Original Article

p21 is Responsible for Ionizing Radiation-induced Bypass of Mitosis*

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Abstract

Objective To explore the role of p21 in ionizing radiation-induced changes in protein levels during the G2/M transition and long-term G2 arrest.

Methods Protein expression levels were assessed by western blot in the human uveal melanoma 92-1 cells after treatment with ionizing radiation. Depletion of p21 was carried out by employing the siRNA technique. Cell cycle distribution was determined by flow cytometry combined with histone H3 phosphorylation at Ser28, an M-phase marker. Senescence was assessed by senescence-associated-β-galactosidase (SA-β-gal) staining combined with Ki67 staining, a cell proliferation marker.

Results Accompanying increased p21, the protein levels of G2/M transition genes declined significantly in 92-1 cells irradiated with 5 Gy of X-rays. Furthermore, these irradiated cells were blocked at the G2 phase followed by cellular senescence. Depletion of p21 rescued radiation-induced G2 arrest as demonstrated by the upregulation of G2/M transition kinases, as well as the high expression of histone H3 phosphorylated at Ser28. Knockdown of p21 resulted in entry into mitosis of irradiated 92-1 cells. However, cells with serious DNA damage failed to undergo cytokinesis, leading to the accumulation of multinucleated cells.

Conclusion Our results indicated that p21 was responsible for the downregulation of G2/M transition regulatory proteins and the bypass of mitosis induced by irradiation. Downregulation of p21 by siRNA resulted in G2-arrested cells entering into mitosis with serious DNA damage. This is the first report on elucidating the role of p21 in the bypass of mitosis.

Key words: G2/M transition; DNA damage; Ionizing radiation; G2 arrest

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INTRODUCTION

Environmental factors, such as toxic chemicals, oxidative stress, and ionizing radiation, could induce DNA damage in organisms.

Once DNA damage is generated, proliferating cells appear to enter a sustained arrest in the G1 or G2 phase of the cell cycle, which allows the cells to repair the damage. If the damage is repaired, cells reenter the cell cycle and become normal. If the damage is not repaired, cellular senescence will be initiated by activation of the persistent DNA damage response (DDR) pathway[1]. Normally, senescent cells

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contain diploid DNA, a typical characteristic of the G1 phase in somatic or *in vitro* cultured cells[2-3]. However, DNA damage-induced senescent cells contained a sizeable subpopulation with tetraploid DNA content[4-6]. These observations imply that the arrested cells with tetraploid DNA enter senescence at the G2 phase. This is inconsistent with the notion that all intermediate phases are unstable, except for G0 and the early G1 phase[7-9]. Our previous work[10] and other studies[4,11] suggested that these G2-arrested cells with DNA damage bypassed mitosis and directly slipped into G1 phase, then underwent senescence.

In this progression, proteins essential for G2/M transition, such as cyclin B1[10,12-14], cyclin-dependent kinase 1 (CDK1)[10], aurora kinase A (Aurora A)[10], and polo-like kinase1 (PLK1)[10] declined dramatically. cyclin B1/CDK1 is a master mitotic regulator[15]. In cycling cells, cyclin B1 accumulated during G2 is degraded at the metaphase to anaphase transition, and is predominantly localized in the cytoplasm until the beginning of mitosis[4,16]. Rapid nuclear translocation of cyclin B1/CDK1 is a key event for nuclear envelope breakdown. Aurora A and PLK1 are two other key mitotic kinases[15]. Aurora A is required for mitotic entry. The initial activation of Aurora A in late G2 phase is essential for recruitment of the cyclin B1/CDK1 complex to centrosomes, where it becomes activated and commits cells to mitosis[17-19]. PLK1 plays an essential role in both the onset of the G2/M transition and cytokinesis[15]. The overexpression of PLK1 is strongly correlated with a wide spectrum of human cancers and poor prognosis[20]. In normal cell cycle progression, these kinases are accumulated at G2 phase and degraded at anaphase by the anaphase-promoting complex/cyclostome (APC/C) to promote the exit of cells from mitosis[21]. The unscheduled degradation of these kinases is believed to result in long-term G2 arrest. However, the types of mechanisms that drive the unscheduled degradation of these mitotic kinases remain unknown.

Interestingly, accompanying the degradation of mitotic kinases, tumor protein p53 (TP53/p53) and cyclin-dependent kinase inhibitor 1A (CDKN1A/p21) were stably accumulated after ionizing radiation (IR)[10]. p21 is a multifunctional protein and a key player in regulating different cellular processes. The transcription of p21 is regulated by p53-dependent and -independent pathways[22]. It is clear that p53 and p21 are required to maintain G2 arrest in human cells[23-24]. Earlier and recent studies strongly suggested that p21 upregulation mediated cyclin B1 degradation by driving nuclear translocation of cyclin B1 in response to DNA damage[4,14,16]. However, it is still unclear whether p21 is responsible for the degradation of these mitotic kinases during G2 arrest after irradiation. In this study, the relationship between the upregulation of p21 and the changes in the protein levels of these mitotic kinases was investigated after exposure of human uveal melanoma 92-1 cells to ionizing radiation, the most common treatment method in cancer therapy.

MATERIALS AND METHODS

Cell Culture and Irradiation

Human uveal melanoma 92-1 cells[25] and normal fibroblast GM-08398 cells were cultured in RPMI-1640 medium (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, Life Technologies, NY, USA), 100 μg/mL streptomycin and 100 units/mL penicillin in a humidified atmosphere with 5% CO_2. Cells were seeded in 12-well plates (1×10^5 cells per well), 35-mm culture dishes (2×10^5 cells per dish) or 60-mm culture dishes (5×10^5 cells per dish), incubated for 48 h to 70% confluence, and irradiated at room temperature with X-rays generated by a Faxitron RX650 (Faxitron RX650, Faxitron Bioptics, Lincolnshire, IL, USA) at a dose rate of 1 Gy/min. Iron ion (LET 400 KeV/μm) irradiation was performed at the HIRFL (Heavy Ion Research Facility of Lanzhou, Institute of Modern Physics, Lanzhou, China). The dose rate ranged from 0.2 to 0.3 Gy/min.

Cell Cycle Assay

After various periods of post-treatment incubation, cells were harvested and fixed with 70% pre-chilled ethanol for over 24 h at -20 °C. The fixed cells were washed twice with PBS, treated with 100 μg/mL RNase A, and stained with 50 μg/mL propidium iodide mixed buffer (BD Biosciences, San Jose, CA) for 30 min at 37 °C. Cell cycle distribution was analyzed with FlowJo. DNA content was measured with CellQuest (Benton Dickinson).

Immunofluorescence

For immunostaining, cells were seeded on sterile coverslips at a density of 4×10^5 cells per dish in 60-mm culture dishes, cultured for 24 h, then irradiated and incubated (37 °C, 5% CO_2) for the indicated time. The irradiated cells were fixed with