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Monitoring of high glucose-induced cell metabolic alteration of retinal microvascular endothelial cells with fluorescence lifetime imaging microscopy

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Retinal microvascular endothelial (RME) cells play a significant role in retinal function as the inner blood-retinal barrier (BRB) whose disruption is largely relevant to the early pathological alterations of diabetic retinopathy (DR). Therefore, it is of great importance to elucidate the mechanisms of metabolic and functional alterations of RME cells under diabetic condition, namely under high glucose condition. Although many *in vitro* and *in vivo* basic studies have been performed and reported based on this motivation to date, it is still a challenging to monitor the cell metabolisms and functions with living cells. Fluorescence lifetime imaging microscopy (FLIM) is a technique to measure and map the fluorescence lifetime of the fluorophores. Fluorescence lifetime is a fluorophore-intrinsic and moreover, may be influenced by the molecular environments such as temperature, pH, viscosity and molecular protein binding status. FLIM coupled with two-photon microscopy (TPM) enables to measure the fluorescence intensities as well as fluorescence lifetimes of the auto fluorescence of two different coenzymes that are important in cell metabolisms, nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH). With TPM-FLIM, the redox ratio of NADH/FAD as well as fluorescence lifetimes of NADH (free/protein bound) and FAD (free/protein bound) can be measured. Since these values are largely related to the status of cell metabolisms, it can be used as a non-invasive monitoring method of cell metabolisms.

In this study, I will introduce our *in vitro* experimental results with cultured human RME cells showing the altered intracellular status of NADH and FAD detected with TPM-FLIM under different high glucose conditions. Combined with the results of other experimental procedures to detect cellular oxidative stress or stress-induced protein expressions, TPM-FLIM was found to be a very sensitive and non-invasive method to monitor the status of these metabolisms-related coenzymes in the cells under high glucose conditions. This method might expand the possibility of detecting cell metabolic states in diabetes basic research and eventually in clinical diagnosis.

Biography

Yoko Miura has completed her MD in 1997 and PhD in 2002 from Osaka City University in Japan. Clinical training was completed in eye clinic of Osaka City University Hospital and since 2002 she is working as a specialist of ophthalmology. In 2005 she started research work in University of Kiel in Germany and in 2009 moved to Institute of Biomedical Optics, University of Luebeck. Today she engages herself in the research and clinical works in Institute of Biomedical Optics and Department of Ophthalmology, University of Luebeck. Leading some research projects related to retinal cell biology. Since 2010, she is the Adjunct Lecturer of Osaka City University.

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