Low-dose rate of ionizing radiation induces AKT gene expression via NF-κB pathway and cell growth

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Introduction

Exposure to low dose and low-dose rate of ionizing radiation is an important issue in radiation protection. Various studies on the dose-effect relationship of ionizing radiation and cell response have been performed with particular emphasis on the biochemical mechanism involved. Some data were reported that low dose radiation could initiate beneficial effects by stimulating cell growth, DNA repair, activation of transcription factors and gene expression (Calabrese et al., 2004; Li et al., 2004). The induction of cellular protective mechanisms that are distinctively different from high dose and high-dose rate radiation response appears to be involed, but the actual contribution of signaling and molecular mechnism is still unknown. We previously reported that low dose of ionizing radiation induced cell survival through the activation of MAPKs and AKT (protein kinase B, PKB) pathway (Kim et al., 2007; Park et al., 2009). The serine/threonine kinase AKT 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. Therefore, the present study investigates the role of AKT pathway in response to low dose and low-dose rate of ionizing radiation.

Materials and Methods

Human lung fibroblast CCD-18Lu cells were irradiated with γ-ray 5 or 20 cGy at a dose rate of 1 cGy/h. To measure the viability of the irradiation cells, crystal violet assay was used according to the manufacturer's instruction. Immunoblot analysis and electrophoretic mobility shift assay using Light Shift Chemiluminescent EMSA kit (Pierce) were performed.

Results

Low dose radiation induces AKT activation via the increase of gene expression.

To determine whether low-dose rate radiation induces AKT activation in CCD-18Lu cells, the expressions of phosphorylated AKT (p-AKT) and AKT were assessed after irradiation with 5 cGy.

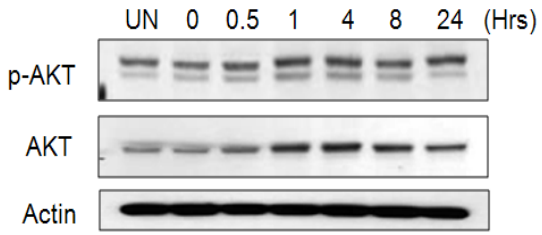

Figure 1. The levels of p-AKT and AKT expression in CCD-18Lu cells which were irradiated with γ-ray of 5 cGy at a dose rate of 1 cGy/h.

The 5 cGy induced AKT phosphorylation in CCD-18Lu cells. AKT expression was also increased by 5 cGy (Fig. 1).

AKT activation induces cell growth after low dose of ionizing radiation.

We examined the role of AKT on cell growth in response to low dose of ionizing radiation. We established stable cell lines from CCD-18Lu cells infected with retrovirus expressing constitutively active AKT (CA-AKT). CCD-18Lu.CA-AKT cells were irradiated with γ-ray of 5 or 20 cGy and cell viability was assessed after 48 (A) or 72 hours (B) of irradiation. CA-AKT overexpression significantly increased low dose radiation-induced cell growth compared to that of the controls (Fig. 2).

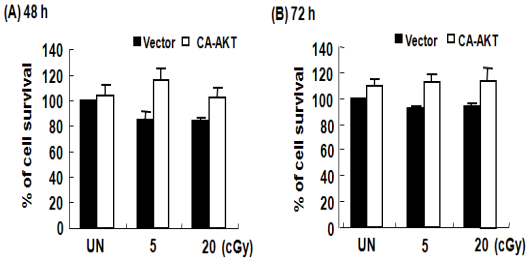

Figure 2. Effect of AKT activation on low dose radiation-induced cell growth in CCD-18Lu cells overexpressing CA-AKT.

NF-κB activation regulates AKT and acinus gene expression.

To investigate whether NF-κB has a role in AKT, acinus L and acinus S expression, we produced stable CCD-18Lu cells overexpressing the NF-κB repressor mutant of IkBα (IκBαM). We investigated AKT and acinus expression in CCD-18Lu.IκBαM cells. The overexpression of Iκ BαM inhibited the increases of p-AKT and AKT expression induced by low dose radiation compared to vector control. The increases of acinus L and acinus S expression by low dose radiation were also blocked in CCD-18Lu.IκBαM cells (Fig. 3).

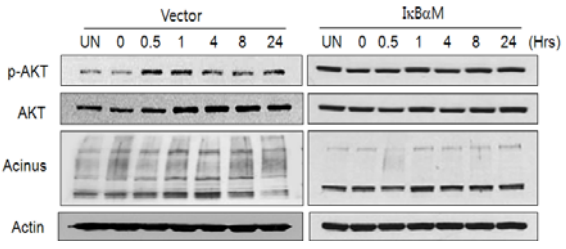

Figure 3. Expression of AKT and acinus expression in CCD-18Lu.IκBαM cells. The cells were irradiated with 5 cGy at a dose rate of 1 cGy/h and incubated for the indicated time periods.

Conclusion

In the present study, we examined the regulatory mechanism responsible for cellular response induced by low-dose rate of ionizing radiation in normal human cells. We found that AKT activation is closely associated with the cell growth induced by low-dose radiation and NF-κB activation by low dose radiation regulates AKT activation via gene expression and acinus expression. In conclusion, our data demonstrate that low dose radiation induces AKT activation and AKT gene expression via NF-κB pathway and then enhances cell growth in normal human lung fibroblast CCD-18Lu cells.

Referances

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