Oxygen imaging of living cells and tissues using phosphorescence lifetime imaging microscopy

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Oxygen imaging of biological cells and tissues is becoming increasingly important in cell biology and in the pathophysiology of various hypoxia-related diseases. Among various techniques for biological oxygen sensing, optical methods using phosphorescent probes have received much attention because of the low invasiveness, high oxygen sensitivity, the feasibility of quantitative oxygen measurements, and high spatial resolution at the single-cell level. Recently, we have been developing a method for measuring the oxygen status of living cells and tissues using phosphorescent iridium(III) complexes.[1-4] One of the excellent properties of iridium(III) complexes as biological oxygen probes lies in the high cell-penetrating ability. A cationic iridium complex BTPDM1 is efficiently taken up into cells when loaded into the media of cultured cells.

Utilizing the high cell-penetrating ability of BTPDM1, we investigated the oxygen distribution in 3D-spheroid cell cultures of HT-29 colon cancer cells. As expected, BTPDM1 penetrated cell membrane and stained whole spheroids. Phosphorescence lifetime imaging microscopy (PLIM) measurements allowed the phosphorescence lifetime imaging of spheroids, which can be converted to oxygen distribution images of cell spheroid. The interior of the spheroid showed longer lifetimes compared with those of the peripheral region, and the lifetime was the longest at the bottom. This suggested that diffusion limited supply of oxygen induced hypoxia near the bottom of the spheroid because of oxygen consumption in the spheroid.

Then we investigated the oxygen status of mouse kidney in vivo. BTPDM1 (250 nmol) was intravenously administered into an anesthetized mouse. Next, the abdomen was opened, and the kidney was placed on the stage of an inverted microscope to obtain PLIM images. The PLIM image clearly indicated that BTPDM1 distributed inside tubular cells and visualized the oxygen status of tubular cells in a living mouse.

References:

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