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Original Article

Low-dose ionizing radiation suppresses the apoptosis-induced by serum-removal culture

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ABSTRACT — Low-dose ionizing radiation (LDIR) at a dose reported in the radiation hormesis effect and adaptive response enhances antioxidant abilities and DNA repair abilities. In this study, we investigated the effects of radiation at 0.1–3 Gy on apoptosis induced by serum removal. Irradiation of 1 Gy at the timing of apoptosis induction improved cell viability. The inhibitory effect on apoptosis was strongly observed at 1 Gy of radiation rather than 0.1 Gy, which is the dose reported in the radiation hormesis effect and adaptive response. To study the effect of irradiation on cell cycle and DNA damage repair system, we investigated the activation of cyclin A and the phosphorylation of KAP1, and results showed that the irradiation decreased cyclin A and activated KAP1, suggesting cell cycle arrest and the activation of DNA damage repair system after the irradiation. Next, we investigated the increased phosphorylation of p38 and Jun-N-terminal kinase (JNK), stress response factors, that occurs during the progression of apoptosis by serum removal, and results showed that 1 Gy of irradiation increased p38 but decreased JNK. We investigated the effect of the irradiation on the regulation of DUSP1, which is a substrate specificity for MAPK, and results showed that the irradiation maintained the expression level for the transient induction of DUSP1 expression by serum removal. The results suggest that in LDIR at 1 Gy, apoptosis was suppressed by the activation of DNA damage repair signaling and crosstalk signaling between the p38 and JNK pathways mediated by DUSP1 induction.

Key words: Low-dose irradiation, Apoptosis, DNA damage repair, Stress response, PC12 cell

INTRODUCTION

High-dose irradiation causes damage such as directly and indirectly cleaving DNA, which results in aging and cell death for various cell types (Adjemian *et al*., 2020). Irradiation to the central nervous system is used as a treatment for tumors, but reports have indicated the occurrence of irradiation-induced central nervous system damage and complications such as cognitive impairment (Tofilon and Fike, 2000; Fike *et al*., 2009). Meanwhile, it has been reported that low-dose irradiation has a beneficial effect as a radiation hormesis effect and adaptive response. It has been shown that mammalian cells exposed to specific LDIR had beneficial effects on

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the ability of maintaining genomic integrity and repairing damaged DNA (Kelsey *et al*., 1991; Feinendegen *et al*., 1996). It has been reported that cell adaptive response occurs in response to low levels of stress due to LDIR exposure, and that there is an increase in antioxidant and DNA repair abilities (Eldridge *et al*., 2012). Reports of the radiation hormesis effect and adaptive response by this LDIR often used doses of 0.1 Gy or less. Based on these reports, it is thought that LDIR has beneficial effects on cells and animal species. It has also been reported that LDIR exposure from brain CT scans could activate brain functions and has the possibility of alleviating symptoms of both Alzheimer's disease and Parkinson's disease, which are neurodegenerative diseases that cause cognitive dysfunction (Cuttler *et al*., 2016). The doses affected by radiation vary depending on animal species and cells, and there are research reports on various doses in addition to those of 0.1 Gy or less. For example, it has been reported that Aβ plaque, which forms in the brain of Alzheimer's disease patients, decreased in Alzheimer's disease model mice at doses of 1 Gy × 10 times and 2 Gy × 10 times (Marples *et al*., 2016). Irradiation at doses of 0.05 Gy and 4 Gy by ^{137}Csγ rays in Alzheimer's disease model Drosophila has been reported to suppress cell death by suppressing AKT signaling pathway and p38 activation (Hwang *et al*., 2019). Based on these reports, it is thought that radiation at doses above those used for the hormesis effect has a suppressing effect on nerve cell death.

PC12 cells have been reported to undergo DNA fragmentation between nucleosomes that is characteristic of apoptosis by serum removal (Batistatou and Greene, 1993). Serum-free PC12 cell cultures are useful for researching the mechanism of neuronal cell death after neurotrophic factor deficiency. The existing studies so far in the areas of LDIR are limited due to insufficient experimental data on accurate serum free cell culture and the neuronal cell death.

Hence, an attempt has been made in this study to systematically investigate the effect of LDIR on cell death in PC12 cells induced by serum removal cultures.

MATERIALS AND METHODS

Cell culture

The rat pheochromocytoma (PC12) cells were cultured in a 75 cm^2 flask at 37°C in a mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium (DF medium 1: 1, vol/vol) containing 15 mM HEPES buffer, pH 7.4, 5% Fetal Bovine Serum and 5% horse serum, in 5% CO_2 humidified atmosphere.

For the experiments in serum-free medium, cells were washed with DMEM/Ham's F-12 medium as described previously (Batistatou and Greene, 1993). For the experiments in serum-free medium, cells were washed with DMEM/Ham's F-12 medium as described previously (Batistatou and Greene, 1993). Experiments in the serum-free cultures involved seeding in a serum-containing medium, after which the cells were adhered for at least four hr, washed once with serum-free DMEM/Ham's F-12 medium, and maintained in a serum-free state.

LDIR treatment

X-ray irradiation involved a dose rate of 1.0 Gy/min at 160 kV and 6.0 mA using an X-ray irradiation device (Faxitron CP-160).

After 24 hr of serum-deprivation culture, the cells (2.5×10^4/cm^2) were irradiated with 100 mGy, 1 Gy, or 3 Gy X-rays, incubated at 37°C for a wide range of hours. Afterwards, the cells were retrieved. The retrieved cells were used in each experimental system.

Figure 1 shows the time schedule for irradiation by serum removal.

Cell viability assays

The cells were seeded on a 35-mm dish at a density of 2.5×10^5 cells/dish. The cells that were treated with LDIR were re-suspended in phosphate-buffered saline and stained with trypan blue. The final concentration of trypan blue (NACALAI TESQUE, Tokyo, Japan) used in this assay was 0.1%. The cell viability was calculated using the following equation.

Cell viability = number of living cells/total number of cells

Detection of DNA fragmentation between DNA nucleosomes

The PC12 cells were seeded on a 100-mm dish at a density of 5×10^6 cells / dish, replaced with serum-free medium, irradiated with X-rays 24 hr later, and then incubated from another 24 hr. Then, the cells were retrieved and made into pellet form. After lysis, an ApopLadder EX™ (TaKaRa Bio, Tokyo, Japan) was used to extract the fragmented DNA according to the manufacturer protocol. Electrophoresis was performed with a 3% agarose gel, GR Green Loading Buffer (GRG-1000, Bio Craft, Tokyo, Japan) was added to the DNA, and the band was visualized under blue LED light. The signal intensity was quantified using ImageJ (Schneider *et al*., 2012).

Apoptosis assay

Measurements of the number of apoptotic cells were analyzed according to the Muse™ Annexin V&Dead Cell Kit (Merck Millipore, Tokyo, Japan) user guide. The cells

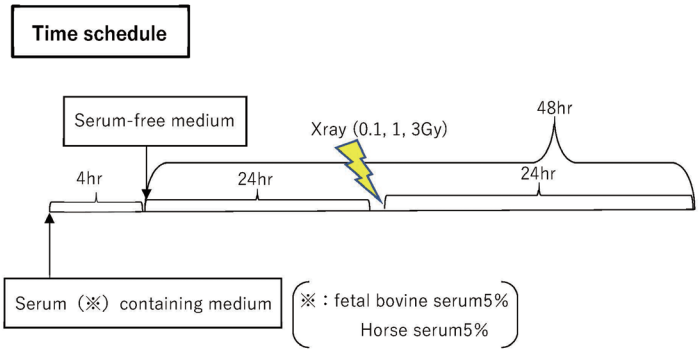

Fig. 1. Experiment schedule.

were incubated in a 35-mm dish (2.5×10^5). The cells were then treated with LDIR, after which they were collected and stained with the Muse™ Annexin V&Dead Cell reagent. Apoptosis was identified using a Muse Cell Analyzer (Millipore Corporation, Tokyo, Japan).

JC-1 fluorescence measurement of mitochondrial membrane potential

JC-1 Mito MP Detection Kit (Dojindo Molecular Technologies, Tokyo, Japan) was used according to the manufacturer protocol to evaluate the mitochondrial membrane potential (MMP). All images were taken with a fluorescence microscope (Keyence, Tokyo, Japan) and processed using BZ X Viewer software.

Western blot

The cells were seeded on a 100-mm dish with a density of 5×10^6 cells/dish, after which the total protein was extracted with an RIPA lysis buffer and quantified with the BCA protein assay kit. Each sample was separated by 10% SDS-PAGE and transferred to a PVDF membrane. The membrane was then blocked with Ez Block Chemi (ATTO, Tokyo, Japan) for one hr, after which it was incubated with the primary antibody overnight at 4°C.

The primary antibodies (Elabscience, Tokyo, Japan) used were AIF1, cytochrome c1, γH2AX, phospo-KAP1 (pKAP1), cyclinA, Bcl-2, p38, phospho-p38, JNK1/2/3, phospho-JNK1/2/3, DUSP1, β-actin. After rinsing, the membrane was incubated with a secondary antibody marked with horseradish peroxidase for one hour at room temperature. Images were taken using an Amersham Imager 680.

Quantification of fluorescence images and band signals

The band signals from DNA, JC-1, and Western blots were quantified using ImageJ (Schneider *et al*., 2012).

Statistical analysis

The data are expressed as mean ± standard error. The statistical significance between the control and irradiation groups was determined using Dunnett's test. Statistical tests and analysis were conducted using Excel 2010 software (Microsoft, Tokyo, Japan). Differences with a P-value < 0.05 were considered to represent a statistically significant difference.

RESULTS

Effect of irradiation on serum removal-induced cell death

Changes in cell viability due to serum removal were confirmed using the trypan blue method. Serum removal reduced survival after 48 hr from 96% to 70% and after 72 hr from 86% to 60% (Fig. 2A, B). Irradiation was conducted with either 0.1 Gy or 1 Gy 24 hr after serum removal; when the survival rate was measured 48 hr after serum removal, it was confirmed that decreases in serum removal-induced survival rate were suppressed (Fig. 2A). This effect was also observed 72 hr after serum removal (Fig. 2B). Meanwhile, when irradiation was similarly conducted immediately after serum removal and the survival rate was measured after 48 hr, there were no differences from that of the non-irradiated group (Fig. 2C). A 3 Gy irradiation also enhanced the decrease in serum removal-induced survival rate regardless of the irradiation timing (Fig. 2A, C). These results show that serum removal-induced cell death could be suppressed by low-dose (0.1 Gy or 1 Gy) irradiation 24 hr after serum removal.

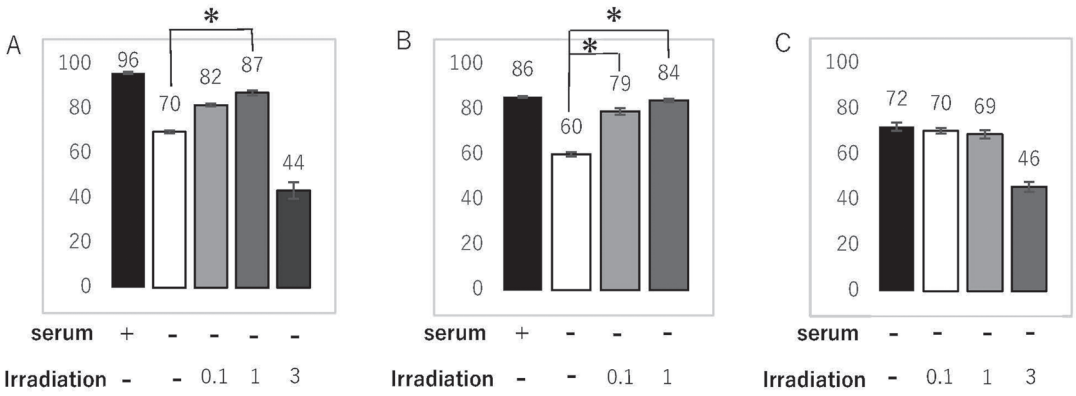

Fig. 2. Effects of X-rays on PC12 cell apoptosis induced by serum removal. X-ray irradiation was conducted either 24 hr (A, B) or immediately (C) after serum removal, and the cells were cultured for 24 hr (A, C) or 48 hr (B). Cell viability was expressed as a percentage with the trypan blue method. Each experiment was performed three times independently. mean ± SE (n = 12). *: $p < 0.05$

Suppressive effect of LDIR on apoptosis-induced DNA fragmentation and mitochondrial membrane potential reduction

PC12 cells have been reported to exhibit DNA fragmentation patterns that are characteristic of apoptosis when cultured in a serum-free medium (Batistatou and Greene, 1993). This DNA fragmentation has been reported to be characteristic of late apoptosis (Collins *et al*., 1997). To investigate whether the cell death-induced by serum removal was apoptosis, DNA was extracted from PC12 cells incubated for 24 hr after X-ray irradiation. When the extracted DNA was visualized on an agarose gel, after serum removal, DNA ladder was detected in non-irradiated-cells (Fig. 3, lane a) but not in the irradiated cells (Fig. 3, lane b and c), indicating the irradiation suppressed DNA fragmentation-induced by serum removal.

Next, the effect of irradiation on the progress of apoptosis was investigated over time using a Muse Cell Analyzer in order to further evaluate the apoptosis-inducing ability of serum removal in PC12 cells. Apoptotic cells were detected using the fluorescent label Annexin V which binds to phosphatidylserine and 7-AAD which is a DNA-binding dye. Phosphatidylserine, which normally resides inside the cell membrane, is exposed to the cell membrane surface in the early stages of apoptosis since 7-AAD is not membrane permeable. The cell membrane has not yet been damaged at this stage, so 7-AAD is not taken up into the cell and does not bind to nuclear DNA, and early apoptosis is detected as a result. Late apoptotic cells are stained using both Annexin V and 7-AAD, so late apoptosis can be detected, and apoptosis progress can be confirmed.

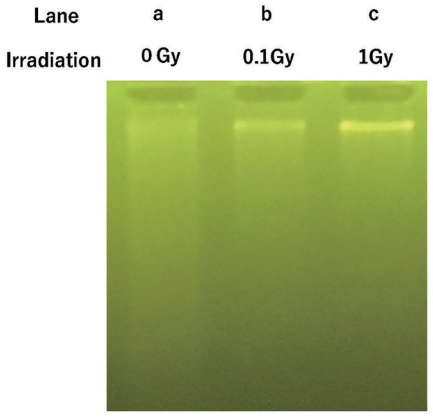

Fig. 3. Inhibition of DNA fragmentation between nucleosomes by X-ray irradiation. The cells underwent X-ray irradiation 24 hr after serum removal and then cultured for 48 hr. Afterwards, DNA was extracted from the cells and observed with agarose gel electrophoresis. Each experiment was performed three times independently.

Results showed that the progress of apoptosis after irradiation was suppressed 24 hr after irradiation (Fig. 4A-1 and 4A-2). For cells after 48 hr, 1 Gy of irradiation suppressed the progress of apoptosis from early to late apoptosis (Fig. 4B-1 and 4B-2). This result supports the patterns of DNA agarose gel electrophoresis.

Decreased mitochondrial membrane potential (MMP) is an early response to apoptosis by the mitochondrial pathway and has been reported to result in mitochondrial membrane permeability and the release of cytochrome

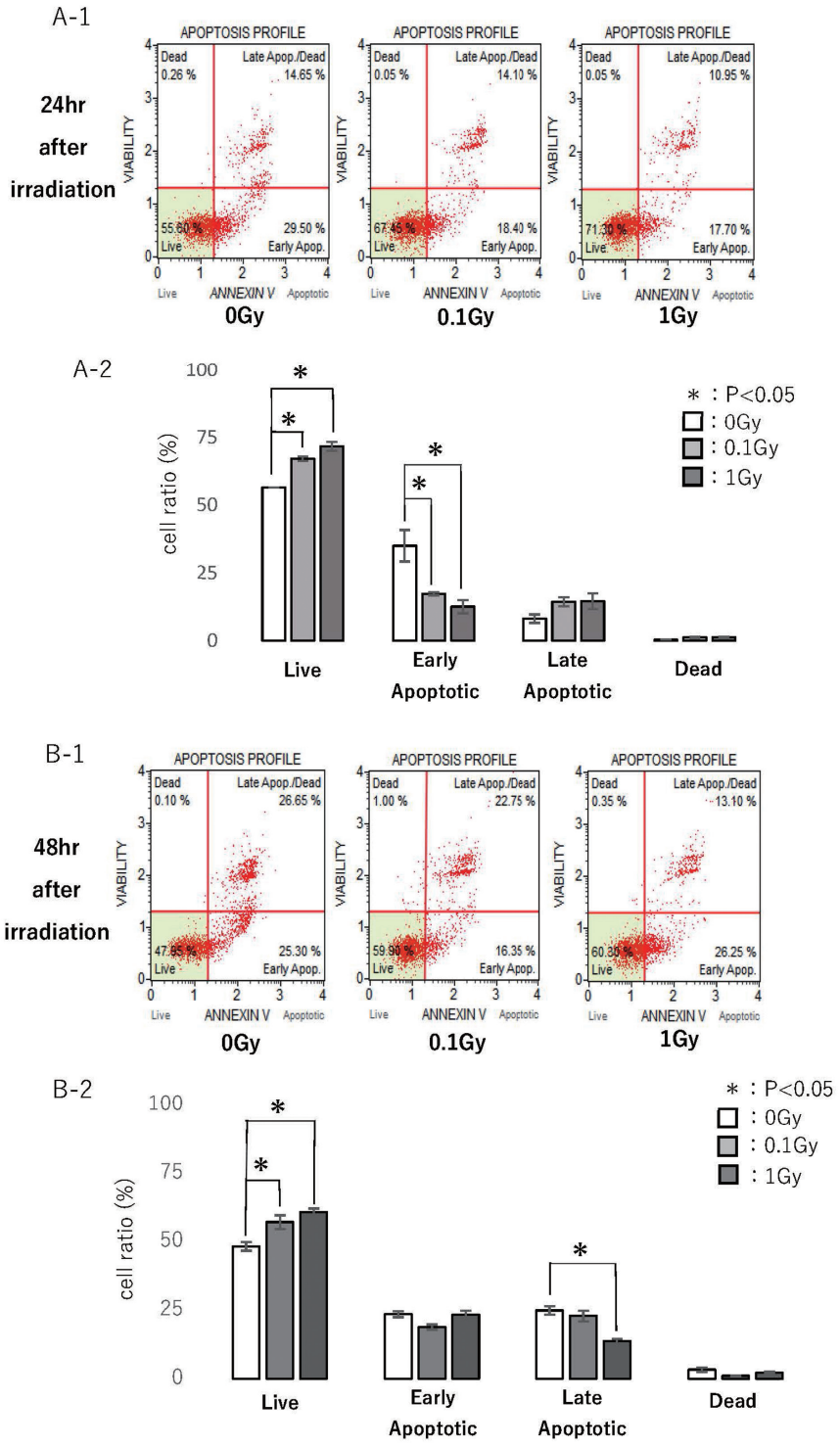

Fig. 4. Effect of X-ray irradiation by serum removal-induced apoptosis. X-ray irradiation was conducted 24 hr after serum removal (A, B), and cells were cultured for 24 hr (A) or 48 hr (B). A-1 or B-1 shows the apoptosis profile (Live cells, Cells in early apoptosis, Cells in late apoptosis or Dead cells), and A-2 or B-2 shows it in a bar graph, respectively. The progress of apoptosis was expressed as percentages in bar graphs. Each experiment was conducted three times independently. mean ± SE (n = 12). *: $p < 0.05$

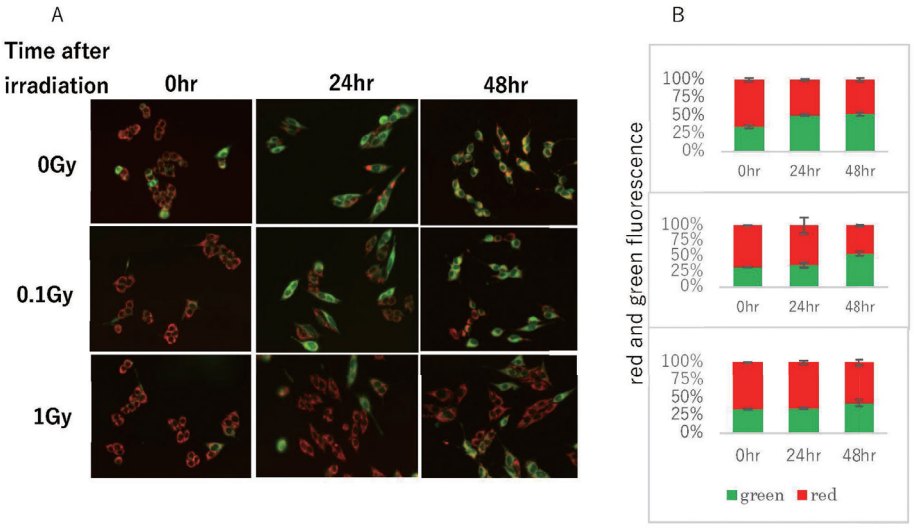

Fig. 5. Suppressive effect of decreased mitochondrial membrane potential by X-ray irradiation. X-ray irradiation was conducted 24 hr after serum removal, and MMP was observed over time with JC-1 staining. Membrane potential changes were quantified and expressed with a percentage. Each experiment was performed three times independently. A shows a fluorescence microscope image, and B shows the red / green fluorescence intensity ratio obtained from the image as a bar graph.

c from the mitochondria to the cytoplasm (Susin *et al*., 1999a). Therefore, JC-1 fluorescence measurements were conducted to detect the disappearance of MMP over time in order to further evaluate the effect of irradiation on the induction of apoptosis by serum removal in PC12 cells. Results showed a decreased red / green fluorescence intensity ratio, which indicates mitochondrial membrane depolarization, due to serum removal; it was also confirmed that decreased MMP induced apoptotic cell death. No differences in the progress of apoptosis after irradiation were observed between the cells immediately after irradiation and the non-irradiated cells; however, suppression of decreases in MMP were confirmed with 1 Gy of irradiation in cells after 24 and 48 hr (Fig. 5A and 5B). This result supports the pattern of DNA agarose gel electrophoresis. Analyses in subsequent experiments were conducted using PC12 cells that were irradiated with 1 Gy.

Effect of irradiation on apoptotic mechanism

It has been reported that the anti-apoptotic protein Bcl-2 acts to suppress apoptosis by maintaining the integrity of the mitochondrial membrane, and it plays a role in protecting cells (Tzifi *et al*., 2012). Therefore, Western blotting was used to confirm whether Bcl-2 is involved in the apoptosis suppression effect of X-ray irradiation. Results showed that slight Bcl-2 production was observed due to serum removal (Fig. 6, ① lane a-c), with irradiation increasing this.

Cyclin A combines with Cdk2 to form a Cdk2-cyclin A complex, which is required for S phase progression (Maddika *et al*., 2008). Therefore, cyclin A expression is reduced and proliferation signals are suppressed due to the progress of serum removal-induced apoptosis. Therefore, Western blotting was used to investigate changes in cyclin A expression amount in order to confirm the activation status of proliferation signals at the time of serum removal-induced cell death induction and at the time of X-ray irradiation-induced cell death suppression effects. Results showed that cyclin A expression was observed up to 24 hr after serum removal, but that its expression significantly decreased after 48 hr (Fig. 6, ② lane a-c). These results are thought to reflect the suppression of proliferation signals associated with the progression of serum removal-induced apoptosis. Meanwhile, this decreased cyclin A expression became even more significant due to irradiation (Fig. 6, ② lane d and e). The Cdk2-cyclin A complex has been reported to act upstream of the mitochondrial cytochrome c release in the apoptotic pathway and promote Rad9-induced apoptosis by phosphorylation of Rad9 (Zhan *et al*., 2012). Therefore, decreased cyclin A expression may indicate its involvement in apoptosis suppression by irradiation.

Cytochrome c and AIF have been shown to be released from mitochondria, translocated to the nucleus, and involved in DNA aggregation and fragmentation. AIF is a flavin protein present in the mitochondrial intermembrane

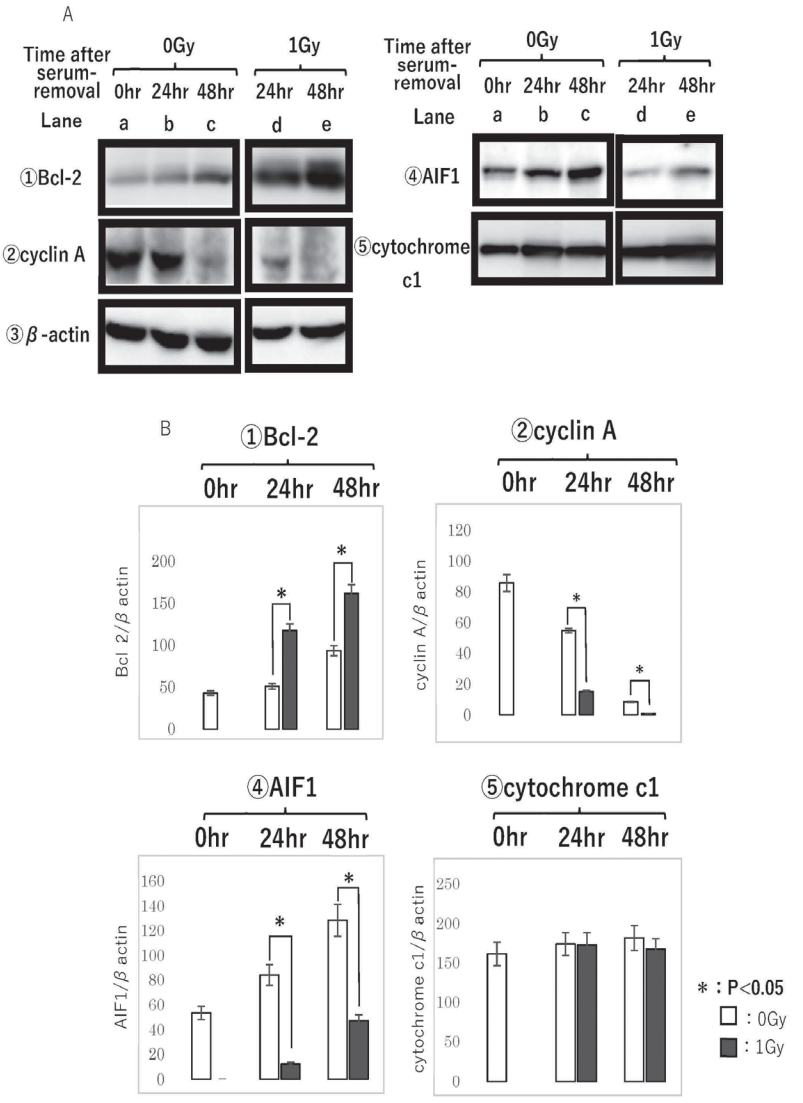

Fig. 6. Effect of 1 Gy of X-ray irradiation on serum removal-induced apoptosis mechanism. Western blot analysis of apoptosis regulators. PC12 cells were extracted 0, 24, and 48 hr after serum removal. X-ray irradiation was conducted 24 hr after serum removal, after which extraction was conducted. β actin was used as the loading control. A shows a typical pattern of electrophoresis, and B shows the average value obtained by quantifying the pattern obtained from three independent experiments. mean ± SE (n = 3). *: $p < 0.05$

space and is an important caspase-independent apoptotic regulator in multiple nerve injury pathways. Cytochrome c1 is a caspase-dependent apoptotic regulator. In response to an apoptosis-promoting signal, AIF and cytochrome c1 translocate from the mitochondria to the nucleus, where they interact with DNA and induce chromatin condensation and DNA fragmentation (Daugas *et al.*, 2000). Therefore, Western blotting was used to confirm whether AIF1 and cytochrome c1 are involved in the apoptosis effect of X-ray irradiation. Results showed that AIF1 production amount increased due to serum removal (Fig. 6, ④ lane a-c), but irradiation decreased this amount (Fig. 6, ④ lane d and e). No effects of irradiation were confirmed for cytochrome c1 (Fig. 6, ⑤ lane a-e).

Effect of irradiation on DNA damage signal analysis

To investigate the effect of irradiation on DSBs production, the production of γH2AX, which is an index an

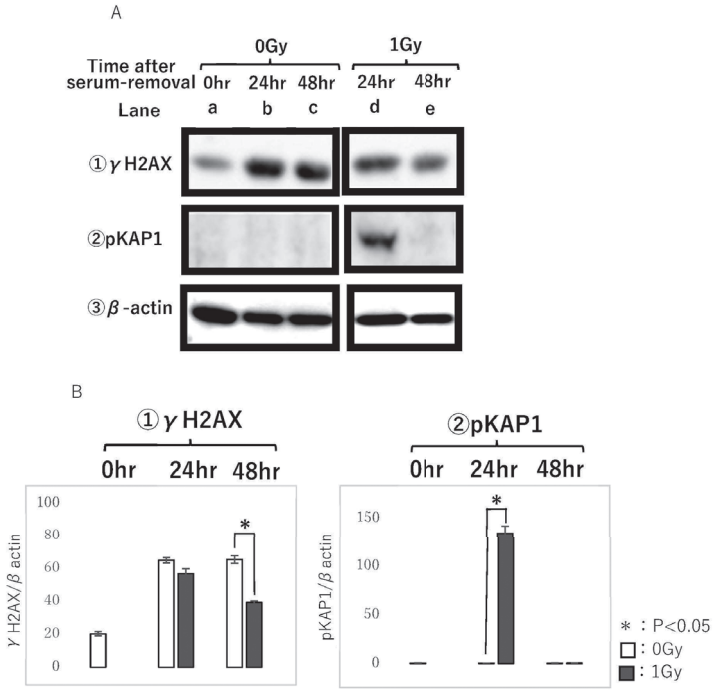

Fig. 7. Effect of 1 Gy of X-ray irradiation on DNA damage repair mechanism by serum removal. Western blot analysis of apoptosis regulators. PC12 cells were extracted 0, 24, and 48 hr after serum removal. X-ray irradiation was conducted 24 hr after serum removal, and extraction was conducted. β-actin was used as the loading control. A shows a typical pattern of electrophoresis, and B shows the average value obtained by quantifying the pattern obtained from three independent experiments. mean ± SE (n = 3). *: $p < 0.05$

indicator production, was confirmed by Western blotting. Serum removal increased the production of γH2AX (Fig. 7, ① lane a-c), and irradiation suppressed this (Fig. 7, ① lane d and e). This result suggests that irradiation may have suppressed DNA damage induced by serum removal through activation of the DNA damage repair system.

ATM, ATR, and DNA-PKcs, which are serine / threonine kinases of the PI3K-related kinase family, have been reported to be involved in the DNA damage response and repair (Yang *et al*., 2003). ATM is activated when DSBs are generated with irradiation; as a result, phosphorylation of a protein called KRAB-associated protein 1 (KAP1) occurs near the C-terminal. Relaxation of the chromatin structure occurs as a result, after which a system that repairs damaged DNA is known to be activated (Ziv *et al*., 2006). Therefore, the activation status of the DNA repair mechanism by the ATM mechanism can be confirmed from the phosphorylation status of KAP1.

There is also the possibility that DSB suppression by irradiation is the result of activation of the KAP1-mediated radiation-induced DNA repair mechanism. Western blotting was used to investigate the phosphorylated KAP1 (pKAP1) production amount at the time of serum removal-induced cell death induction and at the time of X-ray irradiation-induced cell death suppression effects. Results showed that pKAP1 expression was hardly observed in serum removal (Fig. 7, ② lane a-c). In contrast, phosphorylation of KAP1 was clearly observed due to irradiation (Fig. 7, ② lane d). However, its expression was not persistent, and this disappeared 48 hr after serum removal (Fig. 7, ② lane e). This result suggests that the activation of the irradiation-induced DNA damage repair mechanism may suppress serum removal-induced DSBs.

Effect of LDIR on stress response MAPK pathway

The Western blotting method was used to investigate the effect of X-ray irradiation on stress response signals due to trophic factor deficiencies in serum removal.

MAPK regulates apoptosis through transcriptional and post-transcriptional mechanisms. The activation of p38MAPK and JNK is normally called the stress response MAPK pathway; and it has been reported that apoptosis is induced in response to various intracellular and extracellular stresses such as UV, radiation, oxi-

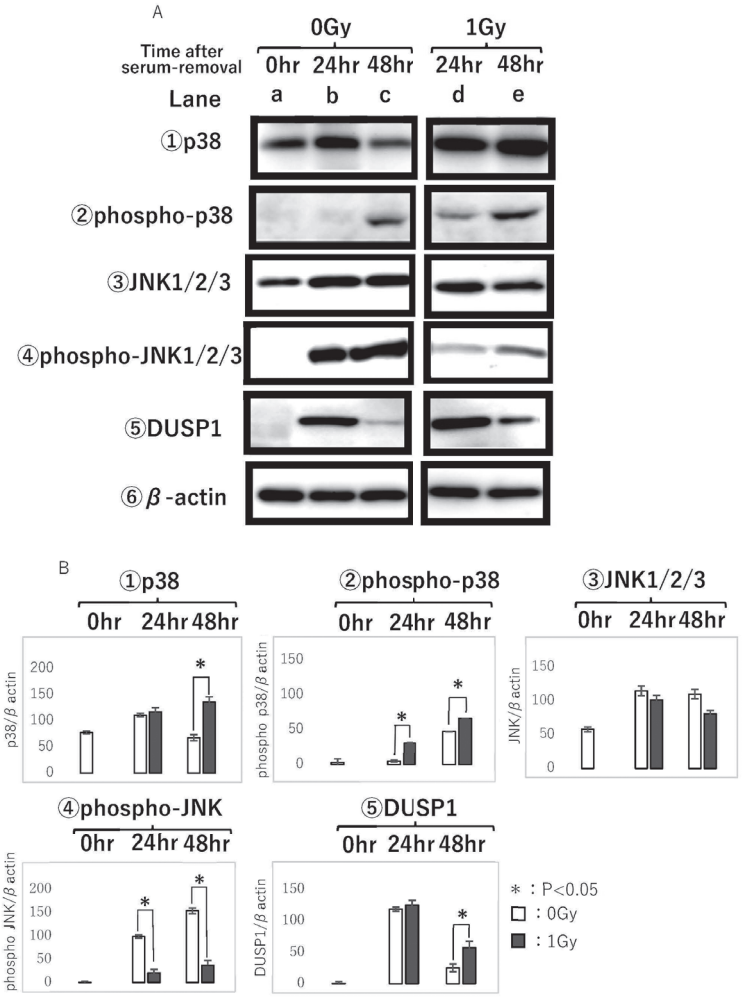

Fig. 8. Effect of 1 Gy of X-ray irradiation on stress response MAPK pathway mechanism by serum removal. Western blot analysis of apoptosis regulators. PC12 cells were extracted 0, 24, and 48 hr after serum removal. X-ray irradiation was conducted 24 hr after serum removal, and extraction was conducted. β-actin was used as the loading control. A shows a typical pattern of electrophoresis, and B shows the average value obtained by quantifying the pattern obtained from three independent experiments. mean ± SE (n = 3). *: $p < 0.05$

dation, heat shock, and hyperosmotic pressure (Yue and López, 2020). It has also been reported that p38 induces the expression of DUSP1, which is an inactivating factor for JNK, and crosstalk signaling that negatively regulates JNK. When p38 induces DUSP1, DUSP1 inactivates JNK and exhibits an anti-apoptotic effect. It has been reported that this crosstalk signaling produces cellular heterogeneity in JNK activity (Miura *et al*., 2018). Therefore, under lethal conditions due to serum removal, signals mediated by the stress response MAPK are activated, and the properties of cells may be altered so as to suppress cell proliferation and adapt to the environment. It is expected that confirming this point will allow for the collection of information on the mechanism of the cell death suppression effect caused by LDIR. Therefore, Western blotting was used to investigate p38 and phosphorylated p38 expression levels in order to confirm the activation status of p38 MAPK. Results showed a decrease in p38 and phosphorylated p38 48 hr after serum removal (Fig. 8, ①lane c, Fig. 8, ② lane c). In contrast, it was confirmed that X-ray irradiation after serum removal promoted the phosphorylation of p38 MAPK immediately after irradiation (Fig. 8, ② lane d and e).

Western blotting was used to investigate the JNK and

phosphorylated JNK expression amounts in order to confirm the effects on apoptosis, similar to that of p38MAPK. Results confirmed an increase in the phosphorylation of JNK 24 hr after serum removal (Fig. 8, ③ lane a-c, Fig. 8, ④ lane a-c). In contrast, X-ray irradiation after serum removal resulted in the suppression of JNK phosphorylation, and the increase over time due to serum removal was also greatly suppressed with irradiation (Fig. 8, ④ lane d and e).

Western blotting was used to investigate the DUSP1 expression amount in order to confirm the negative regulation of p38MAPK and JNK by DUSP1. Results confirmed transient DUSP1 expression 24 hr after transfer to a serum-free medium, with DUSP1 expression decreasing over time (Fig. 8, ⑤ lane a-c). In contrast, DUSP1 expression was induced by X-ray irradiation after serum removal, and expression was sustained up to 24 hr after irradiation (Fig. 8, ⑤ lane d and e).

DISCUSSION

In this study, PC12 cell death induced by serum removal was used as a means of investigating neuronal cell death. It was confirmed that shifting to a serum-free medium induced the cell death mechanism due to trophic factor deficiencies and decreased cell viability. An investigation on the characteristics of this cell death confirmed DNA fragmentation and phosphatidylserine exposure, which are characteristic of apoptosis. Therefore, the experimental system of this study was thought to be suitable for elucidating the mechanism of the apoptosis suppression effective of 1 Gy of irradiation.

It has been reported that X-ray irradiation may cause a hormesis effect at doses of less than 0.2 Gy as a low LET (Kelsey *et al*., 1991; Feinendegen *et al*., 1996; Eldridge *et al*., 2012). Irradiation was conducted with 0.1 Gy and 1 Gy in order to verify the hormesis effect, but it was found in this experimental model that the 1 Gy irradiation was the dose with a high survival rate improvement effect. Furthermore, improved survival rate was confirmed as a result of delaying the irradiation time, so it is thought that the effect of irradiation depends on the progress of deficiencies of nutritional factors and the increase of stress on cells. This may differ by cell or animal species, but it is thought that the decrease in survival rate due to 1 Gy of irradiation was improved as a result of a mechanism that is different from the hormesis effect.

Investigating the serum removal-induced apoptosis mechanism confirmed that AIF1 was induced by the lack of survival factors, which then promoted the apoptosis mechanism. AIF1 releases cytochrome c from the mitochondria and eliminates the membrane potential (Daugas *et al*., 2000; Susin *et al*., 1999b). Therefore, AIF1 indirectly affects the integrity of the mitochondrial membrane. It is thought that the mitochondrial membrane function was reduced as a result. It is thought that 1 Gy of irradiation caused the maintenance of the mitochondrial membrane potential and induction of Bcl-2 expression, and suppression of AIF1 expression is thought to have suppressed serum removal-induced apoptosis induction.

The imbalance caused by DNA damage and repair results in the progression of apoptosis. Confirming the balance between damage and repair enables the confirmation of the progress of apoptosis.

It has been reported that serum removal in PC12 cells results in RNA loss, and apoptosis causes cell damage at each stage of the cell cycle and is independent of arrested growth (Lindenboim *et al*., 1995). LDIR has been reported to cause cell cycle arrest in the G_0/G_1 phase, causing cell proliferation and protein increases (Truong *et al*., 2018). Cyclin A combines with cdk2 to form a cdk2-cyclin A complex in the S phase, and this is needed for S phase progression. Furthermore, cyclin A can bind with CDK1 (CDC2) and is involved in the transition from G2 to M. Given this, cyclin A was used to confirm the progression of the S phase, which is one stage of the cell cycle. Results showed that apoptosis progressed due to cell damage by serum removal and cyclin A expression decreased, and it is thought that proliferation signals were suppressed. It is thought that suppressing the progress of the cell cycle with 1 Gy of irradiation strongly suppressed decreased cyclin A expression from immediately after irradiation. Additionally, the apoptosis suppression effect associated with activation of the DNA damage repair mechanism in LDIR may be occurring as a result of the occurrence of the DSB repair mechanism associated with ATM activation due to pKAP1.

The DNA damage response (DDR) is mediated primarily by the PI3K kinase family ATM and Rad3-related protein (ATR), DNA-dependent protein kinase (DNA-PK), and poly-ADP ribose polymerase (PARP). ATM and DNA-PK are primarily activated by DSBs. When irradiation is conducted, phosphorylation of KAP1 occurs immediately after irradiation, and it is thought that the radiation-induced repair mechanism is induced by ATM activation. This is thought to be due to DNA damage as a result of the apoptosis mechanism being induced 24 hr after serum removal, and the repair mechanism due to ATM was not affected by the induction of serum removal alone. ATM activation associated with stress from high-dose radiation has been reported, but this may indicate that 1 Gy of irradiation affects the DNA repair mecha-

nism by KAP1 activation.

It is thought from these results that the suppression of the proliferation signal by cell cycle arrest was strongly caused by irradiation, and the radiation-induced repair mechanism was induced by ATM activation; therefore, the improvements in the balance between damage and repair are thought to have further suppressed the progress of apoptosis.

Investigation of the serum removal-induced stress response MAPK pathway confirmed that the phosphorylation of stress response factors p38 and JNK was induced due to deficiencies in nutritional factors caused by serum removal. Decreases in p38 and phosphorylated p38 expression amount have been reported with cell senescence of neural progenitor cells (Kase *et al*., 2019). Decreased p38 reduces neurogenesis through the inhibition of Wnt signaling. Therefore, fluctuations in the p38 protein amount are thought to affect cell proliferation ability.

Decreases in p38 protein expression due to serum removal is thought to have advanced cell senescence and decrease proliferative ability. Irradiation of 1 Gy resulted in an increase in p38 proteins and induction of phosphorylation. Furthermore, irradiation was confirmed to have suppressed JNK activation.

DUSP1 has the ability to dephosphorylate MAPK, so it is thought of as a regulator of the stress pathway. DUSP1 functions through DNA damage and may further function through JNK-MAPK signaling, thereby reducing cytotoxicity. The progression of apoptosis by serum removal resulted in regulation of stress response factors by p38 and JNK. It is suggested that 1 Gy of irradiation generated an anti-apoptosis effect as a result of increasing proliferative capacity associated with increased p38 proteins and negative regulation of JNK by crosstalk signaling of DUSP1 associated with p38 activation. It has been reported that p38 induces DUSP1 expression and thereby suppresses JNK (Kim *et al*., 2008; Heinrichsdorff *et al*., 2008). It has also been confirmed that DUSP1 has an apoptosis suppression effect by regulating the DNA damage repair mechanism (Fang *et al*., 2018). It is thought from these results that the activation of the DNA damage repair mechanism by 1 Gy is also involved in the induction of DUSP1 expression by p38. It is thought that dephosphorylation of p38 by 1 Gy of irradiation was not observed because DUSP1 caused p38 dephosphorylation but the p38 protein amount decreased due to serum removal. Further detailed study is needed for the involvement of DUSP1 in irradiation.

The results of this study suggest that the inhibitory effect of 1 Gy irradiation on trophic factor deficiency-induced apoptosis is mediated by the activation of DNA damage repair mechanisms and the cross-talk signaling involving DUSPs that regulate the stress response MAPK pathway. It is also suggested that X-rays may have a beneficial effect even at doses higher than those reported as hormesis effects given that the cell death suppression effect occurred even at a dose of 1 Gy.

In conclusion, the results of this study suggested that 1 Gy of X-ray irradiation functions against DNA damage due to trophic factor deficiencies. It is also suggested that the progression of the DNA damage repair mechanism due to ATM activation by pKAP1 and induction of DUSP1 by p38 activation generated crosstalk signals of the stress response MAPK pathway to JNK and regulated apoptotic signal transduction.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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