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学位(専攻分野) 博士(理学)

学位記番号 総研大甲第 2096 号

学位授与の日付 平成 31年 3 月 22 日

学位授与の要件 先導科学研究科 生命共生体進化学専攻
学位規則第6条第1項該当

学位論文題目 Genetic basis of human-specific skin characteristics in
comparison with other primates

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博士論文の要旨

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論文題目

Genetic basis of human-specific skin characteristics in comparison with other primates

霊長類におけるヒト特異的皮膚形質の遺伝的基盤について

Skin is the largest organ of a body and located at interface between the inside and outside of an organism. It protects the inside of the body from external stresses, such as physical, chemical, and microbial insults. Skin phenotypes have evolved to protect the body and to allow the species to adapt to their habitat environments. Actually, human skin is morphologically and physiologically different from the skin of other primates. The reduced amount of hair and the high number of sweat glands are well-known examples of human-specific skin characteristics. It has been proposed that these human-specific characteristics allowed for efficient thermoregulation and adaptation to the savannah environment after our human ancestors abandoned the forest.

There are many phenotypic characteristics that are unique to the human lineage, including large brain size, bipedalism, and language development as well as skin characteristics. However, genetic causes that underlie human-specific characteristics remain poorly understood. Because skin phenotypes have evolved to protect the inside of the body and to adapt the species to their habitat environments, human-specific skin characteristics are likely to have significant roles in human evolution. In my PhD thesis, I therefore focused on human-specific skin characteristics and studied the genetic basis that underlies these characteristics.

Chapter 1 is a general introduction. I briefly explain the function of skin to protect the inside of the body from external environments. I also describe the main structure of mammalian skin including the epidermis, dermis, subcutaneous tissue, and epidermal basement

membrane (BM) zone. Especially, I focus on the epidermal BM zone, which forms adhesion between the epidermis and dermis, for better understanding of the subsequent parts in this thesis. In addition, I provide overviews of representative cases of the association between genetic elements and human-specific characteristics.

In chapter 2, I quantitatively distinguished histological skin differences between humans and other primates to investigate human-specific characteristics in skin structure. I found that the epidermis and dermis in human skin were significantly thicker than those in the three Old World monkey species examined. I also indicated that the epidermal BM zone topography in humans was undulating, which is known as a rete ridge, while that in the three Old World monkey species was flat. These results, together with previous qualitative studies, suggest that the thicker epidermis and rete ridge may be human-specific skin characteristics, although additional quantitative histological comparison between human and great ape skin is required.

In chapter 3, I then comprehensively compared gene expression levels between human and great ape skin using next-generation cDNA sequencing (RNA-seq) to investigate genes associated with human-specific skin characteristics. I found that the expression levels of four structural protein genes, *biglycan (BGN)*, *collagen type XVIII alpha 1 chain (COL18A1)*, *CD151 molecule (CD151)*, and *laminin subunit beta 2 (LAMB2)*, in skin were significantly higher in humans than in great apes. *COL18A1*, *LAMB2*, and *CD151* are genes that encode proteins structurally associated with the epidermal BM zone. BGN regulates the formation of elastin, which is one of the components of elastic fibers in the dermis. According to previous studies of qualitative histological comparison between human and other primate skin, an abundance of elastic fibers seems to be human-specific skin characteristics. The human-specific expression patterns of the four structural protein genes identified may contribute to the rete ridge formation and rich elastic fibers in human skin.

Humans have a low amount of hair on their body compared with other primates, which gives humans a high level of thermoregulation. However, it is believed that human skin

has lost the ability to protect the internal tissues from external physical stresses by hair. Compared to flat topography of the epidermal BM zone, a rete ridge increases the area where the epidermis and dermis connect, which may make strong adhesion between these two layers. The rete ridge, thick epidermis, and rich elastic fibers might contribute to physical strength of human skin. Although additional quantitative histological comparison between human and great ape skin is required to clarify human-specific skin characteristics, the human-specific expression patterns found in this chapter may contribute to adaptive skin characteristics specific to humans with less hair.

In chapter 4, I inferred substitutions responsible for the human-specific expression patterns of the four structural protein genes (*COL18A1*, *LAMB2*, *CD151*, and *BGN*) in their transcriptional regulatory regions. I first estimated transcriptional regulatory regions for each gene by identifying conserved noncoding regions around the genes with taking histone modifications for active regulatory regions in skin cells into consideration. The human-specific substitutions in putative transcriptional regulatory regions were estimated to be candidate substitutions responsible for the human-specific expression patterns in the genes of interest, resulting in two to ten candidate substitutions for each of the genes. These candidate substitutions, especially those located in the expected binding sites of transcription factors functioning in skin, may give humans adaptive skin characteristics through human-specific gene expression patterns.

Chapter 5 is a general discussion. I suggest that the candidate substitutions in the putative transcriptional regulatory regions inferred may cause the human-specific gene expression patterns that possibly lead to adaptive skin characteristics specific to humans with less hair. In the near future, I am planning to conduct a promoter assay in cultured skin cells to examine whether these candidate substitutions are responsible for the expression differences between humans and great apes. In addition, for the candidate substitutions with expression changes in promoter assay, I will investigate whether these substitutions influence the expression of the genes of interest, but not other genes, in cultured skin cells by genome editing

technique using CRISPR/Cas9 system. Identifying substitutions that may give humans adaptive skin characteristics through human-specific gene expression patterns will contribute to the understanding of how human-specific characteristics have been genetically acquired. Finally, I hope that my PhD study will provide further insight into the human evolution.

博士論文審査結果

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Title
論文題目 Genetic-basis of human-specific skin characteristics in comparison with other primates

ヒトは他の霊長類にない様々な形質を進化過程で獲得した。二足歩行、脳容量の拡大、言語の発達などと共にヒトにはヒト特有の皮膚構造がみられる。本論文で出願者は、他の霊長類との比較の中でヒトの皮膚の構造的・形態的特徴を明確にし、その上でヒトの皮膚において発現様式が異なっている遺伝子を網羅的に探査し、ヒト特異的な皮膚形質の獲得に至った進化遺伝学的な原因を追究した。主な結果は次のとおりである。

1) ヒト、アヌビスヒヒ、サイクスモンキー、ベルベットモンキーの皮膚切片を比較し、ヒトの皮膚はこれらの霊長類と比べ表皮および真皮に統計的に有意に厚みがあること、またヒトのみが表皮基底膜領域において表皮と真皮の境界面が波打つ表皮突起 rete ridge と呼ばれる特徴的な構造をもつことを示した。先行研究も考慮し、表皮の厚みや表皮突起の存在がヒトの皮膚の特異的形質であることを確認した。

2) ヒト、チンパンジー、ゴリラ、オランウータンの皮膚で発現している遺伝子を RNA-seq により網羅的に解析し 31 の遺伝子で発現量が有意に異なることを明らかにした。この中にはパイグリカン BGN、XVIII 型コラーゲンアルファ 1 COL18A1、CD151、ラミニン $\beta 2$ LAMB2 等の構造タンパク質をコードする遺伝子が含まれていた。また統計的に有意ではないものの表皮基底膜領域で発現するコラーゲンやパイグリカンと結合するコラーゲンも概して発現量が高い傾向にあることを示した。これらの遺伝子の発現の違いがヒトの表皮基底膜領域での特徴的な形質を生み出し、ひいてはヒトの皮膚の強度・弾力を高めることに関与している可能性を掲げた。

3) 塩基配列の保存性およびヒストンの修飾を考慮し BGN、COL18A1、CD151、LAMB2 の 4 遺伝子の転写活性に関わる領域を推定、さらにこれらの領域の中で生じたヒトの系統に特異的な塩基置換を検出した。in silico の解析で転写因子が結合する可能性のあることが類推されるこれらの塩基置換は、各遺伝子での転写制御に変化をもたらした可能性があり、今後の研究においてヒトの特有の皮膚構造をもたらした遺伝的要因の有力な候補とみなされるものである。

これらの結果は、ヒトに特有の皮膚の形態学的形質の獲得が King & Wilson が提唱したように主に遺伝子の転写制御の変化にあることを強く示唆するもので、今後その進化過程を探るための鍵となる情報を提供するものである。ヒトには様々な特徴的な形質があるものの、その多くの至近要因は明らかにされていない。本研究は生物学者のみならず多くの人々が興味を持つヒトの進化を探る中で重要な知見となる研究で、その学術的な意義は高いとみなされる。以上により、審査委員会は、本論文が学位の授与に値すると判断した。

(備考)

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