Examination of the direct visualization toward adverse events caused by space radiation

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The cellular response to DNA damage varies according to the cell types and the cell cycle phases as well as the extent of DNA damage. In the early 1960s, Terasima *et al.*[1] employed a method of selectively harvesting cells in M-phase (mitotic shake-off) to examine cell cycle-dependent responses to DNA damage caused by X-ray irradiation in HeLa cells. They concluded that cell survival was minimal when irradiation was executed during the mitotic (M) and late G1 or early DNA synthesis (early S) phases whereas it was maximal during the early postmitotic (early G1) and premitotic (S to G2) phases. This view has been widely accepted to date. However, conventional methods that use pharmacological reagents and the above-mentioned mitotic shake-off method should have artifactual effects on cells or may be incapable of complete cell-cycle synchronization, especially of highly proliferative tumor cells.

Recently, various cell-cycle visualization methods using fluorescent proteins (FP) have been developed, making it possible to directly analyze the cell cycle stages without synchronization and thus without affecting normal cell functions. Our original Fucci (fluorescent ubiquitination-based cell cycle indicator) technique skillfully utilizes the degradation properties of Cdt1 and Geminin (which undergo cell cycle-dependent proteolysis), two important proteins that contribute to normal cell cycle progression. When the degradation domains of Cdt1 and Geminin are conjugated to Red or Green FP, respectively, the cell cycle transition from G1 to S phase is highlighted with high color contrast, like a traffic light: red to green which mean "stop" and "go," respectively (Fig. 1).[2] We will not go into details here, *i.e.* Fucci (SA) highlights nuclei of cells in G1 phase in red and those of cells in S-G2-M phases in green. Furthermore, the improved Fucci (CA) is able to identify clear interphase boundaries between G1, S, and G2 phases (it highlights G1 phase in red, S phase in green, and G2-M phases in yellow). Then we have successfully demonstrated S phase maximal sensitivity of HeLa cells to UV radiation.[3]

In the coming era when humans are once again traveling to the Moon and Mars as represented by "Artemis mission," it should be necessary to reevaluate the impact of space risks such as radiation and microgravity.[4] Various FP-based approaches have been initiated to directly study the effects of space radiation on living cells.[5,6] Live imaging technology using FPs is expected to make significant contributions to the direct visualization and detailed understanding of radiation-related adverse events.

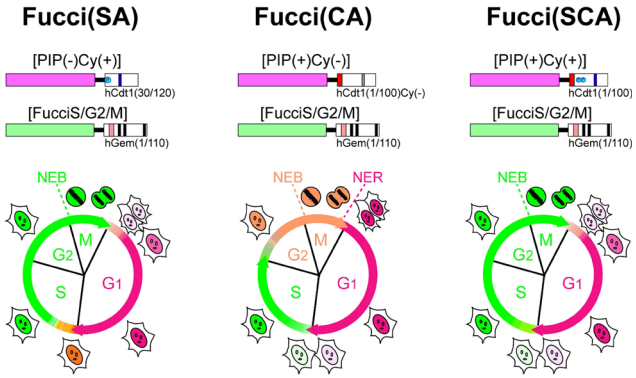

Fig. 1. Cell cycle-phasing capabilities of the Fucci technology. Domain structures of each Fucci repertoire (upper) and cell-cycle phasing capabilities (lower) are shown. For reasons of space, we will not go into details, but please review the original paper to understand Fucci's technology in general. Data were adapted from Sakaue-Sawano *et al.*[3]

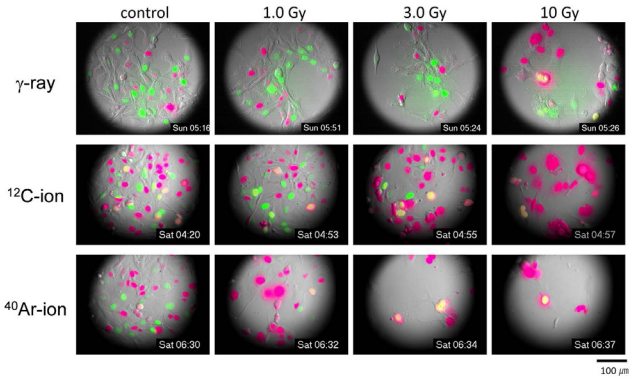

Fig. 2. Example of an experiment to evaluate the effects of heavy-ion irradiation using Fucci Live Imaging. The human Fibrosarcoma cell line; HT1080/Fucci (CA) 5 cells were irradiated with indicated doses for γ-ray, carbon-ion (LET = 50.9 keV/μm) or argon-ion (LET = 185 keV/μm), respectively. Live cell imaging was immediately performed using an LCV100 computer-assisted fluorescence microscope capable of visualizing eight samples simultaneously.[2,3] The data are superimposed snapshots of DIC, Green FP and Red FP 36 hours after irradiation.

We planned to use Fucci (CA) to visualize responses

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to different types and doses of heavy-ion irradiation to understand the heterogeneity or plasticity of cultured tumor cells or non-tumor cells. However, the establishment of a seamless experimental system for time-lapse imaging of living cells before and after a series of irradiations is too challenging due to various physical limitations. Accordingly, we have compromised to use the E5 beamline at the RIKEN RRC for visualizing live cell behavior immediately after heavy-ion irradiation. We proceeded to evaluate the response patterns of cell-cycle populations, their heterogeneity and reproducibility (Fig. 2). Due to the insufficient number of experiments to verify biological differences, we would like to carry our conclusions to the next report. We will conduct further replicated experiments for a comprehensive understanding based on quantitative analysis.

References

1) T. Terasima *et al.*, Nature **190**, 1210 (1961).
2) A. Sakaue-Sawano *et al.*, Cell **132**, 487 (2008).
3) A. Sakaue-Sawano *et al.*, Mol. Cell **68**, 626 (2017).
4) E. Afshinnekoo *et al.*, Cell **183**, 1162 (2020).
5) S. Furukawa *et al.*, Biomed. Res. Int. **2020**, 4703286 (2020).
6) K. Takahashi *et al.*, NPJ Microgravity **7**, 2 (2021).