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Observation of cellular temperature of X-ray irradiated and unirradiated human cells using a chemical thermo-probe

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1 Introduction

Temperature of living system was thought to be uniform. Recent thermometric techniques, such as chemical thermo-probes, have altered this classical image and provided some novel aspects of live-cell temperature which is widely diverse among cells or sub-cellular components. This might strongly reflect of energetic metabolism of the cells. To reveal whether cellular temperature is affected by radiation stress should be one of important issues to be addressed in terms of radiobiological effects, because various biochemical reactions would work in the irradiated cells to repair damage molecules such as DNA, emit intracellular and intercellular stress signals, and upregulate ATP production for supplying those reaction with energy. Cellular temperature measurement might provide a new perspective on thermodynamics for understanding radiobiological endpoints, such as mutation induction or carcinogenesis. For the first step of the study, we tried to reveal change of cellular temperature of human cells exposed to X-rays.

2 Experiment

Human cancer cells, HeLa, were cultured overnight in a 35 mm ϕ dish in an incubator at 37ºC. After removing medium from the culture dish, the dish was put on the stage of microbeam irradiator at BL-27B. To directly irradiate the cells with X-ray beam from bottom of the irradiator guided by Bragg reflection of a Si(311) crystal, the dish was placed upside down. The X-ray energy was 5.35 keV and dose was estimated to be about 10 Gy. The beam size was exactly eliminate within a rectangle area of 1×2mm. The cells in both areas of irradiated and non-irradiated area were observed under microscopic fields.

A fluorescent chemical probe, Cellular Thermoprobe for Fluorescence Ratio (Funakoshi), was used to take red and green fluorescent images. The temperature was determined from the ratio of the two fluorescent intensities based on a calibration curve which was obtained using cell extracts of known temperature beforehand.

3 Results and Discussion

Typical fluorescent images of HeLa cells exposed to X-rays were shown in Fig 1. We tracked the cells for 4h with an off-line fluorescent microscope equipped an incubator for live-cell imaging. The time course of the cellular temperatures calculated using the red and green fluorescence intensities of each cell were shown in Fig. 2.

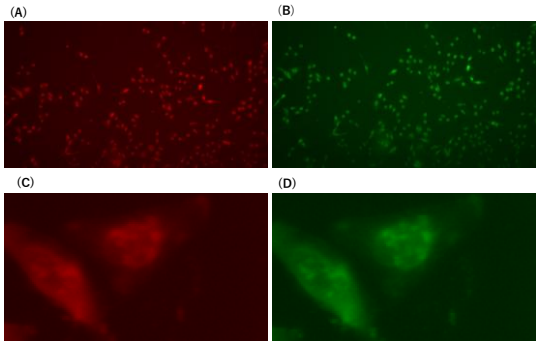

Fig. 1: Image of HeLa cells for red (A) and green (B) fluorescence taken using a 4× objective lens. Two of these cells were enlarged in (C) and (D).

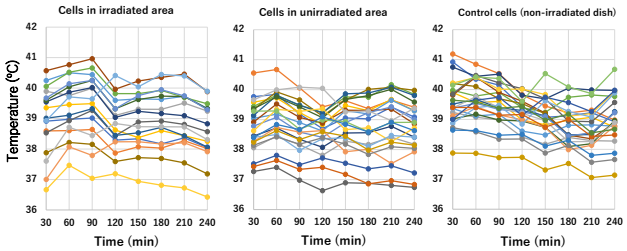

Fig. 2: Time course change of cells in X-ray irradiated (left), unirradiated area (center) and non-irradiated dish (right). Each line shows individual single cells.

The intracellular temperature was significantly different even among the cells in the same dish, distributed in higher temperature, 37−41ºC, than incubation condition of 37ºC. This shows that the cells are “heat generators”. For the irradiated cells, the

dispersion was much larger than that of and prone to be statically higher than that of the control cells, indicating various stress responses for each cell.

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