AKT activation inhibits radiation-induced apoptosis via acinus gene regulation

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Introduction

The serine/threonine kinase AKT (protein kinase B, PKB) promotes cell survival by phosphorylating and inhibiting components of the intrinsic cell death machinery. AKT has been shown to be potently activated in response to a wide variety of growth factors and ionizing radiation (Soderlund et al., 2005). AKT predominantly localizes to the cytoplasm and translocates to the nucleus upon cellular stimulation (Lu et al.,, 1998). AKT regualtion of apoptosis is correlated with the control of key downstream substrates within the nucleus. Acinus predominantly locates within the nucleus and is known to be a member of the apotosiss-and slicing-associated protein (ASAP) complex (Schwerk et al., 2003). It was reported that AKT phosphorylated acinus and inhibits acinus proteolytic cleavage by caspases (Hu et al., 2005). Therefore, we investigated the role of AKT activation on cell survival and acinus regulation in response to ionizing radiation. Here, we show that AKT activation inhibits cell death during radiation-induced apoptosis through the regulation of acinus gene expression in normal human cells.

Materials and Methods

Human lung fibroblast CCD-18Lu cells and B lymphoblast IM-9 cells were irradiated with γ-rays at a dose of 0.05 to 10 Gy. Colony formation assay and MTT assay were used to measure radiation-induced cell death. Immunoblot analysis and quantitative RT-PCR were also performed.

Result

AKT activation protects cells from radiation-Induced cell death

We examined the role of AKT on the radiation induced cell death, we established stable cell lines from CCD-18Lu cells infected with a retrovirus expressing constitutively active AKT (CA-AKT). CCD-18Lu.CA-AKT cells were irradiated with γ-rays at a dose in the range of 0.05 to 10 Gy and cell viability was assessed after 48 hours of irradiation. CA-AKT overexpression significantly inhibited radiation-induced cell death compared to that of the controls (fig. 1).

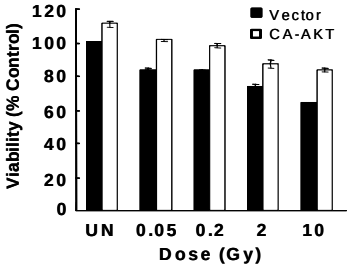

fig 1. Effect of AKT activation on radiation-induced cell death.

AKT activation causes upregulation of Acinus expression

We further investigated whether acinus expression is regulated by AKT activity. CA-AKT overexpression induced an increase in acinus expression in both untreated control and irradiated cells (fig. 2).

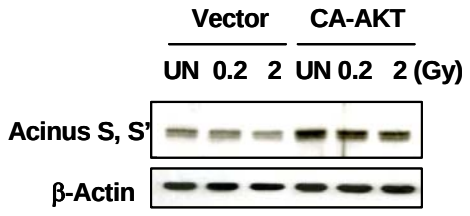

fig 2. Acinus expression in CCD-18Lu.Vector and CCD-18Lu.CA-AKT, which were irradiated with 0.2 and 2 Gy of γ-rays.

NF-kB activation regulates acinus gene expression

To investigate whether NF-κB has a role in acinus L and acinus S expression, we produced stable CCD-18Lu cells overexpressing the NF-κB repressor mutant of IkBα (IκBαM). We investigated acinus mRNA levels in CCD-18Lu.IκBαM cells by using quantitative RT-PCR analysis. The mRNA levels of acinus L and acinus S were decreased in CCD-18Lu.IκBαM cells when compared to controls. This result shows that NF-κB regulates both acinus L and acinus S expression at the transcriptional level (fig. 3).

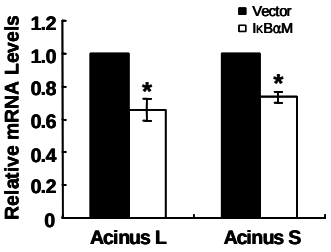

fig 3. The mRNA levels of acius L and acinus S were measured by quantitative RT-PCR

Discussion

In the present study, we examined the novel regulatory mechanism responsible for the radiation induced cell death in normal human cells and found that AKT activation is closely associated with the inhibition of ionizing radiation-induced cell death. In addiation, we showed that AKT activation regulates acinus gene expression via NF-κB pathway. In conclusion, our data demonstrate that the AKT activation increases the expression of acinus via NF-κB pathway and then blocks ionizing radiation-induced cell death.

Referances

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