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Regulation of radiation-induced protein kinase Cδ activation in radiation-induced apoptosis differs between radiosensitive and radioresistant mouse thymic lymphoma cell lines

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Abstract

Protein kinase Cδ (PKCδ) has an important role in radiation-induced apoptosis. The expression and function of PKCδ in radiation-induced apoptosis were assessed in a radiation-sensitive mouse thymic lymphoma cell line, 3SBH5, and its radioresistant variant, XR223. Rottlerin, a PKCδ-specific inhibitor, completely abolished radiation-induced apoptosis in 3SBH5. Radiation-induced PKCδ activation correlated with the degradation of PKCδ, indicating that PKCδ activation through degradation is involved in radiation-induced apoptosis in radiosensitive 3SBH5. In radioresistant XR223, radiation-induced PKCδ activation was lower than that in radiosensitive 3SBH5. Cytosol PKCδ levels in 3SBH5 decreased markedly after irradiation, while those in XR223 did not. There was no apparent change after irradiation in the membrane fractions of either cell type. In addition, basal cytosol PKCδ levels in XR223 were higher than those in 3SBH5. These results suggest that the radioresistance in XR223 to radiation-induced apoptosis is due to a difference in the regulation of radiation-induced PKCδ activation compared to that of 3SBH5. On the other hand, *Atm*$^{-/-}$ mouse thymic lymphoma cells were more radioresistant to radiation-induced apoptosis than wild-type mouse thymic lymphoma cells. Irradiated wild-type cells, but not *Atm*$^{-/-}$ cells, had decreased PKCδ levels, indicating that the Atm protein is involved in radiation-induced apoptosis through the induction of PKCδ degradation. The decreased Atm protein levels induced by treatment with *Atm* small interfering RNA had no effect on radiation-induced apoptosis in 3SBH5 cells. These results suggest that the regulation of radiation-induced PKCδ activation, which is distinct from the Atm-mediated cascade, determines radiation sensitivity in radiosensitive 3SBH5 cells.

Keywords: Mouse thymic lymphoma; Protein kinase Cδ; Ataxia telangiectasia; Radiation-induced apoptosis; Radiation sensitivity

1. Introduction

Radiosensitivity is a major topic in the evaluation of the effects of radiation. Radiation-induced apoptosis has an important role in the mechanism of radiosensitivity [1]. Radiation-induced apoptosis is a protective cellular function to eliminate damaged cells. Many pathways in radiation-induced apoptosis involving TP53, protein kinase C (PKC), and BCL family proteins have been investigated [2–4]. The PKC family of serine/threonine kinases consists of multiple distinct subtypes [5]. A number of studies indicate that PKC is involved in radiation-induced apoptosis and determines radiosensitivity [6–10]. These studies examined PKC inhibitors

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and PKC overexpression [6,8–10]; however, not all functions of the PKC subtypes in radiosensitivity are fully understood.

PKCδ is involved in the apoptosis that is induced by DNA-damaging agents [11–16]. Radiation induces translocation of PKCδ to membrane fractions and subsequent degradation of PKCδ [13,14], which results in PKCδ activation. The degraded catalytic domain interacts with several factors, and then causes apoptosis [14,15]. In contrast, PKCδ enzyme activity is not required for PKCδ-induced apoptosis [17], and in some cases, PKCδ promotes cell survival [18,19]. Thus, the involvement of PKCδ in radiation-induced apoptosis is unclear and detailed analysis is necessary to evaluate the role of PKCδ in apoptosis. Overexpression of PKCδ renders cells highly sensitive to radiation-induced apoptosis [8]. However, the differences in endogenous PKCδ activity and its regulation between radiosensitive and radioresistant cells remain to be determined. In addition, the effect of relatively low-dose (<2 Gy) radiation on PKCδ regulation has not been evaluated in radiation-induced apoptosis. Radiosensitive cells and their radioresistant derivatives are useful for these studies. We previously isolated a radioresistant variant, XR223, from a radiosensitive mouse thymic lymphoma cell line, 3SBH5, and demonstrated differences in its sensitivity in radiation-induced and drug-induced apoptosis [20]. In this study, we examined the regulation of PKCδ activation in radiation-induced apoptosis in these cell lines and determined that the regulation of PKCδ in apoptosis differs between 3SBH5 and XR223. *ATM*, the gene mutated in the human genetic disorder ataxia telangiectasia, participates in radiation-induced apoptosis [21,22] and is involved in PKCδ regulation in response to radiation [12,14]. We examined the involvement of Atm in PKCδ-regulated radiation-induced apoptosis signals using small-interfering RNA (siRNA) down-regulation of Atm protein and $Atm^{-/-}$ mouse thymic lymphoma cells.

Radiation-induced apoptosis in the radiosensitive cells occurs through PKCδ activation, and further, the difference in the regulation of PKCδ activation in response to radiation might determine the difference in radiosensitivity in 3SB cell lines.

2. Materials and methods

2.1. Cells and culture conditions

The murine cell line 3SBH5 was selected as a stable radiation-sensitive subclone from the thymic lymphoma cell line 3SB of B10.thy1.1 mice [20]. The radiation-resistant cell lines XR223 and XR316 were derived from the 3SBH5 cell line as described previously [20]. Cells were incubated in 5% CO_2 in air at 37 °C in a suspension culture in Dulbecco's modified Eagle medium supplemented with 10 mM HEPES, 150 μM asparagine, 100 nM minimal essential medium non-essential amino acids, 50 U/ml penicillin, 50 μg/ml streptomycin, and 8% fetal bovine serum. Under the culture conditions, the X-ray sensitivities of the cell lines were retained for more than 8 wk after inoculation. $Atm^{-/-}$ thymic lymphoma cells were established as described previously [23] using 129 $Atm^{-/-}$ and wild-type mice [24].

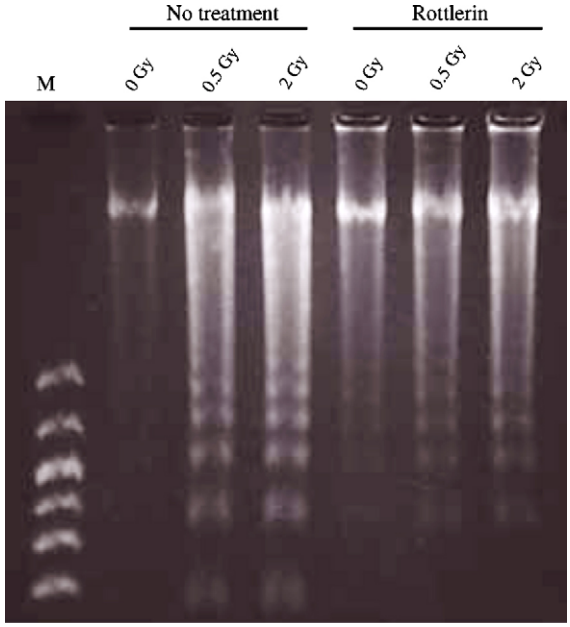

Fig. 1. Effect of rottlerin on radiation-induced apoptosis in 3SBH5 cells. The cells were treated with rottlerin (5 μM) 30 min before irradiation. DNA was extracted for the analysis of DNA laddering 4 h after irradiation. Lane M contains DNA marker (50–1000 bp; Cambrex Bio Science Rockland Inc., Rockland, ME).

2.2. X-irradiation and chemical treatment

Cell lines were irradiated with X-rays (dose rate, 0.4 Gy/min) as described previously [20]. Thirty minutes after treatment with rottlerin (Biomol Research Laboratories Inc., Plymouth Meeting, PA), the cells were irradiated with X-rays and cultured for the times indicated in Figs. 1 and 2B.

2.3. Measurement of apoptosis

Apoptosis was evaluated by DNA fragmentation in cells. The cells were harvested and washed twice with ice-cold phosphate-buffered saline (PBS), and DNA samples were extracted using a TACS™ Apoptotic DNA Laddering Kit (R&D Systems, Minneapolis, MN). DNA samples (1 μg/lane) were electrophoresed on 1.5% TreviGel 500 gel at 100 V and