1. **No.**

   FP01812181

2. **Title**

   Elucidation of Molecular Mechanisms for Protein Functional Changes Caused by Unsaturated Carbonyl Compounds in Cigarette Smoke Gas Phase

3. **Period**

   2016-2018

4. **Summary**

Acrolein (ACR) and methyl vinyl ketone (MVK) are major cytotoxic compounds in the gas phase of cigarette smoke. The effects of ACR and MVK in vascular cell injury were examined in this study. In vascular system, the vascular smooth muscle cells were highly sensitive to ACR and MVK, while endothelial cells were resistant to them. In A7r5 cells, ACR- and MVK-induced cell injury was PKC-dependent. PKCα and PKCδ was activated by ACR and MVK in A7r5 cells. I have also shown that PKCα and PKCδ was carbonylated by ACR, a representative α,β-unsaturated carbonyl compound. Protein carbonylation assay by immunoprecipitation in combination with Aldehyde Reactive Probe using PKCα mutants revealed that PKCα was carbonylated at its five cysteine residues in C-terminal kinase domain. To elucidate the effects of ACR-induced PKCα modification on PKCα function, I have tried constructing PKCα expression and purification system. PKCα was successfully expressed by using *in vitro* expression system. The functional analyses of PKCα and its mutants can be carried out in the future. I have also revealed that intracellular glutathione is a critical factor for deciding ACR and MVK sensitivity of the cells.