氏　　名	NGUYEN THI THU HA
授与した学位	博　士
専攻分野の名称	歯　学
学位授与番号	博甲第６１５９号
学位授与の日付	令和２年３月２５日
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	Type XVIII Collagen Modulates Keratohyalin Granule Formation and Keratinization in Oral Mucosa （XVIII 型コラーゲンは口腔粘膜においてケラトヒアリン顆粒の形成と角化を制御する）
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学位論文内容の要旨

Introduction: Epithelial keratinization involves complex cellular modifications that provide protection against pathogens and chemical and mechanical injuries. In the oral cavity, keratinized mucosa is also crucial to maintain healthy periodontal or peri-implant tissues. Basement membrane (BM) which is a thin layer between epithelial cells and underlying connective tissue has been demonstrated to not only provide mechanical support and divide tissues into compartments, but also decisively influence cellular behavior. Our previous study indicated that the type IV collagen α6 chain, one major BM component, plays essential roles in the keratinization of oral mucosal epithelial cells. Based on these studies, it is highly possible that other BM constituents could also play important roles in oral mucosal epithelial keratinization. In this study, we hypothesized that type XVIII collagen, a collagen-glycosaminoglycan featuring an extracellular matrix component present in the basement membrane, could contribute to the regulatory role of the BM on the keratinization of oral mucosa. The aims of this study were to investigate the difference of the distribution of type XVIII collagen between the keratinized and non-keratinized mucosa and to clarify the role of type XVIII collagen in oral mucosa epithelial keratinization by analysis of the effects of *COL18A1* gene down-regulation *in vitro* using TR146 cells, and *Col18a1* gene deletion *in vivo* using a *Col18a1*-knockout (KO) mouse.

Methods: Expression of type XVIII collagen in basement membrane of keratinized mucosa and non-keratinized mucosa was investigated by immunohistochemical (IHC) staining in wild-type (WT) mice. Additionally, a three-dimensional culture system using human squamous carcinoma cells (TR146) was used to evaluate and correlate changes in the expression of *COL18A1* and epithelial keratinization-associated genes, e.g., *Keratin-1* (*KRT1*) and *Keratin-10* (*KRT10*). Differences in the histological structure and ultra-structure of keratinized mucosa between WT and *Col18a1*-KO mice were evaluated by IHC staining and transmission electron microscopy, respectively. Each experiment was repeated independently at least three times.

Results: Histological analysis of keratinized and non-keratinized mucosa showed that type XVIII collagen was observed in both BMs of both tissue; however, interestingly it was highly expressed in keratinized mucosa. *In vitro* results showed that *COL18A1* gene expression increased significantly after 3 days and followed the concomitant increase in *KRT1* and *KRT10*

mRNA levels after 7 days (p<0.05, one-way ANOVA, Turkey multiple comparison test, n=3). Additionally, loss-of-function analyses using silencing RNA targeting *COL18A1* mRNA could induce a dramatic decrease in the expression of keratinization-associated genes (*KRT1* (60%), *KRT10* (30%), *Involucrin* (30%) (p<0.01, Student's t-test, n=3). Moreover, the deletion of *Col18a1 in vivo* led to an inadequate keratinization phenotype of oral mucosa in the *Col18a1*-KO mice. In details, IHC and quantitative analysis of KRT10 expression in the keratinized gingiva from WT and *Col18a1*-KO mice revealed that the percentage of positive signal area for KRT10 in the oral mucosa epithelium was significantly weaker and sparser and two-fold lower in *Col18a1*-KO mice than in the WT mice (p<0.05, Student's t-test, n=3). The lack of type XVIII collagen could also lead to the marked decrease in the number and size of keratohyalin granules (four-fold smaller) in the granular layer of the epithelium of *Col18a1*-KO mice (p<0.001, Student's t-test, n=4).

Conclusion: Together, the results of this study demonstrate that type XVIII collagen is a modulator of keratohyalin granule formation and oral mucosal keratinization.

論文審査結果の要旨

Epithelial keratinization involves complex cellular modifications that provide protection against pathogens and chemical and mechanical injuries. In the oral cavity, keratinized mucosa is also crucial to maintain healthy periodontal or peri-implant tissues. Basement membrane which is a thin layer between epithelial cells and underlying connective tissue has been demonstrated to not only provide mechanical support and divide tissues into compartments, but also decisively influence cellular behavior, therefore it could also play important roles in oral mucosal epithelial keratinization. In this study, we investigated the roles of type XVIII collagen, a collagen-glycosaminoglycan specifically present in the basement membrane, in oral mucosal keratinization.

Expression of type XVIII collagen in basement membrane of keratinized mucosa and non-keratinized mucosa was investigated by immunohistochemical (IHC) staining in C57BL/6J mice (wild-type, WT). Additionally, a three-dimensional culture system using a human oral squamous carcinoma cell line (TR146) was used to evaluate and correlate changes in the expression of *COL18A1* and epithelial keratinization-associated genes, e.g., *Keratin-1* (*KRT1*) and *Keratin-10* (*KRT10*). Chimeric mice with knocked-out (KO) *Col18a1* gene were backcrossed with C57BL/6J for over fifteen generations to generate the C57BL/6J inbred knockout lines (or *Col18a1*-KO mice). Differences in the histological structure and ultra-structure of keratinized mucosa between WT and *Col18a1*-KO mice were evaluated by IHC staining and transmission electron microscopy, respectively.

Histological analysis of keratinized and non-keratinized oral mucosa showed that type XVIII collagen was highly expressed in keratinized mucosa. *In vitro* results showed that *COL18A1* expression increased significantly after 3 days and followed the concomitant increase in *KRT1* and *KRT10* mRNA levels after 7 days. Additionally, loss-of-function analyses using silencing RNA targeting *COL18A1* mRNA could induce a dramatic decrease in the expression of keratinization-associated genes (*KRT1* by 60%, *KRT10* by 30%, *Involucrin* by 30%). Moreover, the deletion of *Col18a1 in vivo* led to an inadequate keratinization phenotype of oral mucosa in the *Col18a1*-KO mice, as demonstrated by a two-fold suppressed KRT10 expression and remarkably smaller size of keratohyalin granules (four-fold smaller) in the granular layer of epithelial tissue in those mice.

Together, the results of this study demonstrate that type XVIII collagen is a modulator of keratohyalin granule formation and oral mucosal keratinization.

These findings are novel in oral biological science, providing useful knowledge that may promote the advance in regenerative dentistry. Therefore, the dissertation examining committee acknowledged the value of this thesis as a doctoral dissertation.