Mitochondrial ROS signaling regulated by protein S-guanylation
（タンパク質 S–グアニル化によるミトコンドリア活性酸素シグナルの制御機構）

**Background and Purpose**: Modification of protein thiols by reactive oxygen species (ROS) and electrophiles is an important process in redox signal transduction.
Although mitochondrial proteins undergo redox-based thiol modifications, the specificity and biological impacts of such modifications caused by diverse ROS/electrophiles remain largely elusive. 8-Nitroguanosine 3′ ,5′ -cyclic monophosphate (8-nitro-cGMP) is a nitrated derivative of cGMP of which formation is regulated by crosS-talk regulation of reactive oxygen species formation between NADPH oxidase 2 and mitochondria. This nitrated nucleotide can function as a unique electrophilic second messenger in the regulation of redox signaling via modification of protein cysteine thiols to produce a unique posttranslational modification by cGMP adduction (protein S-guanylation). In the present study, we investigated protein S-guanylation occurring in mitochondria.

**Methods**: In this study, a mass spectrometry-based proteomic method 'S-guanylation proteomics', was developed by consisting of two different approaches, (i) direct protein digestion followed by immunoaffinity capture of S-guanylated peptides that can be subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) identification of S-guanylated sites, and (ii) 2D-gel electrophoretic separation of Sguanylated proteins that are extracted and subjected to in-gel digestion, followed by LC-MS/MS identification.

**Results**: By using these approaches, several mitochondrial proteins that are Sguanylated endogenously during immunological stimulation were identified, including stresS-70 (mortalin) and 60 kDa heat shock protein (HSP60). It has recently been reported that mortilin and HSP60 regulate mitochondrial permeability transition pore (mPTP) opening, at least in part, via interacting with cyclophilin D (CyPD), a component of mPTP. These data revealed that mPTP opening was induced by immunological stimulation as well as 8-nitro-cGMP treatment. Such mPTP opening was CyPD-dependent as suggested by cyclosporine A inhibition.

**Conclusion**: This study suggests that mitochondrial heat shock proteins may be novel targets for redox modification via protein S-guanylation that participate in the regulation of mPTP and mitochondrial redox signaling.