Premature Senescence Induced by Ionizing Radiation Requires AKT Activity and Reactive Oxygen Species in Glioma

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1. Introduction

Loss of PTEN, a tumor suppressor gene has frequently observed in human gliomas [1], which conferred AKT activation and resistance to ionizing radiation (IR) and anti-cancer drugs. Recent reports have shown that AKT activation induces premature senescence [2] through increase of oxygen consumption and inhibition of expression of ROS scavenging enzymes [3]. In this study, we compared cellular response to IR in the PTEN-deficient U87, U251, U373 or PTEN-proficient LN18, LN428 glioma cells.

2. Methods and Results

2.1 Senescence was induced in PTEN mutant or deficient U87, U251, U373 glioma cell lines but not in LN18 and LN428 PTEN wt glioma cell lines by IR

To address the responses of the various glioma cell lines to IR, we first examined cell phenotype after treatment with various doses of IR. Interestingly, while PTEN-deficient U87, U251, and U373 glioma cell lines underwent senescence, LN18 and LN428 PTEN-proficient glioma cell lines showed apoptosis. Microscopic analyses showed that only U87, U251, U373 cell lines exhibited the morphology of senescent cells (flattened large cells) and were positive for senescence–associated β-galactosidase (SA-β-Gal), a hallmark of senescent cells as IR dose dependent manner (Fig. 1). In addition CDK inhibitor p21 was upregulated by IR only in PTEN deficient glioma cell lines. In contrast, LN18 and LN428 cell lines showed apoptotic phenotype and stained by trypan blue as IR dose dependent manner.

2.2 The apoptotic pathway could be activated by anti-cancer chemotherapy drug, doxorubicin in U87, U251, and U373 glioma cell lines

To assess whether apoptotic pathway can be activated in U87, U251, and U373 glioma cell lines, we treated these glioma cell lines with high doses of IR or genotoxic-drug, doxorubicin. U87, U25 and U373 cells underwent senescence and showed SA β-Gal positivity as well as p21 upregulation even in 20 and 40 Gy of IR treatment. However, LN 18 and LN428 cells also underwent apoptosis and showed PARP cleavage.

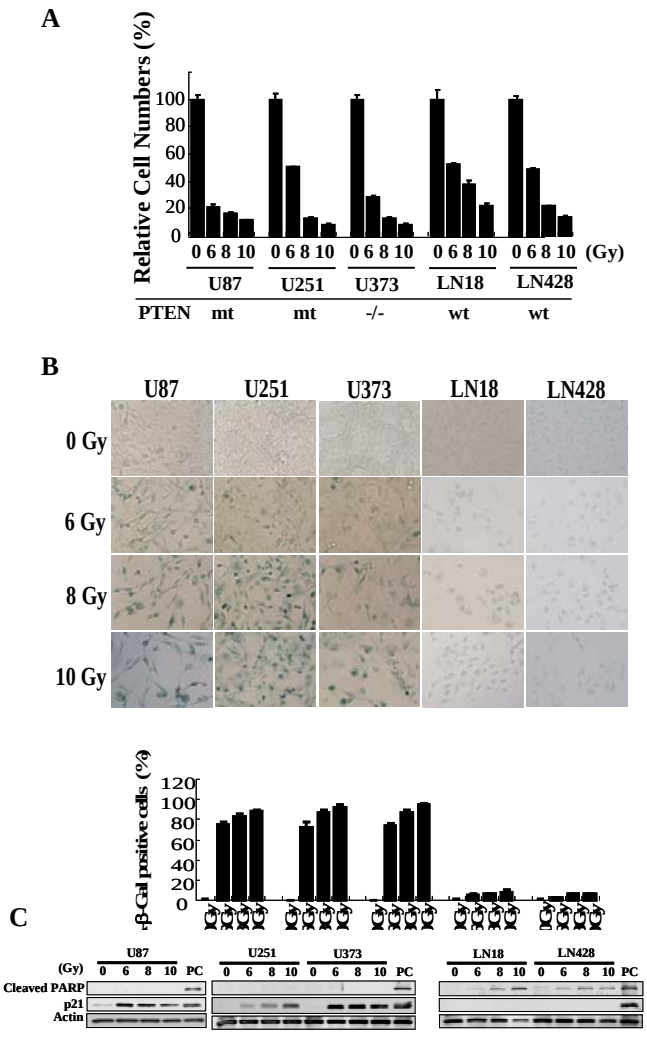

Fig. 1. IR induced senescence in PTEN mutant U87 and U251 or PTEN deficient U373 but apoptosis in PTEN wt LN18 and LN428 glioma cell lines. Cell numbers and SA-β-Gal activities after treatment IR were examined.

In contrast, 10 μg/ml or 20 μg/ml of doxorubicin treated PTEN-deficient or –progficient ells were led to apoptosis (Fig. 2A).

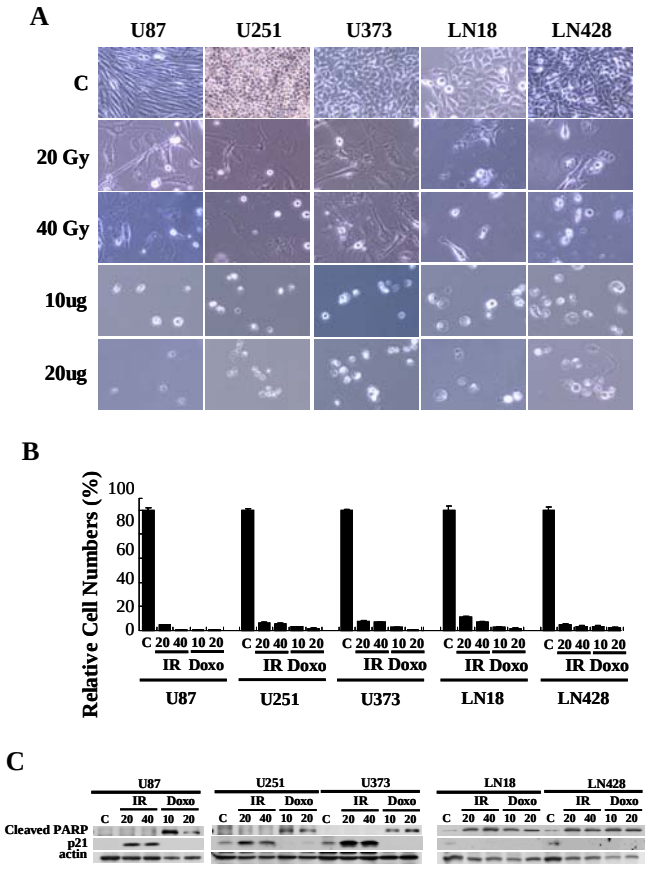

Fig. 2. The apoptotic pathway could be activated by genotoxic drug in PTEN-deficient glioma cells. Cell death was measured with trypan blue staining.

2.3 ROS was required to induce senescence by IR in U87

To verify ROS role in senescence and apoptosis induced by IR, we treated ROS scavenger NAC prior to IR treatment in U87 and LN18 cell lines and examined morphological changes and SA-β-Gal positivity. Interestingly, while NAC blocked induction of senescence by IR in U87, it did not inhibit apoptotic cell death by IR in LN18.

2.4 Upregulation of p21 is essential for Akt-induced senescence by IR

Next we assessed the status of the p53 senescence pathway. To address the function of p53 in the cellular senescence induced by IR in U87, which have a wt p53, we examined the cell phenomenon using siRNA for p53. p53 siRNA prevented senescence which were induced by IR. Induction of p21 through p53 activiation was downregulated . However, PARP cleavage was not detected by the treatment of siRNA against p53.

To confirm the role of p21 in Akt-induced senescence pathway, we transfected siRNA against p21 to U87. Senescence induced by IR was prevented in p21siRNA treated U87 cells and SA-β-Gal positivity was reduced. Conclusively, both p53 and p21 were required to induce senescence by IR.

3. Conclusions

In this study, we examined the responses of five glioma cell lines, U87, U251, U373, LN18, and LN428 to IR. U87, U373, and U252 glioma cell lines harboring PTEN mutation or deficiency showed AKT activation and high level of ROS. In contrast, LN428 and LN18 cells harbor wild type PTEN showed no AKT activation and low level of ROS. We found that U87, U252, U373 cells underwent senescence via AKT activation, p21 induction, and ROS accumulation. However, LN428 and LN18 choose apoptosis pathway rather than senescence after IR exposure. Our data indicate that different cellular context can determine final cell fate and induction of premature senescence via Akt can be alternative antitumor mechanism in glioma cells which are resistant to apoptosis.

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