様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Determination of the functions of specific CD44 proteins in human skin and epidermis and their relation to skin diseases, cancer and autoimmunity

研究課題名（英文）Determination of the functions of specific CD44 proteins in human skin and epidermis and their relation to skin diseases, cancer and autoimmunity

研究代表者
ＴＥＹＥ　ＫＷＥＳＩ（TEYE, Kwesi）

久留米大学・付置研究所・助教

研究者番号：３０５９９３０３

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研究成果の概要（和文）：本研究では皮膚の表皮ケラチノサイトにおいて、CD44の18種類の転写を初めて同定し、免疫染色で様々な皮膚癌、特に有棘細胞癌でCD44の発現が低下していることを明らかにした。乾癬やアトピー性皮膚炎のような炎症性疾患では、CD44の発現は低下していなかった。CRISPR/Cas9システムを用いてCD44の発現をなくしたケラチノサイトのRNAをマイクロアレイで解析し、CD44の発現をなくしたケラチノサイトでは、人皮膚の構造を保つ上で重要な働きをするラミニンの3つのサブユニットの発現がすべて減少していることを明らかにした。またCD44を有さないケラチノサイトは移動が遅くなることも明らかにした。

研究成果の学術的意義や社会的意義
CD44の皮膚での役割はよく分かっていない。本研究ではCD44の発現が有棘細胞癌で減少していること、CD44の発現をなくした表皮細胞において、皮膚の構造を保つ上で重要な働きをするラミニンの3つのサブユニットの発現がすべて減少していることを明らかにした。またCD44を有さない表皮細胞は移動が遅くなることも明らかにした。以上の結果からCD44は創傷治癒において重要な役割を果たしている可能性が示唆された。CD44はある種の皮膚疾患において、治療の標的になり得るかもしれない。

研究成果の概要（英文）：We studied functions and roles of CD44 proteins in human skin. We for the first time identified 18 transcripts of CD44 in epidermal keratinocytes and studied their roles in skin development. To gain insight into CD44 function, we performed immunostaining of normal skin and various skin diseases. We found decreased expression of CD44 variants in various skin cancers, particularly squamous cell carcinoma but not in inflammatory diseases psoriasis and atopic dermatitis. Using CRISPR/Cas9 system, we successfully disrupted CD44 expression in keratinocyte cell line and performed Microarray analysis using RNA from cells with and without CD44. We found that expression of many important genes are controlled by CD44. Importantly, we found that expression of all 3 subunits of a certain laminin, which is important for maintaining structural integrity of human skin, are reduced in cells without CD44. Cells without CD44 migrated slower. Our study revealed important functions of CD44 in human skin.

研究分野：Dermatology, Oncology, Molecular Biology

キーワード：CD44　Skin　Skin disease　Keratinocyte　Squamous cell carcinoma　migration

様　式　C－１９、F－１９－１、Z－１９、CK－１９（共通）

１．研究開始当初の背景

CD44 is a cell surface proteoglycan implicated in multiple cell functions, including adhesion, migration, activation, recirculation and homing of lymphocytes, hematopoiesis, tight-junction assembly and tumor metastasis (Gunthert *et al.*, 1991; Kirschner *et al.*, 2011; Nagano and Saya, 2004; Ponta *et al.*, 2003). CD44 is recognized as the principal receptor for hyaluronic acid (HA) (Aruffo *et al.*, 1990) and CD44-HA interactions have been demonstrated to affect many physiological processes in normal and diseased states (Ahrens *et al.*, 2001; Bourguignon, 2014; Hiraga *et al.*, 2013; Miyake *et al.*, 1998). Transcripts of *CD44* undergo complex alternative splicing of at least 9 of the 18 coding exons, resulting in many functionally distinct isoforms (Screaton *et al.*, 1992). To date, however, the characteristics and pathological roles of different CD44 variants have not been extensively studied in most tissues including skin. Thus many aspects of the functions CD44 are yet to be fully addressed and therefore a more thorough investigation of the roles of these CD44 variants are required to fully understand CD44 function both in normal and disease states.

２．研究の目的

Many aspects of CD44 are still lacking because alternative splicing results in nearly 800 functionally distinct proteins whose expression and function are not well understood. Having cloned and designated all 18 transcripts of *CD44* found in human epidermis and skin, the aim of this project is to determine the functions of CD44 proteins in human skin and epidermis and to determine the role of CD44 molecules in normal and disease skin development.

３．研究の方法

(1) Immunofluorescent staining of various skin tissues

FFPE sections were deparaffinized and dehydrated. After antigen retrieval, sections were blocked with 1% BSA/PBS for 30 min and incubated with 4 μg/ml of anti-CD44v6 monoclonal antibody in 1% BSA/PBS at room temperature for 1 hour. After washing, sections were incubated with Alexa Fluor 488-conjugated anti-mouse IgG (Invitrogen) diluted 1: 1000 at room temperature for 1 hour.

(2) Disruption of CD44 expression in human keratinocytes

Cultured human keratinocytes were transfected with plasmid expressing gRNA against exon 2 of CD44 and CRISPR/Cas9 protein along with a plasmid carrying hygromycin resistant gene as well as CD44 homolog sequences around exon 2. Cells were selected with 20ug/ml hygromycin and colonies were screened for absence of CD44 expression by Western blotting or immunofluorescence. Cells without CD44 expression were used in this study.

(3) RNA isolation, Microarray analysis and RT-qPCR

Both cells with and without CD44 were cultured in 6 well plates coated with collagen type I. When the cells reached 80-90% confluency, RNA was isolated and submitted for Microarray analysis. cDNA was synthesized with SuperScript III First-Strand Synthesis System (Invitrogen) and quantitative PCR were performed with various primers to determine gene expression in response to loss of expression of CD44.

(4) Cell migration assay

Cells with or without CD44 were cultured in 6 well plates coated with collagen type I. When the cells reached 90-100% confluency, the plastic surface was scratched with 200ul pipette tip. Cell migration to fill the scratched area was monitored and photos were taken after a certain period of time.

４．研究成果

(1) **Differential expression of CD44 in skin with various conditions**

We investigated the expression of a CD44 variant in skin with various conditions. We found that CD44v expression is reduced in BCC and SCC (Fig1). The expression was patchy in EMPD and was slightly increased in atopic dermatitis and psoriasis. The analysis suggested that deregulated expression of CD44 may be associated with some skin disease development and CD44 may be an important protein in human skin.

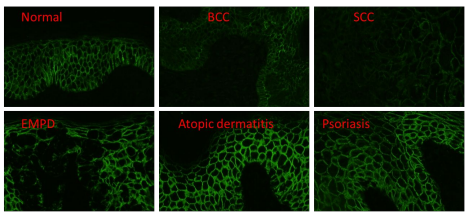

Fig 1. Detection of CD44 variant expression in skin with various conditions

(2) Generation of keratinocytes without CD44

Next, we successfully established human keratinocyte cell line in which CD44 expression was completely disrupted by CRISPR/cas9 system. Analysis by Immunofluorescence revealed that the cells have completely lost expression of CD44 (Fig2).

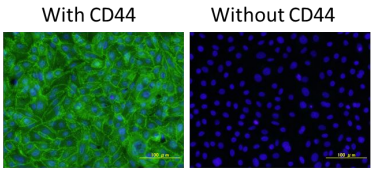

Fig2. Analysis of CD44 in Keratinocytes with (Left) or without (Right) CD44

(3) Results of Microarray analysis

We performed Microarray analysis to determine if loss of CD44 will affect gene expression. Microarray analysis revealed that out of 13799 successful detected genes, 251 show increased expression and 588 showed decreased expression in cells without CD44 (Fig3), as compared with cells with CD44. We then focused on important skin genes that may be controlled by CD44. We found that cells without CD44 showed reduced expression of all 3 subunits of a certain laminin (Fig4, C, I and K), which is important in keeping the skin healthy. Importantly, expressions of other laminins were not affected by loss of CD44 (Fig4), as also confirmed by qPCR, suggesting that the effect is specific.

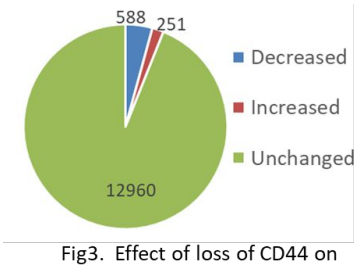

Fig3. Effect of loss of CD44 on expression of human genes

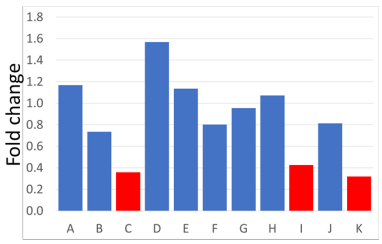

Fig4. Effect of loss of CD44 on expression of human laminins

(4) Effect of loss of CD44 on cell migration

We performed cell migration assay using cells with or without CD44. We found that loss of CD44 leads to delayed migration of human epidermal keratinocytes (Fig5), which is important in wound healing.

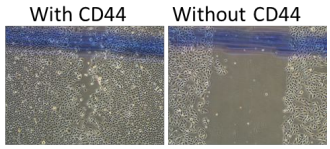

Fig 5. Migration of cells with (Left) and without (Right) CD44

Our study revealed that CD44 proteins have important functions in human skin and loss of CD44 leads to reduced expression of a certain important laminin and may lead to development of certain skin diseases and delayed wound healing due to delayed cell migration. Consequently, CD44 may be used as a target for therapy in certain skin conditions.

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〔図書〕（計 0 件）

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名称：
発明者：
権利者：
種類：
番号：
出願年：
国内外の別：

○取得状況（計　0　件）

名称：
発明者：
権利者：
種類：
番号：
取得年：
国内外の別：

〔その他〕
ホームページ等

６．研究組織

(1)研究分担者
研究分担者氏名：名嘉眞　武国
ローマ字氏名：(NAKAMA, Takekuni)
所属研究機関名：久留米大学
部局名：医学部
職名：教授
研究者番号（8 桁）：50221453

研究分担者氏名：沼田　早苗
ローマ字氏名：(NUMATA, Sanae)
所属研究機関名：久留米大学
部局名：医学部
職名：助教
研究者番号（8 桁）：40599312

研究分担者氏名：石井　文人
ローマ字氏名：(ISHII, Norito)
所属研究機関名：久留米大学
部局名：医学部
職名：准教授
研究者番号（8 桁）：80330827

(2)研究協力者
研究協力者氏名：
ローマ字氏名：

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