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Autofluorescence Lifetime Microscopy in Normal and Cancer Cells and Nanosecond Pulsed Electric Field Effects

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Abstract

Electric field effects on dynamics and function of biological systems depend on the cell membrane capacitance and on the pulse duration of the applied electric field, which enables selective application of pulsed electric field in specific organelles and specific living cells. If electric field whose pulse width is shorter than the charging time of the outer membrane is applied to living systems, the pulsed electric field may penetrate into the cell and induce changes in intracellular function. In fact, we reported the apoptosis induced by application of nanosecond pulsed electric field (nsPEF) with a pulse width of 10–50 ns in HeLa cells.¹

Fluorescence lifetime is independent of experimental condition such as dye concentration, photobleaching, excitation intensity, but dependent on the local environments surrounding fluorophores. This makes fluorescence lifetime microscopy (FLIM) a powerful method for sensitive detection of intracellular environment.² Endogenous fluorophores that exhibit autofluorescence exist in each cell, and microscopy using autofluorescence enables label-free observation without spending time on dye staining and also without considering the toxicity of the fluorophores, and physiological conditions of cells can be preserved during the measurements.³ Nicotinamide adenine dinucleotide (NADH) is one of the most well-known autofluorescence chromophores, and intracellular condition can be elucidated by monitoring the NADH autofluorescence.⁴

FLIM measurements using autofluorescence of NADH and flavin adenine dinucleotide (FAD) have been applied to cancer and normal cells to investigate intracellular environment. Effects of nsPEF on intracellular function have been also examined with a microelectrode by measuring the NADH fluorescence.⁵ In the present study, special attention has been paid to the difference in fluorescence lifetime and in effect of nsPEF between normal and cancer cells, i.e., human normal lung fibroblast and human lung large carcinoma. It is shown that autofluorescence lifetime of NADH and FAD in cancer cells is different from the one in normal cells, indicating that intracellular environments of cancer and normal cells are different from each other.⁶ It is also shown that application of nsPEF induces apoptosis and that nsPEF more efficiently affects cancer cells than normal cells in cell viability, suggesting the possibility of drug-free cancer therapy by nsPEF without any damage to normal cells.^{7,8}

Keywords: Fluorescence Lifetime Spectroscopy, Autofluorescence, Effect of Nanosecond Pulsed Electric Field, Apoptosis, Normal and Cancerous Cells

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