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DNA damage response following X-irradiation in oral cancer cell lines HSC3 and HSC4

Sirimanas Jiaranuchart[a,b], Atsushi Kaida[a], Yusuke Onozato[b], Hiroyuki Harada[b], Masahiko Miura[a,*]

[a] Department of Oral Radiation Oncology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan
[b] Department of Oral and Maxillofacial Surgery, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo, 113-8549, Japan

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ABSTRACT

Objective: The objective of this study was to characterize the DNA damage response in two human oral cancer cell lines following X-irradiation.

Design: To visualize radiation-induced cell cycle alterations, two human oral cancer cell lines, HSC3 and HSC4, expressing fluorescent ubiquitination-based cell cycle indicator (Fucci) were established in this study. G2 arrest kinetics following irradiation were obtained from two-color flow cytometric analysis and pedigrees of Fucci fluorescence. DNA double strand break repair kinetics were obtained from immunofluorescence staining for phosphorylated histone H2AX, p53-binding protein 1, phosphorylated DNA-dependent protein kinase catalytic subunit, and breast cancer susceptibility gene 1.

Results: Both cell lines showed apparent G2 arrest after 10 Gy of irradiation, but it was more enhanced in the HSC3-Fucci cells. Radiosensitivity was higher in the HSC3-Fucci cells than in HSC4-Fucci cells. Pedigree analysis of Fucci fluorescence revealed that the HSC3-Fucci cells exhibited a significantly longer green phase (normally indicating S/G2/M phases, but here reflective of G2 arrest) when irradiated in the red phase (G1 phase) than HSC4-Fucci cells irradiated in either red or green phases. Non-homologous end joining was marginally suppressed during the G1 phase and markedly more likely to be impaired during the S/G2 phases in HSC3-Fucci cells. When G2 arrest was abrogated by checkpoint kinase 1 or Wee1 inhibitors, only HSC4-Fucci cells exhibited radiosensitization.

Conclusions: We characterized DNA damage response in HSC3-Fucci and HSC4-Fucci cells following irradiation and the former demonstrated inefficient non-homologous end joining, especially during the S/G2 phases, resulting in enhanced G2 arrest. These findings may have clinical implications for oral cancer.

1. Introduction

Ionizing radiation induces varying types of DNA damage (Ward, 1988). Among them, DNA double strand breaks are thought to be crucial in cell death. Non-homologous end joining and homologous recombination are two major repair pathways for DNA double strand breaks; the former occurs throughout the cell cycle while the latter occurs only in the S and G2 phases. In mammalian cells, DNA double strand breaks are repaired mainly via non-homologous end joining, which is a faster, but error-prone process. DNA-dependent protein kinase catalytic subunit and Ku70/86 are known to be core factors of non-homologous end joining. The initial step involves binding of the Ku70/86 heterodimer to the DNA double strand break end, followed by recruitment of DNA-dependent protein kinase catalytic subunit to Ku70/86 (Bakkenist & Kastan, 2004). On the other hand, the use of sister chromatids as a template makes homologous recombination more complicated and slower than non-homologous end joining, but its fidelity is high. Even though many key factors such as breast cancer susceptibility gene 1/2 and Rad51 have been revealed, its detailed repair mechanism still remains elusive (Bakkenist & Kastan, 2004; Hall & Giaccia, 2012a).

In response to DNA double strand breaks, cell cycle checkpoints are activated to stop the cell cycle progression, which allows DNA double strand breaks time to repair. G1/S and G2/M checkpoints are well

Abbreviations: Fucci, fluorescent ubiquitination-based cell cycle indicator; FRPD, first red phase duration; FGPD, first green phase duration

* Corresponding author.
E-mail address: masa.mdth@tmd.ac.jp (M. Miura).

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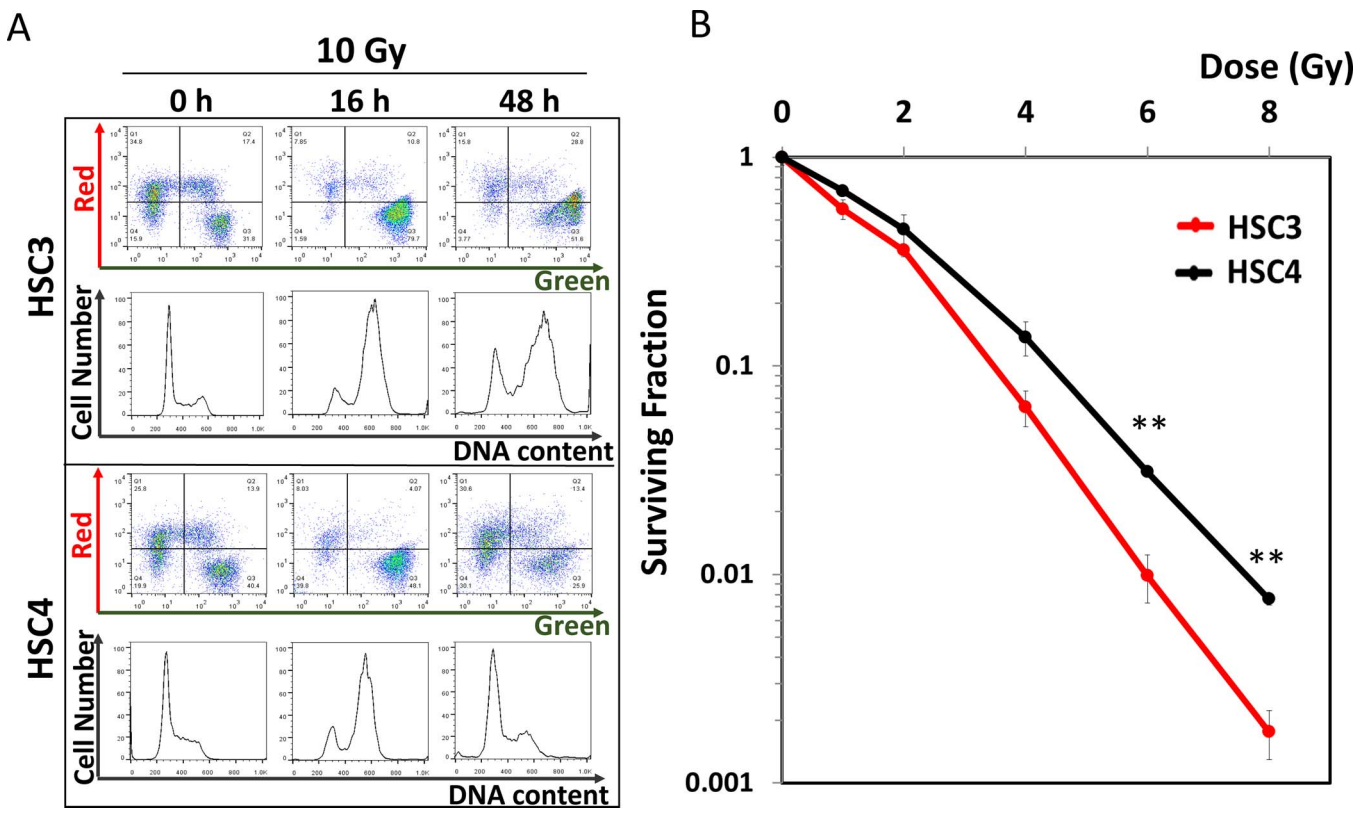

Fig. 1. Radiation-induced cell cycle alterations in HSC3-Fucci and HSC4-Fucci cells and their radiosensitivities. A, Cell cycle alterations following irradiation. Cells were irradiated at 10 Gy and fixed at the indicated times. Flow cytometric analysis was performed on cells prepared for DNA content and two-color Fucci fluorescence analysis of Fucci. B, Dose-cell survival curves. Cells were irradiated and the surviving fractions were obtained via colony formation assay. Data represent means ± S.E. of triplicate determinants. **, $p < 0.01$ (HSC3 vs. HSC4).

investigated, the former requiring normal p53 function, but not the latter. Therefore, tumor cells with deficient p53 functions exhibit apparent G2 arrest. In this process, factors including ataxia telangiectasia mutated, ataxia telangiectasia and Rad3-related protein, checkpoint kinase 1/2, and cell division cycle 25A/C play important roles (Waldman, Kinzier, & Vogelstein, 1995; Sancar, Lindsey-Boltz, Unsal-Kaqmaz, & Linn, 2004). After adequate repair of the damaged DNA, cells are released from the G2 arrest. This phenomenon of DNA repair and cell cycle checkpoints triggered by DNA damage is referred to as DNA damage response. It is believed that DNA damage response is a pivotal deterministic factor for intrinsic radiosensitivity (Hall & Giaccia, 2012b). Therefore, characterizing DNA damage response in oral cancer cell lines would be useful in designing radiosensitizing strategies for oral cancer.

Fluorescent ubiquitination-based cell cycle indicator (Fucci) is a live cell cycle-visualizing tool that emits red and green fluorescence in the G1 and S/G2/M phases, respectively (Sakaue-Sawano et al., 2008). We previously reported that the system allowed us to visualize radiation-induced G2 arrest kinetics in SAS (a human oral cancer cell line) and HeLa cells (Kaida, Sawai, Sakaguchi, & Miura, 2012; Onozato, Kaida, Harada, & Miura, 2017). In this study, we newly established two human oral cancer cell lines, HSC3 and HSC4, expressing Fucci and characterized DNA damage response in detail using these cell lines. Based on our results, we also tried to propose DNA damage response-dependent radiosensitizing strategies for oral cancer such as HSC3 and HSC4 cells.

2. Materials and methods

2.1. Cell lines and culture conditions

Two p53 mutant type human tongue squamous cell carcinoma cell lines, HSC3 and HSC4 (Sakai & Tsuchida, 1992) were obtained as a generous gift from Dr S. Abe (Department of Maxillofacial Surgery, Tokyo Medical and Dental University). All the cells were grown in Dulbecco's minimum essential medium (Sigma-Aldrich, St. Louis, MO) containing high-glucose (4500 mg/l), supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 mg/ml streptomycin. They were maintained in a humidified incubator at 37 °C containing 5% CO_2.

2.2. Establishing HSC3-Fucci and HSC4-Fucci cell lines

To establish Fucci-expressing HSC3 and HSC4 cell lines, Fucci plasmids (CFII-EF-mKO2-hCdt1 [30/120] and CFII-EF-mAG-hGeminin [1/110] (provided by the Riken Bio-Resource Center via the National Bio-Resource Project of the MEXT, Japan) were transduced and selected using flow cyotmetry as described previously (Onozato et al., 2017). HSC3 and HSC4 cells expressing red fluorescence in G_1 phase and green fluorescence in $S/G_2/M$ phase, respectively, were designated as HSC3-Fucci and HSC4-Fucci cells. This experiment was approved by the Genetically Modified Organisms Safety Committee of Tokyo Medical and Dental University (2012-006C3).

2.3. Colony formation assay

To determine HSC3 and HSC4 radiosensitivity, an appropriate number of cells [100–4000 cells/plate depending on the irradiation dosage (1, 2, 4, 6, and 8 Gy)] were plated in 60 mm dishes in triplicate. For cells treated with G2 arrest-releasing inhibitors, 180 nM Wee1 inhibitor (MK-1775; Axon Medchem, Groningen, Netherlands)(Bridges et al., 2011) or 300 nM checkpoint kinase 1 inhibitor (PF-00477736; Sigma-Aldrich) (Zhang et al., 2009) was used 1 h before irradiation. After a 24 h treatment, the medium was replaced with fresh growth