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研究課題名（和文）エピゲノム解析及び核内３次元構造解析による上皮細胞バリア機能の分子基盤解明

研究課題名（英文）Role of the epigenome and 3D nuclear dynamics in maintenance of skin barrier function

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研究成果の概要（和文）：上皮細胞の持つバリア機能は皮膚の向上性を維持し、細菌感染などから体内を防御する。バリア機能の破綻は、アトピー性皮膚炎（AD）などの疾患の要因となる。そのため、バリア機能を制御する分子メカニズムの解明は、上皮の生物学的な機能を理解することのみならず、ADなどの疾患の治療法開発にも役立つと期待できる。本研究では、ADに類似した表現型を示すマウスモデルを用い、上皮細胞の機能を制御する遺伝子群を同定した。さらに、これらに遺伝子の制御にH3K27me3を介したエピゲノム機構やそれと連動した3次元核内構造の変化が深く関与していることも発見した。

研究成果の学術的意義や社会的意義
My study shows that the barrier function of the epidermis is mediated by the expression a set of genes involved in immune response and cellular differentiation. Gene expression changes are regulated by changes in epigenetic marks (H3K27me3) and 3D nuclear domain.

研究成果の概要（英文）：Epidermis is the outermost layer of the skin that possess dual roles to protect the body from the outer environment (e.g. bacterial infection, temperature, dryness) and maintain of skin homeostasis. Specialized cell layers, namely, the basal layer and the cornified layer, plays a crucial role for regulation of epidermal functions. The molecular mechanisms that mediate the balance and function of these cell layers, however, remain unclear.
To elucidate how epigenetic mechanisms and 3D genome regulation changes between healthy or disease states epidermal cells, I have taken advantage of animal model systems. Using these systems, I have found that the onset of disease phenotype is associated with expression changes of genes involved in inflammation, immune response and keratinocyte differentiation. Importantly, these changes were regulated by H3K27me3 in an epigenetic level, and a shift from B-compartment (repressive state) to A-compartment (active state) revealed by Hi-C analysis.

研究分野：Epigenetics

キーワード：Epidermis　Keratinocyte　Epigenetics　3D nuclear domain　Gene expression

１．研究開始当初の背景

Epidermis is the outermost layer of the skin that possess dual roles to protect the body from the outer environment (e.g. bacterial infection, temperature, dryness) and maintain of skin homeostasis. Specialized cell layers, namely, the basal layer and the cornified layer, plays a crucial role for regulation of epidermal functions. The molecular mechanisms that mediate the balance and function of these cell layers, however, remain unclear. As disruption of the balance between basal and cornified layers has been implicated by human diseases such as atopic dermatitis (AD), it should be important to clarify the molecular mechanisms behind these regulation for understanding the biology of the epidermis and also for designing therapeutic intervention of skin diseases such as AD.

２．研究の目的

Biological phenomena such as cellular differentiation (e.g. differentiation of epidermal basal layer to cornified layer) are regulated by changes in gene expression. These changes, in turn are mediated by higher order regulatory mechanisms such as 3D genome organization, and epigenetic changes imparted via DNA methylation and histone modifications. The objective of this research is to elucidate how 3D nuclear genome architecture and epigenetic mechanisms regulate epidermal functions.

３．研究の方法

To elucidate how epigenetic mechanisms and 3D genome regulation changes between healthy or disease states epidermal cells, I have taken advantage of two animal model systems. The first is a knockout mouse model, in which the Tmem79 gene has been genetically deleted, that shows atopic dermatitis-like phenotypes characterized by skin inflammation and infection. The second is a contact-dermatitis model, in which epidermal inflammation is induced by topical application of a chemical irritant (i.e. hapten). I have purified keratinocytes from healthy (WT or untreated) or disease state (Tmem79-KO or hapten-treated) and performed RNA-seq to reveal the gene expression changes between healthy or disease states. I found that nearly 1000 genes, involved in immune response and keratinocyte differentiation were upregulated in the disease state. Differentiation associated genes are regulated by polycomb group (PcG) of factors. PcG factors mediate methylation of histone H3 lysine 27 (H3K27 mono/di/tri methylation), that contributes to gene silencing via repressive 3D nuclear domains. To ask whether gene expression changes were regulated by changes in the level of H3K27 methylation, I performed ChIP-seq of H3K27me3 in keratinocytes. I further asked if potential changes in H3K27me3 could alter 3D nuclear architecture by performing Hi-C.

４．研究成果

RNA-seq analysis revealed that in the disease state, genes that possess inflammation associated or immune regulatory functions are upregulated. I also found that differentiation associated genes were also upregulated in the disease state. As mentioned above, the Tmem79-KO mice or the hapten-treated mice, both exhibit inflammation related phenotypes which is likely caused by increased immune response. Therefore, the gene expression changes detected by RNA-seq in disease state keratinocytes recapitulate those cellular phenotypes. Further, disruption of the balance between progenitor (keratinocyte) and differentiated (cornified layer) cells is a hallmark of skin diseases such as atopic dermatitis (AD). Therefore, the finding that differentiation associated genes are upregulated in disease state keratinocytes, indicate that Tmem79-KO or hapten-treated mice exhibit aberrant cellular differentiation in the epidermis, reflecting the disease onset mechanism observed in AD.

ChIP-seq analysis for H3K27me3 showed that this epigenetic mark was enriched in the promoter of immune- and inflammation-related genes, and also differentiation-related genes in healthy state keratinocytes. Interestingly, in the disease state (Tmem79-KO or hapten-treated), H3K27me3 levels were decreased in the promoter of the genes that showed transcriptional upregulation. This indicates that H3K27me3, mediated by PcG factors, silence these genes in the healthy state, which are aberrantly upregulated in association with the onset of disease phenotype. Thus, my study uncovers a new role for epigenetic modifications (i.e. H3K27me3) for regulation of epidermal cells in healthy or disease states.

Finally, I asked if H3K27me3 changes in the disease state, were also linked with 3D nuclear architecture changes. To this end, I performed Hi-C in healthy or disease state keratinocytes.

The Hi-C data analysis is still ongoing but the preliminary results show that in the healthy state H3K27me3-marked genes are located in the repressive B-compartment (calculated by PCA1 of the Hi-C data), but shifts to the permissive A-compartment in the disease state. Taken together, these results indicate a model in which epidermal cell functions, especially barrier functions, are compromised with the onset of disease. Notably, disruption of cellular functions appears to be mediated by epigenetic changes and 3D nuclear domain changes.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　0件）

１．著者名 Matsui T, Kadono-Maekubo N, Suzuki Y, Furuichi Y, Shiraga K, Sasaki H, Ishida A, Takahashi S, Okada T, Toyooka K, Sharif J, Abe T, Kiyonari H, Tominaga M, Miyawaki A, Amagai M.	４．巻 118
２．論文標題 A unique mode of keratinocyte death requires intracellular acidification	５．発行年 2021年
３．雑誌名 Proc Natl Acad Sci U S A.	６．最初と最後の頁 e2020722118
掲載論文のDOI（デジタルオブジェクト識別子） 10.1073/pnas.2020722118	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関