

Research Group for Radiation and Biomolecular Science

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The research objective of our group is to elucidate the radiation effect of living systems from molecular to cellular level. To explore the effects of X-ray irradiation on cycle dynamics of human cells (HeLa), single cells were tracked using the fluorescent ubiquitination-based cell cycle indicator (Fucci) technique and the obtained data were interpreted based on systems biology.

Single-cell tracking for cell cycle modulation of HeLa cells

Recent advances in microscopic live cell imaging technologies with cell-labeling indicators have enabled us to easily observe various cellular functions as dynamics. Using HeLa-Fucci cells showing their cell-cycle stages with specific colors, we have tracked individual cell cycle after X-ray exposure by a time lapse technique.

A typical fluorescence image for a single non-irradiated (control) cell is shown in Figure Fig. 1. Both red fluorescence for the G1 phase and green fluorescence for the G2/M phase were alternately observed during the culture period, indicating progression of the cell cycle.

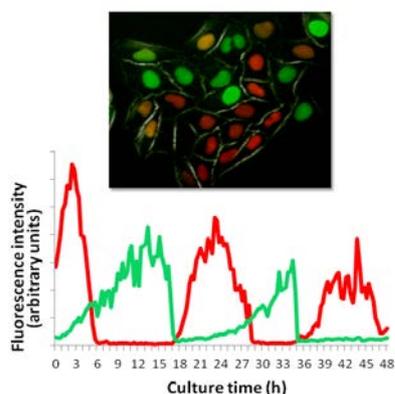


Fig. 1 The time-dependence of fluorescence intensity for a single HeLa-Fucci cell emitting red and green fluorescence, corresponding to the G1 and S/G2 phases respectively, as shown in the photograph. After cell division at 17 h of culture, as shown by the rapid decrease of green (G2) fluorescence, only one of the daughter cells was plotted.

Dynamical analysis of cellular population based on systems biology

The intensity of green fluorescence in G2/M cell nuclei of 21 irradiated cells was monitored for 48 h after X-irradiation, as well as 25 non-irradiated cells. The fluorescent profiles of individual cell are shown as overlapping lines in Fig. 2. To facilitate comparison, the profiles were justified to the time of the first cell division, which was designated as time zero and is the minimum recorded fluorescence value. It should be noted X-irradiation of the HeLa-Fucci cells prolonged the cell cycle, particularly the second G2 phase. Notably, the irradiated cells could be separated into two distinct populations. One population had a similar G2 phase as that of non-irradiated cells that the X-irradiation treatments appear at various points on the fluorescence profiles due to the normalization of cell division.

Generally cell cycle regulated by the cyclin dependent kinase (Cdc2) and cyclin B complex, protein-tyrosine phosphatase

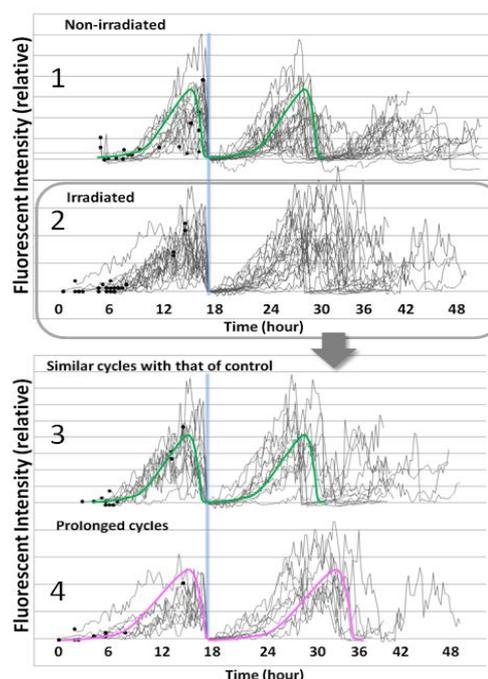


Fig. 2 Time-dependence of green (G2) fluorescence intensity of individual cells. Panel 1: non-irradiated cell. Panel 2: irradiated cells. Irradiated cells with an interval between the first and second cell divisions of shorter and longer than 21 h are shown in Panels 3 and 4, respectively. The points of X irradiation are indicated by the closed circles at the left of each line.

(Cdc25) has been known to be inhibited by "checkpoint proteins", Chk1, Chk2, and a positive-feedback loop regulates entry of HeLa cells into the M phase. Thus we propose the mechanism underlying the appearance of the two types of cell populations is that cell cycle switching from the "ON" to "OFF" state may be sharply induced by increased phosphorylation of Cdc2 when the concentration of phosphorylated Cdc25 falls below a certain threshold value, as shown in Fig. 3 [1].

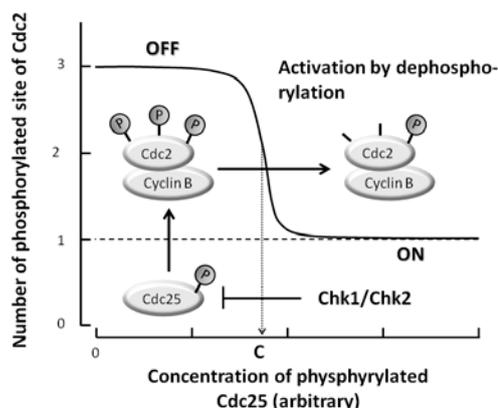


Fig. 3 Proposed switching mechanism of cell cycle regulation.

Reference

- [1] K. Kaminaga, et al., *Radiat. Protect. Dosim.* (Epub ahead of print) doi:10.1093/rpd/nv168.