

# Analysis of Stress fiber formation processes in Photodynamic therapy

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Photodynamic therapy (PDT) is a method in which a photosensitizer is administered in vivo and irradiated with light to generate reactive oxygen species (ROS), thereby causing the selective death of cancer cells. In PDT, despite the importance of its fine control, the mechanism of the reaction process from the generation of reactive oxygen by photoinduction inducing the formation of actin filament and its polymerization to form stress fibers has not yet been clarified. Here, we have combined atomic force microscopy (AFM) with other techniques to reveal the mechanism of the actin filament and stress fibers formation processes that underlies the cell death process due to PDT [1].

Figures 1 show a phase contrast image, a topographic image, and an elastic modulus mapping of typical cells in the sample before and after irradiation.

Figure 1(f) shows a higher elastic modulus than Fig. 1(c), indicating that the actin filament production increases with the irradiation. The relative increase in average elastic modulus is slight for 5 min irradiation, changed greatly for 10 min irradiation. On the other hand, the RhoA concentration decreased significantly after 1 min irradiation and then appeared to decrease only slightly (Fig. 2). Namely, the activation of RhoA reached an equilibrium state in about 1 min; however, the production of actin filaments and its polymerization continued. The elastic modulus hardly changed in 5min and changed greatly in 10 min, corresponding well to the process in which actin filament becomes stress fiber by myosin phosphorylation in 5 min. the mechanism that it takes 5 min to change actin filaments to stress fibers by myosin phosphorylation.

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[1] A. Taninaka et al., *RSC Advances* 2022, 12, 5878-5889.

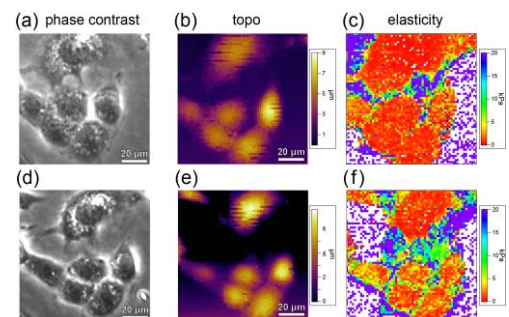


Fig. 1 Results obtained by irradiating RGK1 for 10 min: (a)-(c) before light irradiation and (d)-(f) after light irradiation.

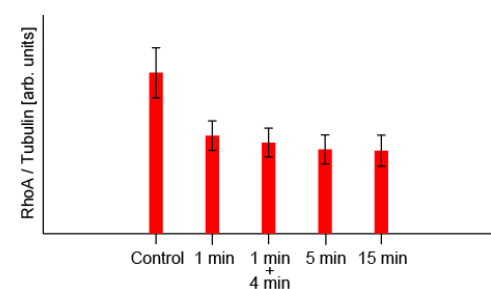


Fig.2 Change in RhoA concentration induced by irradiation. The values are normalized by the amount of  $\beta$ -tubulin antibody.