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Integrative analysis of post-translational protein S-nitrosylation in endothelial cells

Nitric oxide (NO), an endogenous evolutionary gaseous molecule with labile character can bind to cysteine residues (Cys-NO, S-nitrosylation) and then alter the enzyme activity. NO is regarded as a mild reactive oxygen/nitrogen species (ROS/RNS) that can compete with other, more potent ROS/RNS and protects cells from irreversible oxidative stress caused by free radicals. At present available methodologies applied to study the implications of NO in physiological responses include Western blotting to measure the phosphorylation of endothelial nitric oxide synthase (eNOS) at Ser1177 and Ser633 residues and detecting gaseous NO by Griess reagent. However, this reagent is greatly affected by the presence of peroxinitrite (ONOO−). Therefore, the new fluorescent probe - 5-amino-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl) benzoic acid methylester (FA-OMe) - that specifically binds to endogenous NO, was developed and utilized. As a result, the elevated production of NO can be estimated not only by eNOS phosphorylation in Western blots but also by direct quantification using FA-OMe. Once it became possible to confirm the production of NO, the identification of the subsequent protein S-nitrosylation resulted from NO binding to the cysteine residues became important. The utilizations of commercialized antibody and mass spectrometric devices were reported to detect the Cys-NO residue directly. However, for the reason of poor antibody specificity and weak chemical binding of Cys-NO, both two methods were not reliable. We therefore designed a tag-based labeling system on cysteine residue that modified from biotin-switch, (e.g. IAA, IAM and iTRAQ). Cys-NO will be replaced by these tags and was then detected either by 2-DE-based Western blot or mass spectrometry with identical molecular weight shifts. The whole profiles of enzyme activation, gaseous NO molecule production and the subsequent protein S-nitrosylation could be analyzed simultaneously to explain more details about the physiological mechanisms of action in protein S-nitrosylation.

Biography

Bin Huang obtained his PhD degree from the Department of Plant Science, National Taiwan University. He then focused on Cardiology during his Postdoctoral studies. He became experienced in the gaseous molecule-mediated post-translational proteome, particularly in the NO-mediated S-nitrosylation when studying endothelial cells of the vascular system. He became interested in the behavior of mitochondrial fusion/fission when studying cell aging and drug-resistance of cancer cells. In addition to these research interests, he develops the Center for Stem Cell Research of Kaohsiung Medical University as the Vice Director.

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