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Application of Fluorescence Lifetime Imaging as a Diagnostic Tool for Podocytopathies

Yoosun Joo, Hyunji Lim, Hana Choi, Youngyoon Moon, Su Woong Jung, Yang Gyun Kim, Sang Ho Lee, Tae Hoon Lee, Ju Young Moon
Department of Internal Medicine-Nephrology, Kyung Hee University Hospital at Gangdong, Korea, Republic of

Objectives : Kidney biopsy is necessary, but not sufficient, for the diagnosis of glomerular diseases. Among the glomerulopathies, minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) are two podocytopathies that are sometimes difficult to discriminate both clinically and pathologically since both diseases are characterized by massive proteinuria and podocyte foot process effacement. Fluorescence lifetime imaging (FLIM) is a non-invasive technique that utilizes the spectroscopic properties of endogenous fluorophores such as proteins and lipids and is very sensitive for cellular pH, protein binding state, and polarity.
Methods : 22-week-old male Podocin Cre mTmG mice treated with 15mg/kg doxorubicin via tail vein injection. Untreated female mice were used as a control group. Glomerular injury was confirmed with weekly measurements of body weight and proteinuria. Mice were sacrificed after 2 or 4 weeks and kidney tissue were embedded in paraffin for FLIM. Tissue were imaged with two-photon FLIM at an excitation wavelength of 900nm to obtain lifetime data and the results were interpreted with phasor plot analysis. Kidney biopsy specimen of patients diagnosed as FSGS or MCD were analyzed in an identical manner.
Results : FLIM with phasor plot analysis showed different lifetime between injured and normal glomeruli in mouse tissue samples.
Conclusions : Since it is not operator-dependent and does not require special staining to visualize tissue structures, FLIM is a viable imaging technique with potential for digital diagnosis of podocytopathies. More research is needed for identification of precise fluorophores imaged with FLIM and its application for classification and stratification in podocyte injury.