がある。

研究成果の概要（英文）：Skin microbiota of mice deficient for skin basement membrane protein (BMZ-deficient mice) were analyzed. At P1, BMZ-deficient mice had less abundant skin microbiota compared with the control group mice while the quantity of skin bacteria was comparable between BMZ-deficient mice and the control mice at P4. Visualization of skin microbiota using FISH methods revealed that bacterial signals were located mainly in the cornified layers of BMZ-deficient mice and the control mice. The specificity of the probe to detect bacterial signals was confirmed by the negative FISH signals using a probe reacting with fungal species which are not found in skin. Further analyses will be done on microbiota of adult BMZ-deficient mice skin and human epidermolysis bullosa patients' skin.

研究分野：医歯薬学

科研費の分科・細目：内科系臨床医学・皮膚科学

キーワード：皮膚細菌叢

１．研究開始当初の背景

表皮基底膜は、表皮と真皮をつなぐ重要な生体装置であり、この構成蛋白の先天的な欠損によって表皮水疱症が引き起こされる。表皮水疱症患者では、皮膚細菌感染が高頻度で発生することが知られており、皮膚細菌叢の動態が異なっている可能性があると想定されていた。

２．研究の目的

表皮基底膜蛋白欠損によって、皮膚細菌叢の動態が変化するかどうかを明らかとすること。

３．研究の方法

当研究室で樹立した表皮基底膜蛋白の一つである 17 型コラーゲン（COL17）のノックアウト（KO）マウスとその同胞である KO ヘテロマウスを用いて下記の実験を計画した。

（１）皮膚からの細菌叢抽出

（２）抽出した細菌叢を用いて、細菌叢に共通した 16srRNA 遺伝子プライマーによる定量 PCR を施行し、これによって細菌叢の総量を測定する

（３）Fluorescent in situ hybridization による皮膚細菌叢の可視化

４．研究成果

生後１日目の段階では、COL17KO ヘテロマウスのほうが COL17KO マウスよりも皮膚細菌量が多く（図 1）、P4 と P7 では両者で皮膚細菌量が同等であることが確認された（図 2、図 3）。Fluorescent in situ hybridization を用いた皮膚細菌叢の可視化実験では、COL17KO マウス、COL17KO ヘテロマウスともに主に表皮の角層において細菌叢が確認された。陰性コントロールとして用いた、皮膚に存在しない真菌のプローブではシグナルを認めなかった。今後は、長期生存する COL17KO マウスの皮膚や表皮水疱症患者皮膚を用いてさらに皮膚細菌叢の解析を進める必要がある。

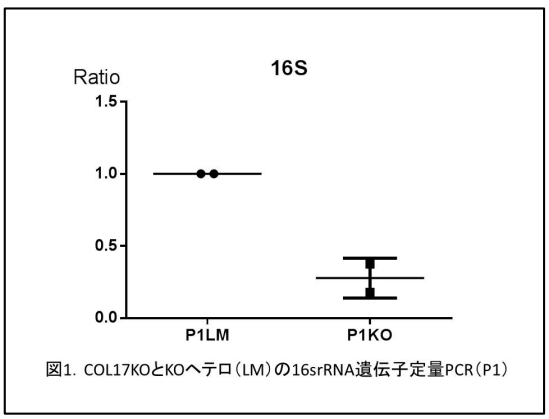

図1. COL17KOとKOヘテロ（LM）の16srRNA遺伝子定量PCR（P1）

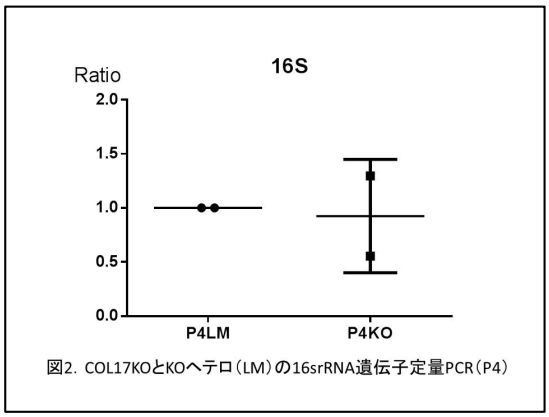

図2. COL17KOとKOヘテロ（LM）の16srRNA遺伝子定量PCR（P4）

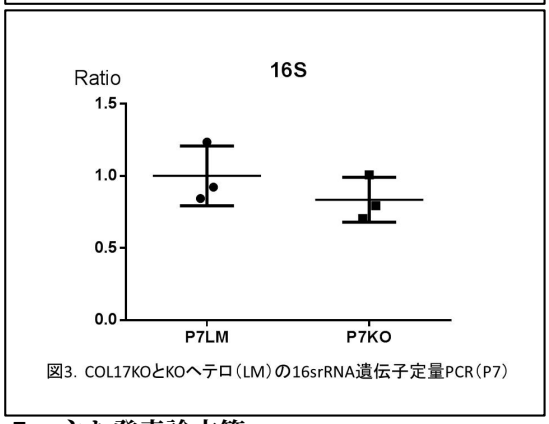

図3. COL17KOとKOヘテロ（LM）の16srRNA遺伝子定量PCR（P7）

５．主な発表論文等

（研究代表者、研究分担者及び連携研究者には下線）

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〔産業財産権〕
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