Appearance of autofluorescence in RPE cells at the rim of photocoagulation

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Purpose: The effects of sub-lethal temperature increase on RPE cells by laser irradiation are of great interest. Therefore, we studied RPE cells by two-photon microscopy in order to evaluate changes of fluorescence characteristics and morphology after laser irradiation.
Methods: RPE-choroid explants were isolated from porcine eyes and cultivated in perfusion culture system. RPE cells were irradiated by laser (λ= 532 nm; power 80 mW; spot diameter 300 µm; irradiation time 100 ms). The tissue in the culture medium was observed with two-photon microscopy 3 hrs to 4 days after laser irradiation. Detection of autofluorescence (AF) intensity and spectrum was combined with fluorescence lifetime imaging.
Results: From 3 to 48 hrs after irradiation, punctated and bright AF (Ø≈ 0.5-1.5 µm) different from melanin AF appeared in some cells around the coagulated area. The fluorescence intensity was maximal at λex= 730-750 nm. Four-channel detection revealed the peak emission wavelength in λem= 450-500 nm at λex= 750 nm. Fluorescence lifetime decay was best-fitted as a three-exponential curve (t_1= 0.19 ns, t_2= 2.58 ns, t_3= 5.23 ns; mean lifetime 0.655 ns). This is similar to previously-reported fluorescence lifetime of A2-E, a precursor of lipofuscin.
Conclusion: Laser photocoagulation induced the appearance of bright AF spots in RPE cells around the coagulation area. We hypothesize that this AF might result from laser-induced oxidation of phagosomes, which include undigested photoreceptor outer segments, leading to the generation of AF substance like lipofuscin. Intracellular distribution and the amount of this substance might indicate cell condition, such as, apoptotic change.