Project number　88

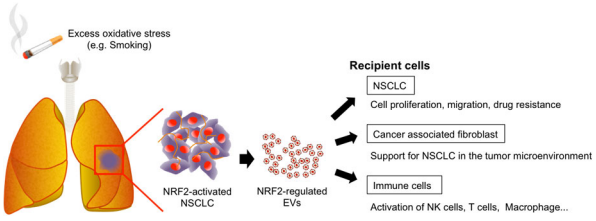
Understanding the Role of Tumor-derived Extracellular Vesicle Cargo in Evading Immune Cell Recognition and Destruction

［1］**Research group**
Principal Investigator (PI)：
Andrew Vonberg
(Cincinnati Children's Hospital)
Host researcher at IDAC：
Tomoki Yagai
(IDAC Tohoku University)

Expenditure report of research funds：
Consumables 200,000YEN

［2］**Research setup**

As the incidence of cancer and stress responses continue to rise, new insights into cell signaling pathways and the induction and prevention of inflammatory responses need to be determined. Our lab is interested in extracellular vesicles (EVs), important signaling molecules whose cargo can influence immune responses, which are secreted from donor cells to recipient cells to modify functions. In particular, we have shown that EVs secreted from liver hepatocytes under stress conditions can stimulate macrophage activation and lead to damaging inflammation culminating in Type 2 Diabetes (T2D) or Non-Alcoholic Steatohepatitis (NASH) conditions. Using our expertise in EV analysis and research, we study whether EVs secreted from non small cell lung cancer (NSCLC) can avoid activation of inflammation and destruction by sentinel immune cells or accelerate proliferation or invasion in NSCLC itself. Nuclear factor erythroid 2-related factor 2 (NRF2) is an transcription factor regulating antioxidant protein expressions, and has significant roles for protecting cells from oxidative stress. A part of NSCLC has robust NRF2 activity and capability for immune escape and drug resistance, resulting in bad prognosis after the surgery. Since previous studies uncovered oxidative stress increase EV secretion in various kinds of cells, we hypothesized that amount of EVs is controlled by NRF2 and also is important not only in silencing or activating cell signaling, but that their cargo is of vital importance in the capacity of EVs to the functions. We looked at these specific cancer EVs using the assistance of Dr. Tomoki Yagai, assistant professor at Tohoku University and IDAC member, who is an expert in isolating EVs from serum and can isolate and prepare samples for us to test using our established *in vitro* co-culture model with NSCLC. Using the EVs from NSCLC would allow to determine if specific cargo of the isolated EVs are important in the function of cell proliferation, invasion, drug resistance and immune activation. Comprehensive analyses would allow us to determine the mechanism by which these cancer EVs were able to induce the phenotypic change. This data could potentially lead to novel therapeutic targets to prevent lung cancer and also other NRF2-activated diseases.



［3］**Research outcomes**

（3－1）**Results**

NSCLC removed by surgery was subjected to qRT-PCR to determine whether NRF2 activation was related with the patient's medical history. The comparative analyses for 6 patients showed that excess smoking experiences resulted in drastic increase of NRF2 activity in the removed NSCLC (Figure 1). In contrast, the NRF2 activity was not correlated with malignancy grade, indicating that high NRF2 activity was derived from oxidative stress but not the malignancy grade.

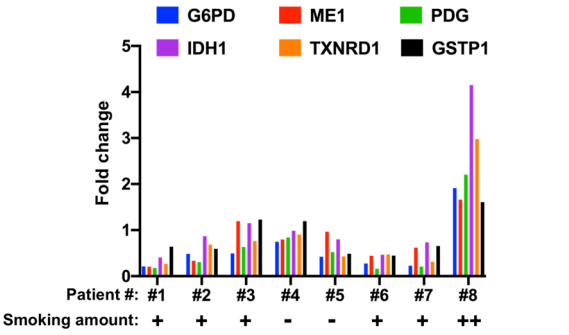


Figure 1. Expression levels of NRF2 target genes in NSCLC related with smoking amount

To analyze the potential autocrine effects of NRF2-regulatory EVs, cell proliferation assay with NSCLC cell line was performed. EVs purified from NRF2 knockdown H460 cells were added to the culture supernatant, and then the cell number was counted after 24 hours culture. The assay showed that EVs from NRF2-activated cells ($NRF2^{high}$ EVs) significantly induced cell proliferation, whereas EVs from NRF2-repressed cells ($NRF2^{KD}$ EVs) did not change the levels (Figure 2).

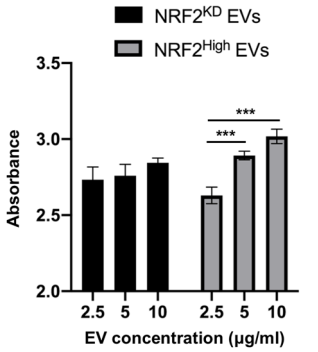


Figure 2. Cell proliferation assay with NRF2-related EVs

（3－2）Future perspectives

These results suggested that EVs secreted from NRF2-activated NSCLC might accelerate lung cancer proliferation and aggravate the disease. We will further analyze the autocrine mechanisms, especially drug resistance, because previous studies reported NRF2-activated NSCLC had potent tumor drug resistance. Paracrine functions of NRF2-regulated EVs also will be analyzed by use of immune cell lines such as T cell and NK cells. Additionally, EV functional assay with human PBMC is being prepared for confirming the responses in primary cells. We continue to collect NSCLC removed by surgery to determine the correlation between NRF2-activated NSCLC and oxidative stress in lung.

［4］List of Papers

The paper is now under preparation