【Grant-in-Aid for Scientific Research (S)】

Broad Section H

Title of Project：Deciphering Molecular Basis for the Anti-Oxidative Stress Response and Application of the Basis for Disease Prevention and Therapy

YAMAMOTO Masayuki
(Tohoku University, Graduate School of Medicine, Professor)

Research Project Number：19H05649　Researcher Number：50166823
Keyword：Stress response, KEAP1-NRF2 system

【Purpose and Background of the Research】

Environmental factors such as dietary poison, ultraviolet, and air pollution often cause redox disturbance and leads to irreversible changes of biomolecules that might explain many types of disorder. The KEAP1-NRF2 system is one of the most important defense mechanisms against the redox disturbance. In addition to the anti-oxidant function of NRF2, we recently clarified that NRF2 has a potent anti-inflammatory function, which is likely to result from direct inhibition of pro-inflammatory cytokine production by NRF2. Considering recent studies describing the increased oxidative stress and smoldering chronic inflammation in the pathological basis of many disorders, including Alzheimer's disease, arthritis and type 2 diabetes, we can expect that NRF2 activation is effective for prevention and treatment of the chronic diseases and achievement of healthy aging. The goal of this research is to clarify new mechanisms of the KEAP1-NRF2 system and to explore the effectiveness of NRF2 activation for anti-disease strategy toward health and longevity. In this research proposal, we will clarify basic molecular mechanisms how the KEAP1-NRF2 system is regulated, contributions of NRF2 to the prevention of stress-related disorders, and relation among the functionality of the KEAP1-NRF2 system and organismal redox balance and health.

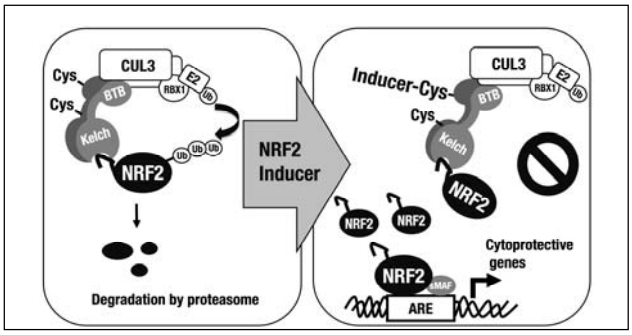

Figure 1 KEAP1-NRF2

【Research Methods】

We will clarify basic molecular mechanisms how the KEAP1-NRF2 system is regulated, contributions of NRF2 to the prevention of various disorders, and relation among the functionality of the KEAP1-NRF2 system and organismal redox balance and health.

1) ROS sensor(s) of KEAP1. To clarify the function of the oxidative stress sensor *in vivo*, we are planning to generate KEAP1 mutant knock-in lines of mice that will be unable to response to hydrogen peroxide.

2) Structure analysis of NRF2-KEAP1-CUL3 complex. To understand this mechanism how KEAP1's structure changes in response to stress to regulate NRF2's activity, structural analysis of full-length KEAP1 must be undertaken. We will endeavor to reveal the structure and function of KEAP1 in complex with NRF2 and CUL3 by combining X-ray crystallography, cryo-EM and NMR spectroscopy analyses.

3) Physiological analysis of NRF2 in prevention of aging related disease. We have developed several lines of mice for targeting KEAP1 or NRF2, and also obtained disease model animal for Alzheimer's disease, arthritis and type 2 diabetes. To clarify contribution of NRF2, we are generating compound mice having loss- or gain-of-NRF2 function in these disease model mice.

【Expected Research Achievements and Scientific Significance】

As an outcome of this research, we will consolidate an idea that NRF2 activation is a general target for an anti-disease strategy. Extension of health span is an urgent need in the current super-aging society. To this end, long-term and preventive intervention with low costs is required. A good thing about the KEAP1-NRF2 system is that NRF2 can be appropriately activated by naturally occurring phytochemicals contained in vegetables and other food. From these social perspectives, we believe that NRF2 is a perfect target for anti-aging strategy with sufficient practicality.

【Publications Relevant to the Project】

- **Yamamoto M**, Kensler TW, and Motohashi H. The Keap1-Nrf2 System: a thiol-based sensor-effector apparatus for the maintenance of redox homeostasis. ***Physiol Rev*** 98, 1169-1203. (2018)
- Suzuki T, **Yamamoto M**. et al, Molecular mechanism of cellular oxidative stress sensing by Keap1. ***Cell Reports*** *in press* (2019)

【Term of Project】 FY2019-2023

【Budget Allocation】 153,000 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.dmbc.med.tohoku.ac.jp/official/index.html
masiyamamoto@med.tohoku.jp

Title of Project：Elucidation of pathogenic immunological memory to understand the pathogenesis of intractable inflammatory diseases

NAKAYAMA Toshinori
(Chiba University, Graduate School of Medicine, Professor)

Research Project Number：19H05650　Researcher Number：50237468
Keyword：Immune systems, Airway inflammation, Allergy, Pathogenic immunological memory

【Purpose and Background of the Research】

The main purpose of our research is to investigate the mechanisms that control the differentiation of memory helper T cells and their induction of allergic airway inflammation (asthma). “Immunological memory” is a major issue in the field of immunology research. Recently, we identified two pathogenic memory Th2 cell populations (IL-5-high-producing and fibrosis-inducing memory Th2 cells) that are harmful to humans (Figure 1).

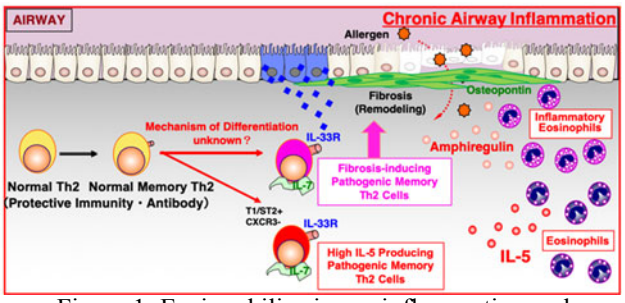

Figure 1: Eosinophilic airway inflammation and fibrosis induced by pathogenic memory Th2 cells

Based on these findings, we proposed a “pathogenic memory Th population disease induction model” in which pathogenic subpopulations induce and control the pathogenesis of various inflammatory diseases (Figure 2). We intend to explore the mechanisms underlying how pathogenic immunological memory T cells differentiate and are maintained in mouse models or human patients for a long time at the molecular and cellular levels.

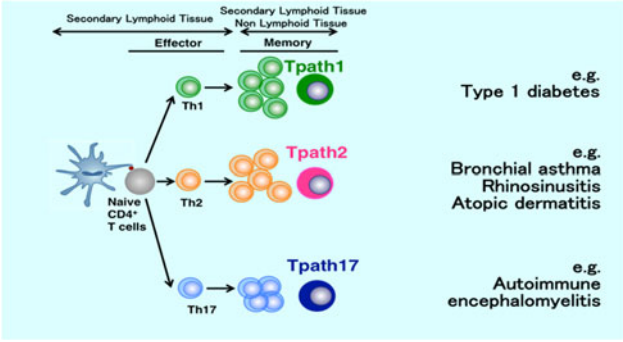

Figure 2: Pathogenic memory Th cells and inflammatory diseases

【Research Methods】

(1) To identify novel functional molecules regulating the pathogenesis and differentiation of "Pathogenic Memory Th2 (Tpath2) cells", we will conduct integrative analyses of Tpath2 cells using single-cell RNA-Seq, ChIP-Seq, or ATAC-Seq. We will also analyze the mechanism underlying the functional transformation and maintenance of Tpath2 cells regulated by Polycomb and Trithorax groups at the chromatin level. (2) In the spatio-temporal mapping of microenvironments responsible for the differentiation and maintenance of Tpath2 cells, we will examine immunohistological and pathological changes of inducible bronchus-associated lymphoid tissue (iBALT) and fibrosis of localized inflammation. (3) To promote research in support of a proof of concept, we will perform analyses using samples of human patients with chronic eosinophilic sinusitis, chronic hypersensitivity pneumonitis, eosinophilic esophagitis, and other diseases.

【Expected Research Achievements and Scientific Significance】

This research aims to clarify the nature of “pathogenic immunological memory”, both at the molecular and cellular levels, and to define the regulation of pathogenesis by immunological memory *in vivo*. These points of view are unique and scientifically significant. We will also focus on human immunology: we plan to analyze the inflamed tissues of several patients as well as human cells in almost all experiments. We additionally intend to examine the concepts derived from animal experiments to see if they can be applied to humans. Once we have determined how to control the number or function of immunological memory cells, this research may help contribute to the development of new treatment strategies for intractable inflammatory diseases.

【Publications Relevant to the Project】

・Morimoto Y, Nakayama T, et al., Amphiregulin-producing pathogenic memory T helper-2 cells instruct eosinophils to secrete Osteopontin and facilitate airway fibrosis. *Immunity* 49:134-150 (2018).
・Nakayama T, et al., Th2 Cells in Health and Disease. *Annu. Rev. Immunol.* 35:53-84 (2017).

【Term of Project】

FY2019-2023

【Budget Allocation】

155,400 Thousand Yen

【Homepage Address and Other Contact Information】

https://www.m.chiba-u.ac.jp/class/meneki/english/index.html

Title of Project：Comprehensive analysis of molecular machineries for mitotic spindle formation in human cells and its application to development of next generation anti-cancer drug.

KITAGAWA Daiju
(The University of Tokyo, Graduate School of Pharmaceutical Sciences, Professor)

Research Project Number：19H05651　Researcher Number：80605725
Keyword：cell division, centrosome, centriole, mitotic spindle, mitotic inhibitor

【Purpose and Background of the Research】

The centrosome is an evolutionarily conserved organelle in eukaryotes, and is important for formation of mitotic spindles, and thus is deeply involved in proper chromosome segregation and maintenance of genome stability. On the other hand, in human cancer cells, it has been reported that cell division proceeds by a centrosome-independent spindle formation mechanism even if the centrosome is physically removed. Recently, we found that in different cancer cell types, the contribution of centrosomes in spindle formation is significantly different. Therefore, in this study, we will identify various spindle formation machineries by performing comparative analysis using many types of human cancer cells as a model. Furthermore, we analyze the molecular basis that controls mitotic spindle formation in an integrated manner, and elucidate the mechanisms of centrosome-dependent and independent mitotic spindle formation in various types of human cancer cells. In addition, by combining the latest cytogenetics, cell biology and chemical biology, it is possible to develop mitotic-phase specific anti-cancer drug.

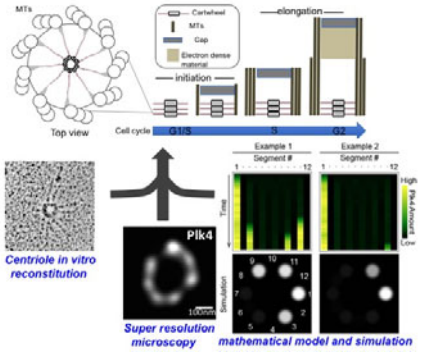

Figure 1　Mechanisms of centriole duplication

【Research Methods】

1) Elucidation of centrosome duplication mechanisms by a combination of super resolution microscopy and *in vitro* reconstruction system. 2) Comprehensive identification and functional analysis of centrosome-independent spindle formation machineries using cytogenetic methods in various human cancer cell types. 3) Develop small molecule compounds that specifically inhibit mitotic spindle formation. 4) Elucidation of the spindle formation mechanism in blood cancer cells.

【Expected Research Achievements and Scientific Significance】

In this research, we use super resolution microscopy technology, simulation, structural biological analysis, etc., and clarify the basic principles that mediate centriole duplication and mitotic spindle formation. Also, we will establish an accurate duplication model of centrosomes using mathematical models and simulations based on raw data. Furthermore, the findings obtained from this study are expected to lead not only to a better understanding of the cell division processes of various cancer cell types but also to the development of new anti-cancer strategies.

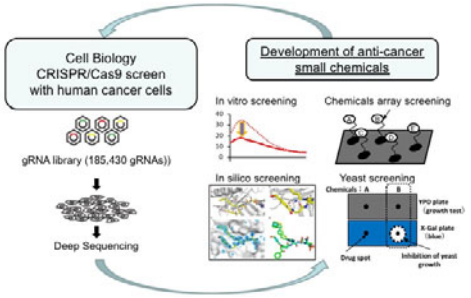

Figure 2　Combination of CRISPR screen and chemical biology

【Publications Relevant to the Project】

・Ohta M., Watanabe K., Ashikawa T., Nozaki Y., Yoshiba S., Kimura A. and Kitagawa D. (2018) Bimodal Binding of STIL to Plk4 Controls Proper Centriole Copy Number. *Cell Reports*, 23, 3160-3169, doi: 10.1016/j.celrep.2018.05.030.
・Tsuchiya Y., Yoshiba S., Gupta A., Watanabe K. and Kitagawa D. (2016) Cep295 is a conserved scaffold protein required for generation of a bona fide mother centriole. *Nature Communications*, doi: 10.1038/ncomms12567.

【Term of Project】

FY2019-2023

【Budget Allocation】

153,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.f.u-tokyo.ac.jp/~seiri/index.html

Title of Project：Genomic origin of chemodiversity in medicinal plants

SAITO Kazuki
(RIKEN, Center for Sustainable Resource Science, Deputy Center Director)

Research Project Number：19H05652　Researcher Number：00146705
Keyword：medicinal plants, genome, metabolome

【Purpose and Background of the Research】

The diversity of the plant metabolome, which is the source of medicine, far surpasses animals and so on, but its genomic origin is an unknown subject and is a fundamental issue. In addition, 90% of physicians still prescribe Kampo, and expectations for botanical medicine are high for extension of healthy lifespan in the aging society. While many herbal medicines depend on imports, the Nagoya Protocol came to seek fair profit distribution, but it is rather a great opportunity for genomic elucidation and intellectual property defense of domestically grown medicinal plants. In addition, in the “Sustainable Development Goals” SDGs (Sustainable Development Goals) adopted by the United Nations in 2015, conservation of biodiversity resources and its sustainable use are issues of global concern. In addition, there is a rapid development of genomic science related technology as a technical background.

In this study, we decipher the genome and metabolome of medicinal resource plants, clarify the origin of their chemical diversity, and apply their findings to the sustainable use of plant resources.

【Research Methods】

Regarding licorice most important as a herbal medicine, which is most frequently used for Kampo prescriptions and so on, we determined high-quality genome sequences of plant species containing and not containing glycyrrhizin, its main active ingredient. Variant strains with different component patterns are resequenced to obtain mutational information. Next, transcriptome and metabolome data are also acquired, and genes, genome regions and mutations that determine component patterns are identified by co-occurrence network analysis or genome wide association study (GWAS) of these. Next, along with functional identification of these genes, biotechnologies such as genome editing and synthetic biology are applied to molecular breeding of licorice and production of active ingredients. At the same time, we will extend this basic method to functional genomics of important medicinal plants other than licorice.

【Expected Research Achievements and Scientific Significance】

It is possible to decipher genomes and metabolomes and clarify the origin of their chemical diversity in medicinal plants of increasing importance in the fields of medicines and medical field. This can be applied to the sustainable use of plant resources that contributes to "Sustainable Development Goals" SDGs, and at the same time, it can extend the horizon of human knowledge. Furthermore, it will open up a new path to medicinal plant resource development in the next 10 to 20 years.

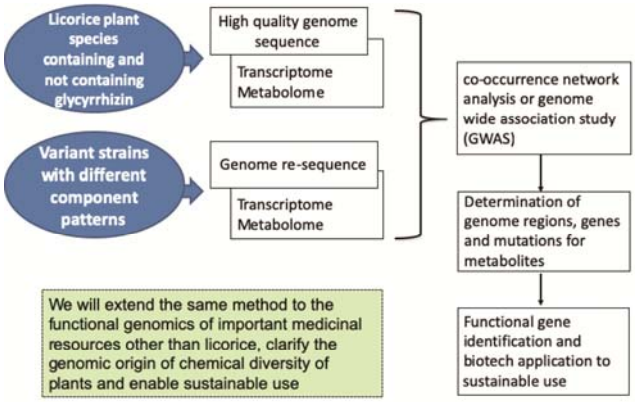

Figure 1 Outline of research

【Publications Relevant to the Project】

・Mochida, K., *et al.*: Draft genome assembly and annotation of *Glycyrrhiza uralensis*, a medicinal legume. *Plant J.*, **89**, 181–194, (2017)
・Rai, A., Saito, K., Yamazaki, M.: Integrated omics analysis of specialized metabolism in medicinal plants. *Plant J.*, **90**, 764-787 (2017)
・Knoch, E., *et al.*: The third DWF1 paralog in Solanaceae, sterol Δ^{24}-isomerase, branching withanolide biosynthesis from the general phytosterol pathway. *Proc. Natl. Acad. Sci. USA*, **115**, E8096-E8103 (2018)
・Tsugawa, H., Nakabayashi, R., *et al.*: A cheminformatics approach to characterize metabolomes in stable-isotope-labeled organisms. *Nature Methods*, **16**, 295–298 (2019)

【Term of Project】

FY2019-2023

【Budget Allocation】

154,600 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.riken.jp/research/labs/csrs/metabolom/
http://metabolomics.riken.jp/