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Transmission of persistent ionizing radiation-induced foci through cell division in human primary cells

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ABSTRACT

Unrepaired DNA double-strand breaks (DSBs) induced by ionizing radiation are associated with lethal effects and genomic instability. After the initial breaks and chromatin destabilization, a set of post-translational modifications of histones occurs, including phosphorylation of serine 139 of histone H2AX (γH2AX), which leads to the formation of ionizing radiation-induced foci (IRIF). DSB repair results in the disappearance of most IRIF within hours after exposure, although some remain 24 h after irradiation. Their relation to unrepaired DSBs is generally accepted but still controversial. This study evaluates the frequency and kinetics of persistent IRIF and analyzes their impact on cell proliferation. We observed persistent IRIF up to 7 days postirradiation, and more than 70% of cells exposed to 5 Gy had at least one of these persistent IRIF 24 h after exposure. Moreover we demonstrated that persistent IRIF did not block cell proliferation definitively. The frequency of IRIF was lower in daughter cells, due to asymmetric distribution of IRIF between some of them. We report a positive association between the presence of IRIF and the likelihood of DNA missegregation. Hence, the structure formed after the passage of a persistent IRI focus across the S and G2 phases may impede the correct segregation of the affected chromosome's sister chromatids. The ensuing abnormal resolution of anaphase might therefore cause the nature of IRIF in daughter-cell nuclei to differ before and after the first cell division. The resulting atypical chromosomal assembly may be lethal or result in a gene dosage imbalance and possibly enhanced genomic instability, in particular in the daughter cells.

1. Introduction

DNA damage is a key event in cell response to ionizing radiation, one involving genetic and epigenetic modifications that may affect the homeostasis of healthy tissues in exposed individuals. In particular, DNA double-strand breaks (DSBs) that are incorrectly or not repaired are associated with lethal effects and genomic instability [1]. DSBs trigger activation of phosphatidylinositol-3′-OH kinase-related kinases (PIKK), such as ataxia telangiectasia mutated (ATM) and DNA-dependent protein kinase (DNA-PK); these, in turn, phosphorylate the histones H2AX located around the break, at serine 139 [2–5]. This phosphorylation of H2AX (γH2AX) quickly spreads over several megabases on the adjacent chromatin [5,6] and results in the formation of microscopically visible nuclear foci, known as ionizing radiation-induced foci (IRIF). One explanation for this extensive phosphorylation postulates that DSBs cause massive relaxation of chromatin coiling [6]. γH2AX initiates the formation of a platform to attract and retain proteins, such as Nijmegen breakage syndrome 1 (NBS1), mediator of DNA damage checkpoint protein 1 (MDC1), breast cancer susceptibility 1 (BRCA1), and p53-binding protein 1 (53BP1); these lead in turn to the recruitment of DNA damage repair proteins [7–10].

The number of γH2AX IRIF peaks at 30 min after exposure, and most of these IRIF disappear within hours of exposure [5,6]. This disappearance is thought to be related to DSB repair and restoration of chromatin integrity and structure [11,12]. However, some IRIF remain 24 h after exposure [13], and their association with residual DSBs is generally accepted since DDR proteins are still present within these persistent IRIF [13,14]. Although the nature, role, and consequences of these IRIF are still unclear, some studies suggest

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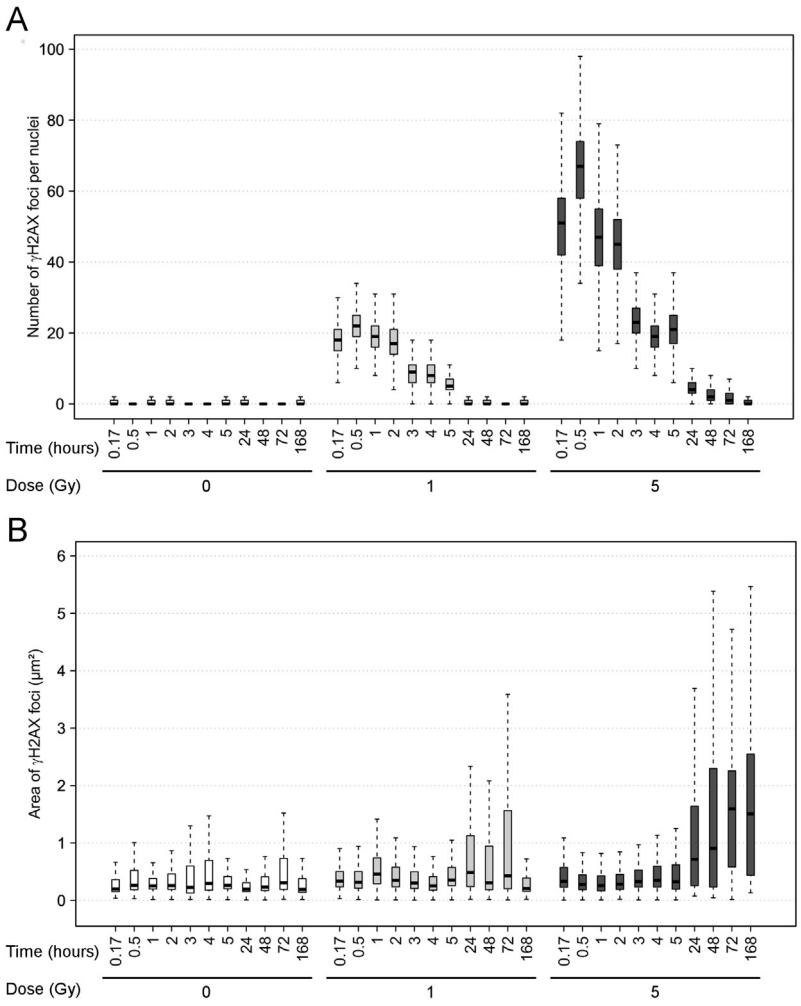

Fig. 1. Numbers and areas of γH2AX foci in G0/G1 primary HUVECs as a function of time after irradiation by 1 Gy (light gray boxes), 5 Gy (dark gray boxes), and in non-irradiated cells (white boxes). Box-and-whisker plots of (A) the number of γH2AX foci per nucleus, and (B) their corresponding areas. Bold black bars of boxplots correspond to medians. The lower and upper borders of the box correspond to the first and third quartiles, respectively, and the upper and lower whiskers to 1.5 times the interquartile distance. The numbers of γH2AX foci per nuclei and their respective areas were evaluated with image analysis software on around 3000 cells for each post-irradiation time, corresponding to one representative experiment. The number of γH2AX IRIF peaked 30 min post-exposure. The area of γH2AX IRIF increased as the number per nucleus decreased. Most early γH2AX IRIF (before 24 h after exposure) were small, but persistent IRIF were characterized by a larger size.

that they are involved in the inhibition of G1/S progression [13] but also in radiation-induced cellular senescence and death [15,16].

In this work, we evaluated the kinetics of the appearance and disappearance of γH2AX and 53BP1 IRIF. We characterized persistent IRIF up to 7 days after exposure to ionizing radiation and evaluated their impact on the resumption of the cell cycle and the division of normal human primary cells irradiated during the G0/G1 phase.

2. Materials and methods

2.1. Cell cultures and irradiation

Primary human umbilical vein endothelial cells (HUVECs, C2519A, lot. 0000087758) were isolated by Lonza from human tissue (from 3 females and 1 male) donated after permission was obtained for its use in research applications by informed consent or legal authorization. All cells tested negative for mycoplasma, bacteria, yeast, and fungi. Cell lots and donors were tested and negative for HIV-1, hepatitis B, and hepatitis C. The HUVECs were cultured at 37 °C, with 95% humidity and 5% CO_2 in EGM-2 media optimized for the proliferation of endothelial cells and supplemented with 5% fetal bovine serum, hydrocortisone, hFGF-B, VEGF, R3-IGF-1, ascorbic acid, hEGF, gentamicin, and amphotericin-B (EGM-2MV BulletKit, CC-3202, Lonza). We used HUVEC primary cells at low passages and evaluated their cytogenetic status by M-FISH during passages 2 (P2) and 4 (P4) [17]. No clonal abnormality was observed, and the proportions of males and females obtained by cytogenetic analysis were consistent with the initial cell pools (3 females and 1 male). To obtain cells in G0/G1 phase of the cell cycle at irradiation, we synchronized cells by contact inhibition induced in confluent culture. Subsequently, synchronized cells were seeded on glass in Nunc® Lab-Tek® II chamber slide systems (Thermo Fisher Scientific) for 5 h before irradiation. The experimental protocol was designed to ensure that the DNA damage took place in cells in G0/G1 (initial synchronization of the cells) but also that nothing other than irradiation could block resumption of the cell cycle. We therefore verified that at 5 h post-seeding, cells were in G0/G1 and almost all adherent and that the density of seeding would allow future cell growth. An Elekta Synergy Platform (linac accelerator) was used to deliver X-rays with a maximum energy of 4 MeV (4 MVp) at a dose rate of 2.3 Gy min^{-1} and with a delivered dose uncertainty rate of 7%. We delivered two different doses: 1 and 5 Gy. Culture media were not renewed after irradiation. To monitor