Graduate School of Advanced Science and Engineering
Waseda University

博 士 論 文 概 要
Doctoral Dissertation Synopsis

論 文 題 目
Dissertation Title

Control of reactive oxygen species (ROS) machinery to maintain the cellular homeostasis

活性酸素種による細胞動態恒常性調節機構の解析

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Synopsis:

Regulation of oxidative stress and redox control processes is one of the most important mechanisms for development, maintenance of characteristics, self-renewal, and reprogramming of stem cells. Regulation of the balance of oxidative stress and antioxidation involves the acryl hydrocarbon receptor–Jun dimerization protein 2–nuclear factor (erythroid-derived 2)-like 2 (AhR–Jdp2–Nrf2) axis through mitochondrial integrity. The reactive oxygen species (ROS) balance within the cells was maintained by oxidative stress and antioxidation, of which verifying a fact that Activation protein 1/Activation transcription factor Jun dimerization protein 2 (Jdp2) was involved. Here we provide a novel insight about the role of the AhR–Jdp2–Nrf2 gene battery in determining the cell plasticity and the cell fate.

In this study, AhR–Jdp2 axis activation initiate through stimulation by dimethyl sulfide (DMSO), a commonly used polar organic solvent. The promoter of the *AhR* gene was significantly upregulated via a Dioxin response element (DRE), which increased ROS production, and resulted in apoptosis of mouse embryonic fibroblasts. The mechanism of AhR promoter upregulation is dependent on the expression of Jdp2. Moreover, the interaction of AhR with Jdp2 and small Maf basic zipper transcription factor K, has been detected by assays of co-immunoprecipitation and chromosome immunoprecipitation on the DRE of the AhR promoter region. Take these results together, Jdp2 forms a complex with the phase I enzyme factor, AhR, and the phase II enzyme factor, Nrf2. Furthermore, this complex plays an important role in the AhR promoter activation in response to DMSO.

To confirm the crosstalk between the AhR–Jdp2–Nrf2 axis further, we identified Nrf2 as one of the factors critical for AhR-promoter activation in response to the phase I enzyme ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin, in a spatiotemporal *cis*-element-dependent manner. These findings identified the role of the AhR–Jdp2–Nrf2 gene battery in modulating ROS production, controlling cytoskeletal remodeling, cell spreading, migration, and tumor progression. The induction of ROS through AhR and Nrf2 batteries can potentially offer a therapeutic strategy for cancer treatment. In pancreatic adenocarcinoma, we also demonstrated that tumor growth is affected significantly by the Jdp2–AhR–Nrf2 gene battery to modulate ROS production.

Regorafenib, one of the most commonly used multi-kinase inhibitors is frequently used as an anticancer drug for the treatment of hepatocellular carcinoma (HCC). Moreover, it activates oxidation stress by promoting ROS to inhibit cancer formation and angiogenesis. Here, we produced a regorafenib-resistant HepG2 HCC cell line HepG2_Rego_R which showed the upregulation of the transcription factor forkhead box protein M1 (FOXM1). The FOXM1 upregulation was crucial for the survival of cancer cells treated with regorafenib; in this case, ROS control was an important factor for drug resistance. By combining the FOXM1 inhibitor thiostrepton with regorafenib, we have succeeded to suppress cancer cells through severe ROS induction. This effect exhibited a significant reduction in the survival of regorafenib-resistant cells.

Taken together, these studies of the AhR–Jdp2–Nrf2 gene battery provide basic and compelling evidence for the application of ROS balance in the search for new therapeutics targeted to the AhR–Jdp2–Nrf2 gene battery in cancers or other diseases in the future.

List of research achievements for application of Doctor of Science, Waseda University

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種類別 (By Type)	題名、 発表・発行掲載誌名、 発表・発行年月、 連名者（申請者含む） (theme, journal name, date & year of publication, name of authors inc. yourself)
○	Dimethyl sulfoxide stimulates the AhR-Jdp2 axis to control ROS accumulation in mouse embryonic fibroblasts. Cell Biology and Toxicology, 38: 203-222 (2022). *Wuputra, K., Tsai, M-H., Kato, K., Yang, Y-H., Pan, J-B., Ku, C-C., Noguchi, N., Kishikawa, S., Nakade, K., Chen, H-L., Liu C-J., Nakamura, Y., Kuo. K-K., Lin, Y-C., Te-Fu, Chan, Wu, D-C., Hou, M-F., Huang, S-K., Lin, C-S., and Yokoyama K.
○	FOXM1-CD44 signaling is critical or the acquisition of regorafenib resistance in human liver cancer cells. International Journal of Molecular Sciences. 23(14): 7782 (2022). *Wuputra, K., Hsiao P-J., Chang, W.T., Wu, P-H., Chen, L-A., Huang, J-W., Su, W-L., Yang, Y-H., Wu, D-C., Yokoyama, K.
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	Deletion of Jdp2 enhances Slc7a11 expression in Atoh-1 positive cerebellum granule cell progenitor in vivo. Stem Cell Research & Therapy, 12, 369 (2021). *Ku, C-C., Wuputra, K., Kato, K., Pan J-B., Li C-P., Tsai, M-H., Noguchi, M., Nakamura, Y., Liu C-J., Chan, T-F., Hou M-F., Wakana, S., Wu Y-C., Lin C-S, Wu D-C., Yokoyama K. The progress in the study of reprogramming to acquire the feature if stem cells in iPSCs and cancers. Recent Advances in iPSC technology. Springer Nature, Chapter 4 (2021). Saito S., Wuputra, K., Koto, K., Yokoyama K. Jdp2-deficient granule cell progenitors in the cerebellum are resistant to ROS-mediated apoptosis through xCT/Slc7a11 activation. Scientific Reports, 10, 4933 (2020). *Ku, C-C., *Wuputra, K., Kato, K., Lin, W-H., Pan, J-B., Tsi, S-C., Kuo, C-J., Lee, K-H., Lee, Y-L., Lin, Y-C., Saito, S., Noguchi, M., Nakamura, Y., Miyoshi, H., Eckner, R., Nagata, K., Wu, D-C., Lin, C-S., and Yokoyama K. Tissue regulation and healing by ROS-mediated NOX2 and Ca2+ ion uptake. Journal of Stem Cells Research, Reviews & Reports, 5; 1026 (2018). Lin, C-S., Wuputra, K., and Yokoyama, K. Multiple function of histone chaperone Jun dimerization protein 2. Gene, 590, 193-200 (2016). Tsai. M-H., Wuputra, K., Lin Y-C., Lin C-S., and Yokoyama K. Environmental-stress-induced chromatin regulation and its heritability. Journal of Carcinogenesis & Mutagenesis 5, 156 (2014). Fang, L., Wuputra K., Chen D., Li H., Huang, S-K., Jin C., and Yokoyama K. Reactive oxygen (ROS) homeostasis in influenza virus infection. BMC Microbiology, 20, 214 (2020). Chen, K-K., Minakuchi, M., Wuputra, K., Ku, C-C., Pan, J-B., Kuo, K-K., Lin, Y-C., Saito, S., Lin C-S., and Yokoyama K.

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