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Abstract of thesis

Hair plays an important role in individual's general appearance making hair problems, such as hair loss and hair graying, two of the major health and psychological concerns in the modern society. These disorders occur in both men and women independent of their age but the mechanism by which they occur is not well understood nor is there an available therapy that is without an unwanted side effect. At present, the US Food and Drug Administration have approved minoxidil and finasteride against hair loss but these drugs causes impotence, dizziness, unwanted grown hair, weakness, headache, skin rash and others. Developing an alternative therapy for promoting hair growth and pigmentation is therefore necessary.

Caffeoylquinic acid (CQA) is a phenylpropanoid compound exhibiting several beneficial properties including anti-oxidant, anti-allergic, neuroprotective, and melanogenesis-regulating effects. 3,4,5-tri-*O*-caffeoylquinic acid (TCQA) is a CQA derivative and chlorogenic acid (CGA) family member compound, that has a stable albumin affinity and is composed of multi-esters formed between quinic acid and three residues of trans-caffeicacids. TCQA has been found to induce powerful inhibitory activities against aldose reductase, hypertension, hyperglycemia, and Alzheimer's disease without unwanted secondary effects. Moreover, TCQA induces neurogenesis, improves learning and memory in aged mice, and promotes the

differentiation of human neural stem cells, however its effect on hair growth and hair pigmentation promotion has not yet been assessed. In this thesis, the author hypothesized that TCQA has hair growth and hair pigmentation effect and elucidated the underlying mechanism for the observed effect. The effect of TCQA on the induction of hair pigmentation and growth through exploring the possible common pathways such as Wnt/β-catenin were investigated.

To demonstrate the function of the hair follicle (HF) as it completes its regular cycle composed of anagen, catagen, and telogen phases and to answer the question whether TCQA has an effect on hair growth and pigmentation, an *in vivo* study was conducted in accordance with the guidelines of the Committee for the Ethical Use of Animals of the University of Tsukuba. C3H male mice (8-weeks-old) back was shaved and TCQA was applied topically for a month after which TCQA-treated mice were observed to have stimulated and accelerated anagen phase. In this part of the study, the author was able to demonstrate that TCQA significantly enhanced hair growth (~120%) *in vivo*. In contrast, for the control group, only 37% hair regrowth in the shaved area was observed. Moreover, the hair shafts plucked from the treated area of TCQA-treated mice were darker in color compared to the control and when the melanin content was measured on these hair shafts, TCQA-treated mice's hair's melanin content was increased by 75%. The author then elucidated the mechanism underlying the effect of TCQA by performing DNA microarray analysis on TCQA-treated mice skin. An upregulation in hair growth- and pigmentation-associated genes was observed while genes significant in β-catenin binding, pigmentation, neural cells differentiation and migration, transcription regulation, and Wnt signaling were upregulated. On the other hand, genes involved in Wnt repression, melanin degradation, and β-catenin degradation complex were downregulated. Immunohistochemical analysis of the skin from the treated area revealed an accumulation of β-catenin in the epidermis and the hair bulb where the dermal papilla (DP) and the active melanocytes are located. An activation of tyrosinase (TYR), that is the rate-limiting enzyme in melanin synthesis, was also observed showing that the melanocytes are differentiated or producing melanin. A staining of CD34, an anagen marker in the skin, showed the stimulation of this protein in TCQA-treated skin compared with the control. These immunohistochemistry results revealed that the HF from TCQA-treated mice are in the anagen phase of the hair cycle, which means that the hair matrix cells are differentiating to form the hair shaft and the melanocytes are active and produce the melanin.

The author further validated TCQA's effect using human hair follicle dermal papilla cells (HFDPC) and human epidermal melanocytes (HEM) and found out that the ATP levels in HFDPCs was increased by TCQA at the same time, TCQA upregulated the protein and the gene expression of β-catenin in HFDPCs. In HEM, TCQA increased the melanin content as well as the gene and protein expression of TYR, TYRP1, and DCT (the melanogenesis enzymes), attributed to the activation of MITF (the master regulator melanogenesis enzymes) via β-catenin. To further demonstrate the role of β-catenin, the author also used XAV939 which is known to inhibit the activation of β-catenin, stimulating its phosphorylation and non-translocation to the nucleus. The author treated HFDPCs first with 10 μM XAV939 for 6 and 12 h and results showed that *CTNNB1* expression was significantly decreased upon treatment confirming the inhibitory effect of XAV939 on Wnt/ β-catenin signaling. The author then treated HFDPCs with 10 μM XAV939 (6 and 12 h) and with 10 μM TCQA (6 and 12 h) and the results showed that TCQA significantly

upregulated *CTNNB1* expression even in the presence of XAV939. Co-treatment with XAV939 and TCQA modulated β-catenin expression, with the level higher than after treatment with just the inhibitor validating the previously observed activation of β-catenin by TCQA in DP that caused the activation of the anagen phase (of the hair growth cycle).

Overall, the author's findings suggest that TCQA triggered the activation of Wnt/β-catenin pathway leading tothe transition from telogen to anagen phase, anagen phase initiation and elongation, hair matrix differentiation, and hair shaft development and pigmentation. Furthermore, the observed stimulation of hair growth cycle was supported by the downregulation of telogen- and aging-associated genes, Wnt signal inhibitors, and melanin degradation-associated genes which contributed to further enhance the effect of β-catenin and Wnt proteins activation. TCQA successfully activated the proliferation of dermal papilla cells often considered they key regulators of hair growth cycle and the melanocytes, melanin-producing cells.

In summary, the author has shown in this thesis that TCQA activates melanocytes via Wnt/β-catenin-dermal papilla cells interaction. Although the author suggests that clinical studies would be necessary to introduce TCQA as a safer drug, the results in this dissertation has provided enough evidence as to the hair growth promotion and hair pigmentation effect of TCQA.

Abstract of assessment result

【Review】

Hair growth problems affect human physical and mental health and are of particular relevance during the aging process. Recently, drugs like minoxidil and finasteride are used to promote hair growth but they showed side effects. Development of a drug with no advertise effects is becoming urgent in order to have an alternative therapy for promoting hair growth. The applicant focused on 3,4,5-tri-*O*-cffeoylquinic acid (TCQA) and assessed its effect on hair growth and hair pigmentation effect using mice model and human cultured cells. The applicant observed that treatment of TCQA causes a complete regrowth of the shaved area of C3H mice and found that TCQA promoted the transition from telogen to anagen phase, anagen phase initiation and elongation, hair matrix differentiation, and hair shaft development and pigmentation. Global gene analysis revealed that these effects were triggered by the activation of Wnt/β-catenin pathway, as well as melanogenesis pathway, upon treatment of TCQA. Thus, TCQA is expected to be a novel candidate for the agent to promote hair growth and hair pigmentation.

【Result】

The final examination committee conducted a meeting as a final examination on Dec 24, 2019. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Science.