3 - 73 Both p21 and Cytoskeleton are Involved in Radiation Induced Cell Cycle Arrest in Uveal Melanoma 92-1 Cells

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One of the main engines that drive cellular transformation is the loss of proper control of the cell cycle. The cyclin-dependent kinase inhibitor p21 (also known as p21WAF1/Cip1) promotes cell cycle arrest in response to many stimuli[1]. Our recent study[2,3] showed that a global and stable cell cycle block was established in 92-1 cells after 10 Gy of X-ray and carbon ion irradiation and cells underwent senescence 5 d later. p21 was detected at high levels from 4 h to 5 d after irradiation in these cells, which implied that p21 played a crucial role in cellular response to irradiation.

To explore whether p21 up-regulation was sufficient to induce cell cycle arrest and cellular senescence in 92-1 cells, we depleted p21 after transient transfection of small interfering RNA (siRNA). Then we treated cells with 5 Gy of X-rays and detected cell proliferation and cellular senescence 5 d later. Interestingly, depletion of p21 in 92-1 cells did not rescue cells from radiation induced cell cycle block. However, the percentage of multinuclear cells was distinctly increased (Fig. 1), while the beta-Gal-positive cells were decreased at 5 d in cells depleting p21. Whereas the growth-inhibitory functions of p21 were associated with its nuclear localization, we detected the p21 localization by immunofluorescence assay in 92-1 cells. Compared with the cells directly irradiated, cells transfected with siRNA-p21 lose its p21 nuclear localization ability.

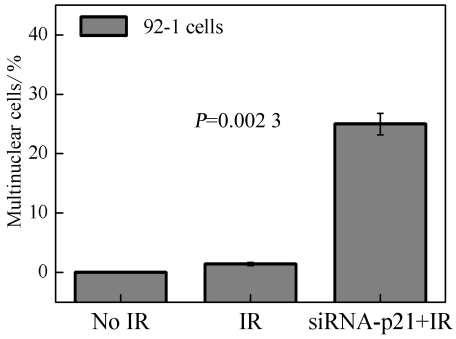

Fig. 1 Multinuclear cells calculated at 5 d after different treatment. No IR: Control; IR: Cells were treated with 5 Gy of X-rays and cultured for 5 d; siRNA-p21+IR: Cells were transient transfected with siRNA to deplete p21, then treated with 5 Gy X-rays and cultured for 5 d.

Since F-actin links to cytokinesis and is also required for maintenance of normal cellular morphology, we examined F-actin in 92-1 cells after irradiation. Depolymerization of F-actin was observed in cells after exposure to ionizing radiation. This depolymerization remodeled the cellular morphology and could destroy cytokinesis in the cells.

These results imply that p21 is important for radiation induced cell cycle block. Depletion of p21 rescues cells from the radiation induced nucleus division suspension. Since cytoskeleton structure was remodeled after irradiation, this may abolish cytokinesis of 92-1 cells. We conclude that both p21 and cytoskeleton remodeling are responsible for multinuclear cells in 92-1 cells.

References

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