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論　文　の　要　旨
Abstract of thesis

The objective of this research was to understand human carcinogenesis, particularly the role of environmental factors and their intervention with natural compounds. The author used human cell culture system in this study and got several new findings. This work is well planned and well executed. Proper standards have been used for each assay. The author established loss of function screening in which cells that escaped 5AZA-dC induced senescence were collected and analyzed in terms of their miR expression by micro array analysis. The strategy is unique and well executed. The author identified miR-451 as overexpressed in cells that escaped senescence have validated its anti-proliferative role by a variety of assays in cultures cells. It is very clear that miR-451 is a tumor suppressor, which causes growth arrest of cancer cells. Being consistent with this function, the author found that it is down-regulated in cancer cells. These results demonstrate that tumor suppressor miR-451 can be used for intervention of pro-proliferative phenotype of cancer cells. miR-451 was claimed to result in the upregulation of growth arrest regulating proteins and identified that p21 is the major player. In addition, the author investigated several possible targets of miR-451 and established CARF as its new target. Conclusively, this report used loss-of-function strategies in human cancer model exposed with environment stresses revealed molecular regulation of growth arrest or senescence/aging and melanogenesis processes and identified miR-451, CARF and Mortalin as key molecular targets to develop interventional approaches.

With cumulating burden of various environmental factors on human health, it has become very important for production of mutagenesis and other health anomalies, precise assessment of their molecular regulatory effects and development of an interventional approach. This research has delineated that loss-of-function approaches are useful models to dissect molecular pathways and regulatory molecules in several phenotypes including carcinogenesis, melanogenesis and growth arrest.

The dissertation is divided into 4 chapters. In the chapter 1, the author presented a literature review on the previous studies relating to cancer, miRNA-451 and environmental stress. This author also addressed the relationship between cancer and environmental stress, microRNAs, and melanogenesis. Research motivations were arrived at the end of this chapter. In the chapter 2, the author investigated and used 5-Aza-dC or shRNA-based loss-of-function strategies in human cancer cells to reveal molecular regulation of growth arrest or senescence/aging and melanogenesis processes, and identified miR-451, CARF and Mortalin as key molecular targets to develop interventional approaches. Future perspectives warrant further studies on (i) the validation of upregulation of miR-451 for cancer therapies, (ii) the elucidation of the molecular mechanism of the role of tyrosinase in mortalin-mediated melanogenesis, and (iii) the validation of mortalin as a target and drug discovery tool for manipulation of skin pigmentation for therapeutic and cosmetic purposes. In the chapter 3, the author described in detail on shRNA-mediated loss-of-function screening in conjunction with induction of melanogenesis by OAG (diacylglycerol 1 -oleoyl-2-acetyl-sn-glycerol) in human melanoma G361 cells. Cells were transfected with shRNA library and assayed for induction of melanogenesis by multidimensional approach, involving quantitative biochemical and visual determination of the melanin content and tyrosinase activity. Gene targets of the shRNAs that led to the loss of OAG-induced melanogenesis were considered as candidate cellular factors crucial for melanogenesis. 40 gene targets were identified. Bioinformatics and pathway analyses revealed that these gene targets are involved in the regulation of cell proliferation, apoptosis, stress response and mitochondrial functions. Based on these data, the role of mitochondrial stress chaperone, mortalin in melanogenesis was identified. This study demonstrated (i) its use as a molecular target for manipulation of melanogenesis, and (ii) the whitening effect of some natural and synthetic compounds in OAG-induced melanogenesis in cell culture models. Finally, in the chapter 4, the author summarized the major results of this study.

審 査 の 要 旨
Abstract of assessment result

This research used loss-of-function screening strategy provided the molecular evidences that how miR451 instigates growth arrest in cells leading to their resistance to 5Aza-dC-induced senescence. miR-451-induced growth arrest at molecular level was found to essentially be mediated by increase in p21WAF1. With enrollment of environmental stress factors, mRNA and 3’UTR reporter assays, Collaborator of ARF (CARF) protein was found to be a new target of miR-451. Being consistent with the loss-of-function screening (shRNA-based approach), results from the effect of environmental and metabolic stresses on the melanogenesis process in the aging skin (using skin cancer/melanoma cell model) revealed mitochondrial stress chaperone, mortalin as a key regulator of melanogenesis.

The results have contributed to the understanding of role of miR-451 in stress and cancer biology, and is deemed to be use for their intervention. Also, the results have shown that mortalin can serve as a molecular target for manipulation of melanogenesis, suggesting its value for cosmetics and therapeutic manipulation of skin color and other characteristics regulating stress, tolerance and pathologies.

The final examination committee conducted a meeting as a final examination on 19 January, 2018. 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.

Therefore, the final examination committee approved that the applicant is qualified to be awarded the degree of Doctor of Philosophy in Environmental Studies.