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Distinct Cellular Calcium Metabolism in Radiation-sensitive RKO Human Colorectal Cancer Cells

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Radiation therapy for variety of human solid tumors utilizes mechanism of cell death after DNA damage caused by radiation. In response to DNA damage, cytochrome c was released from mitochondria by activation of pro-apoptotic Bcl-2 family proteins, and then elicits massive Ca^{2+} release from the ER that lead to cell death. It was also suggested that irradiation may cause the deregulation of Ca^{2+} homeostasis and trigger programmed cell death and regulate death specific enzymes. Thus, in this study, we investigated how cellular Ca^{2+} metabolism in RKO cells, in comparison to radiation-resistant A549 cells, was altered by gamma (γ)-irradiation. In irradiated RKO cells, Ca^{2+} influx via activation of NCX reverse mode was enhanced and a decline of $[Ca^{2+}]_i$ via forward mode was accelerated. The amount of Ca^{2+} released from the ER in RKO cells by the activation of IP_3 receptor was also enhanced by irradiation. An increase in $[Ca^{2+}]_i$ via SOCI was enhanced in irradiated RKO cells, while that in A549 cells was depressed. These results suggest that γ-irradiation elicits enhancement of cellular Ca^{2+} metabolism in radiation-sensitive RKO cells yielding programmed cell death.

Key Words: A549 cells, Inositol-1,4,5-triphosphate receptors, Na^+-Ca^{2+} exchanger, RKO cells, Store-operated Ca^{2+} influx

INTRODUCTION

Radiation therapy is one of common conventional treatment modalities for variety of human solid tumors. Apoptosis plays an important role in cell death after DNA damage caused by radiation. In response to DNA damage caused by irradiation, p53 (53 kDa protein) activates various genes [1-3]. Protein products of pro-apoptotic Bcl-2 gene-family members cause cytochrome c released from the mitochondria into the cytosol and released cytochrome c activates the caspase cascade [4-6]. Mitochondrial apoptotic involvement could depend on signals that originate from other intracellular compartments. Namely, Ca^{2+} released from the endoplasmic reticulum (ER) could induce and/or play a facilitative role in the apoptotic changes [7-9].

Cell death has always been known to be one of the numerous cellular events triggered by an increase in intracellular Ca^{2+} evoked by physiological or pathological stimuli. The role of intracellular Ca^{2+} activity ($[Ca^{2+}]_i$) in apoptosis was appreciated more recently [10]. It has been reported that the expression and/or localization of Bcl-2 can modulate Ca^{2+} fluxes during the course of cell death [11-13].

Increase in intracellular Ca^{2+} causing apoptosis can arise from a variety of sources. Mechanisms for increasing $[Ca^{2+}]_i$ include the entry of extracellular Ca^{2+} via Ca^{2+} channels (voltage-gated channels, receptor-mediated channels) and the transient receptor potential channel of store-operated Ca^{2+} influx (SOCI), or the release of stored Ca^{2+} form intracellular stores via IP_3 receptors and ryanodine receptors in intracellular Ca^{2+} stores [14-17]. Once Ca^{2+} has served its signaling function, $[Ca^{2+}]_i$ is lowered to resting levels to maintain intracellular Ca^{2+} homeostasis. Ca^{2+} is sequestered into intracellular stores by pumps such as the sarcoplasmic/endoplasmic Ca^{2+}-ATPase (SERCA) or is extruded to the extracellular environment by transporters such as the Na^+-Ca^{2+} exchanger (NCX) and the plasma membrane Ca^{2+}-ATPase (PMCA) [14,15,17]. Mitochondria also decrease intracellular Ca^{2+} via the mitochondrial uniporter located in the inner mitochondrial membrane [18], although their role in regulating intracellular Ca^{2+} levels appears to be clearing Ca^{2+} in restricted microdomains such as the microenvironment of IP_3 receptor channels [19].

The role of Ca^{2+} in promoting cell proliferation and cell

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ABBREVIATIONS: RKO cells, RKO Human Colorectal Cancer Cells; $[Ca^{2+}]_i$, intracellular Ca^{2+} activity; NCX, Na^+-Ca^{2+} exchanger; IP_3, Inositol-1,4,5-triphosphate; SOCI, Store-operated Ca^{2+} influx; PMCA, plasma membrane Ca^{2+}-ATPase; SERCA, sarcoplasmic/endoplasmic Ca^{2+}-ATPase; ER, endoplasmic reticulum.

death has been regarded as signaling checkpoints in cancer, which determine how these processes are remodeled in cancer [20]. Many studies have reported that a large influx of Ca^{2+} triggering apoptosis in cancer cells is provided by Ca^{2+} influx mediated by store-operated Ca^{2+} entry channels, which suggest a pivotal role of SOCI in apoptosis and cancer progression [21]. Moreover, the anti-apoptotic protein Bcl-2, which is commonly degraded in cancer, appears to modulate IP_3-receptor Ca^{2+} channel activity on the ER Ca^{2+} stores [10,22,23]. It was also reported that the reduction in ER means that Ca^{2+} release is insufficient to produce apoptosis [10,24]. All these results suggest that the deregulation of cellular Ca^{2+} homeostasis caused under non-physiologic condition such as irradiation can elicit cell death and determine the sensitivity of cancer cells to radiotherapy. It was also suggested that ion transports may contribute to the intrinsic radio-resistance and the survival of the tumor cell, by controlling cell cycle, metabolic adaptations or DNA repair [25]. In this study, to explore the role of cellular Ca^{2+} metabolism in sensitivity of tumor cells to radiation, the effects of gamma (γ)-ray irradiation on cellular Ca^{2+} metabolism in radiosensitive RKO human colorectal cancer cells and A549 human lung cancer cells, one of known radiation-resistant cells, were examined.

METHODS

Cell culture and Irradiation of cell cultures

RKO human colorectal cancer cells and A549 human lung cancer cells were used. The cells were grown in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. The cells were cultured in 25 cm^2 plastic tissue culture flasks at 37°C in a humidified 5% CO_2/95% air atmosphere. When the cells were in exponential growth phase at a cell density of 3×10^6 cells/ 25 cm^2 flasks, cells were irradiated with 10 Gy of γ-rays at a dose rate of 5.0 Gy/min with a 137 Cs irradiator (Cis biointernational IBL437C, France).

Measurements of $[Ca^{2+}]_i$

Intracellular free Ca^{2+} concentration was measured as described previously [26]. Cells were washed with PBS and incubated in 2 ml of buffer (0.05% trypsin and 0.02% EDTA). The cells were then resuspended in Tyrode solution (in mM: 140 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1 NaH_2PO_4, 5 HEPES, 5.5 Glucose and pH 7.4) and incubated at 37°C with 3 μM fura-2 AM (Molecular Probe, Eugene, Oregon, USA) for 30 min and transferred to a recording chamber on an epifluorescence inverted microscope (Nikon Diaphot 300, Tokyo, Japan). Experimental solutions were superfused at a flow rate of 2 ml/min. Fluorescence intensity was measured using a cooled CCD camera (Photometrics PXL37, Tucson, Arizona, USA) and processed using the Axon Imaging Workbench v.2.2 (Axon Instrument, Foster city, CA, USA). $[Ca^{2+}]_i$ was presented as the ratio of flurescence intensities ($R_{340/380}$) excited by alternating illumination of 340 nm and 380 nm. Fluorescence intensity through 510 nm wavelength filter was collected using a cooled CCD digital camera (PXL-37, Photometrics, Tucson, AZ, USA). Experiments were done at 37°C.

Solutions

The composition of Tyrode's solution was 140 mM NaCl, 2.5 mM $CaCl_2$, 5 mM KCl, 1 mM $MgCl_2$, 1 mM NaH_2PO_4, 5 mM N-[2-hydroxyethl] piperazine-N'[2-ethanosulfonic acid] (HEPES), and 5.5 mM glucose at pH 7.4. In the 0 mM Na^+/2.5 mM Ca^{2+} solution (Na^+-free solution), NaCl was isosmotically replaced by *N*-methyl-D-glucamine (NMDG). 140 mM Na^+/0 mM Ca^{2+} solution (Ca^{2+}-free solution) was made by omitting $CaCl_2$. To isolate NCX activity from other Ca^{2+} pathways, 1 μM thapsigargin (ER Ca^{2+}-ATPase inhibitor), 5 mM caffeine (ryanodine receptor inhibitor), and 250 μM La^{3+} (plasma membrane Ca^{2+}-ATPase inhibitor) were added to the superfusing solutions. The 0 Ca^{2+} solution, which was used to empty the internal Ca^{2+} stores, also contained 0.1 mM EGTA and 1 μM thapsigargin.

Statistical analysis

All data were expressed as mean±SD. Statistical analysis was performed by independent *t*-test, with $p<0.05$ as criteria of significance.

RESULTS

Basal level of intracellular Ca^{2+} activity

Basal levels of intracellular Ca^{2+} activities in γ-ray irradiated RKO cells were compared to those in non-irradiated control cells (Table 1). When the cells were incubated for various durations up to 48 hrs after irradiation, basal levels of $R_{340/380}$ in RKO cells were not fluctuated both in the control and irradiated cells. And no difference in $R_{340/380}$ was observed between the control and irradiated cells. Even when the basal levels of $R_{340/380}$ were deviated most, such as in cells incubated for 48 hrs (0.81±0.05 vs, 0.84±0.11), no statistical significance was observed (p=0.14). Based on these findings, further experiments were carried out with cells which were incubated for 48 hrs after γ-ray irradiation.

Table 1. Effects of γ-irradiation on basal level of $R_{340/380}$ in RKO cells

Incubation time		30 min	1 hr 30 min	3 hrs	6 hrs	12 hrs	24 hrs	48 hrs
Basal level of $R_{340/380}$	Control	0.80±0.05	0.80±0.05	0.81±0.05	0.80±0.05	0.81±0.05	0.82±0.05	0.81±0.05
	γ-ray irradiated	0.82±0.05	0.80±0.05	0.81±0.05	0.79±0.05	0.83±0.11	0.83±0.05	0.84±0.11

Note that p=0.1379 at 48 hrs after γ-irradiation. n=30.

Physiological activity of NCX

When the cells were superfused with 0 mM Na^+/2.5 mM Ca^{2+} solution, as described in "METHODS", $R_{340/380}$ in RKO cells increased to a plateau value (from 0.85±0.01 to 1.65±0.03) as shown in Fig. 1. Subsequent superfusion of 140 mM Na^+/0 Ca^{2+} solution lowered $R_{340/380}$ to the resting level with rate of $R_{340/380}$ changes of −0.17±0.05 /min (Fig. 1A and Table 2). In γ-ray irradiated RKO cells, $R_{340/380}$ increased to a plateau value (from 0.92±0.10 to 1.66±0.20) and an additional increase to 1.87±0.40 was followed. Subsequent superfusion of 140 mM Na^+/0 mM Ca^{2+} solution lowered $R_{340/380}$ to the resting level with rate of $R_{340/380}$ changes of −0.25±0.10 /min (Fig. 1B and Table 2). The decay to the basal level of $R_{340/380}$ in irradiated cells was completed faster than that of control cells (230 sec vs. 290 sec) ($p < 0.001$).

In A549 cells, $R_{340/380}$ increased to a plateau value (from 0.73±0.06 to 1.21±0.12). Subsequent superfusion of 140 mM Na^+/0 mM Ca^{2+} solution lowered $R_{340/380}$ to the resting level with rate of $R_{340/380}$ changes of −0.13±0.06 /min (Fig. 1C and Table 2). In γ-ray irradiated A549 cells, $R_{340/380}$ increased to plateau values (from 0.73±0.06 to 1.11±0.12). Subsequent superfusion of 140 mM Na^+/0 mM Ca^{2+} solution lowered $R_{340/380}$ to the resting level with rate of $R_{340/380}$ changes of −0.24±0.05/min (Fig. 1D and Table 2). The decay to the basal level of $R_{340/380}$ was also completed faster than that of control cells ($p < 0.0001$).

Ca^{2+} influx via SOCI

Ca^{2+} influx via SOCI were measured by superfusing cells with the normal Tyrode solution after empting the internal Ca^{2+} stores. When cells were superfused with 0 Ca^{2+} solution containing 0.1 mM EGTA with 1 μM thapsigargin,

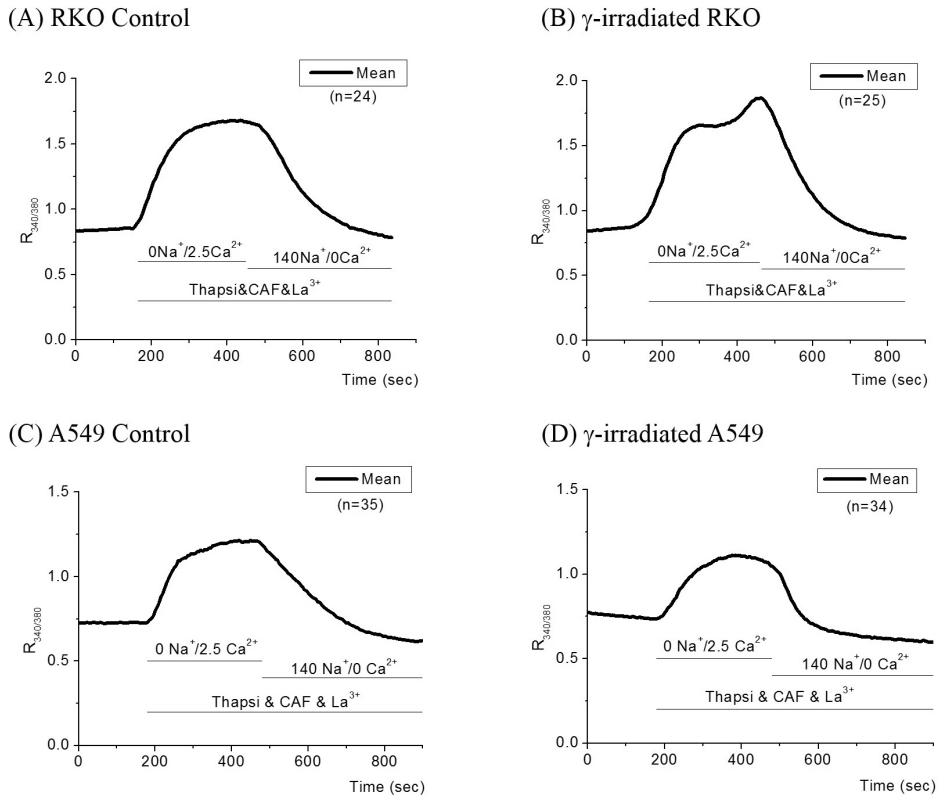

Fig. 1. Effects of γ-irradiation on NCX in RKO and A549 cells. The activity of NCX in cells was measured in the reverse mode of NCX induced by superfusing 0 mM Na^+/2.5 mM Ca^{2+} solution containing 1 μM thapsigargin (Thapsi), 5 mM caffeine (CAF), and 250 μM La^{3+} and in the forward mode of NCX by 140 mM Na^+/0 mM Ca^{2+} solution. (A) In RKO control cells, $R_{340/380}$ increased with the reverse mode of NCX and decreased with the forward mode. (B) In γ-ray irradiated RKO cells, the second peak was observed. The slope of $R_{340/380}$ changes by the forward mode of NCX was increased in γ-rays irradiated cells. (C) In A549 control cells, $R_{340/380}$ changed as in (A). (D) In γ-ray irradiated A549 cells, the slope of $R_{340/380}$ changes by the forward mode of NCX was increased, confirming the activity of NCX was increased by γ-irradiation ($p < 0.0001$). Tracings in (A) to (D) represent the average values of $R_{340/380}$.

Table 2. Comparison of NCX-mediated $R_{340/380}$ changes in RKO and A549 cells

	Cell type	Non-irradiated control	γ-irradiated
Basal level	RKO	0.85±0.05 (n=24)	0.92±0.10 (n=25)
	A549	0.73±0.06 (n=35)	0.73±0.06 (n=34)
Plateau value	RKO	1.65±0.15	1.66±0.20
	A549	1.21±0.12	1.11±0.12
Rate of $R_{340/380}$ changes/min	RKO	−0.17±0.05	−0.25±0.10**
	A549	−0.13±0.06	−0.24±0.05***

**$p < 0.001$, ***$p < 0.0001$.

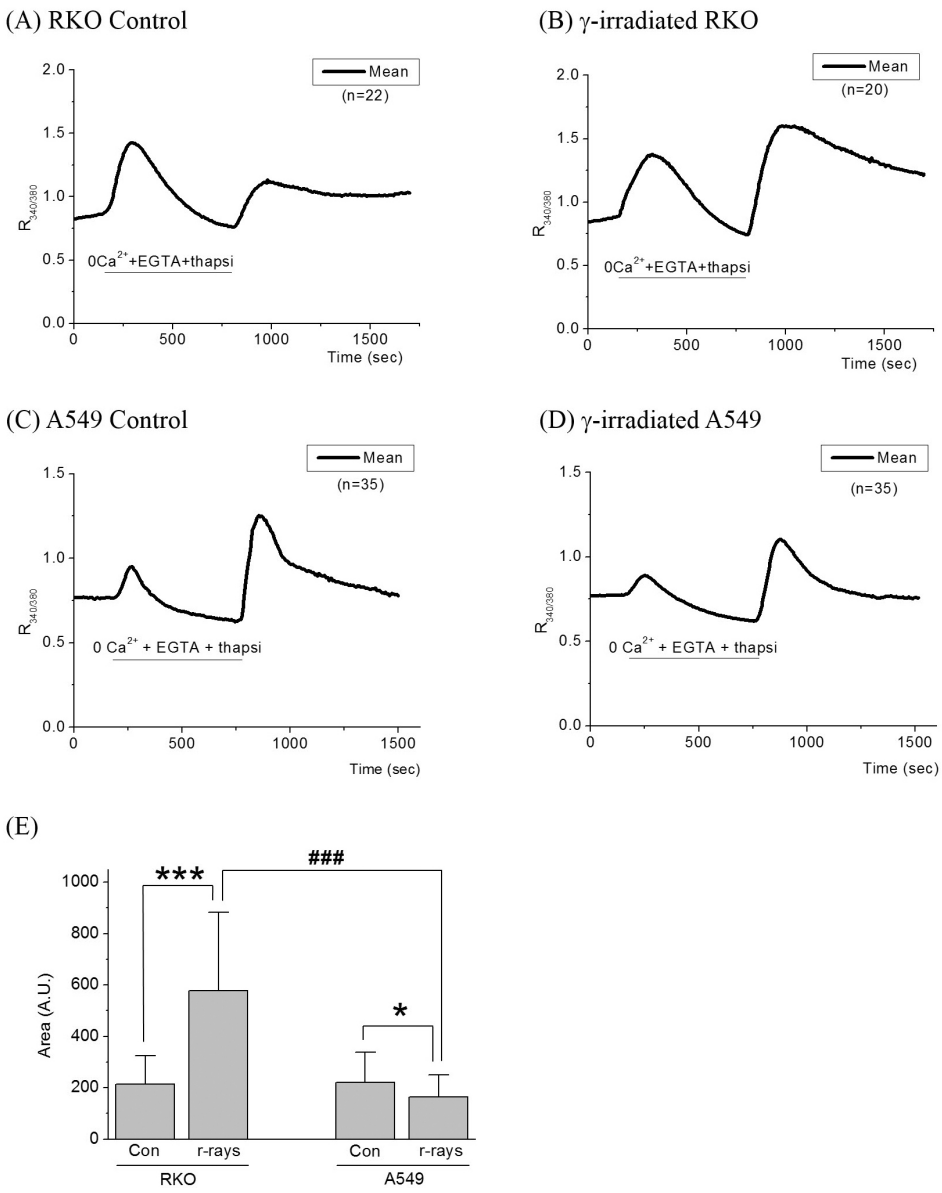

Fig. 2. Effects of γ-irradiation on SOCI in RKO and A549 cells. (A) When RKO control cells were superfused with Tyrode's solution including 2 mM Ca^{2+} after depleting intracellular Ca^{2+} store, $R_{340/380}$ was increased by Ca^{2+} influx via SOCI. (B) Ca^{2+} influx via SOCI was enhanced in γ-ray irradiated RKO cells, compared to control cells (***$p < 0.0001$). (C) In A549 control cells, $R_{340/380}$ was increased by Ca^{2+} influx via SOCI, as in (A). (D) Ca^{2+} influx via SOCI was decreased in γ-ray irradiated A549 cells, compared to (C) (*$p < 0.05$). (E) In RKO cells, areas under SOCI response, which approximate the amount of Ca^{2+} influxed via SOCI, were increased by γ-ray irradiation (***$p < 0.0001$). In A549 cells, areas under SOCI response were decreased by γ-irradiation (*$p < 0.05$). The increment of areas under SOCI response in RKO cells after γ-irradiation was significantly different from that in A549 cells, which was decreased by γ-irradiation ($^{\#\#\#}p < 0.0001$). Tracings in (A) to (D) represent the average values of $R_{340/380}$.

which empties Ca^{2+} out of the ER, $R_{340/380}$ increased and consequently declined below the control level as shown in Fig. 2 and Table 3. In RKO cells, $R_{340/380}$ increased much greater than that of A549 cells (Fig. 2A vs. 2C). In γ-ray irradiated cells, significant changes in these peaks were not observed in either cell. After the internal Ca^{2+} stores were emptied, superfusions of 2.5 mM Ca^{2+} Tyrode solution raised $R_{340/380}$ from 0.76±0.09 to 1.14±0.14 in RKO cells. In γ-ray irradiated RKO cells, with superfusion of 2.5 mM Ca^{2+} Tyrode solution, $R_{340/380}$ increased from 0.74±0.09 to 1.60±0.44 and the increments in $R_{340/380}$ were much larger than those of control cells (0.86±0.44 vs. 0.38±0.14) ($p < 0.0001$), as shown in Fig. 2B. Areas under SOCI response which approximate the amount of Ca^{2+} influxed by SOCI were increased by γ-ray irradiation from 213±112 to 576±304, with statistical significance ($p < 0.0001$), as shown in Fig. 2E.

When A549 cells were superfused with 2.5 mM Ca^{2+} Tyrode solution, $R_{340/380}$ increased from 0.77±0.06 to 1.26±0.30 (Fig. 2C). In γ-ray irradiated A549 cells, $R_{340/380}$ increased from 0.77±0.12 to 1.10±0.24 as shown in Fig. 2D. The increments in $R_{340/380}$ in γ-ray irradiated A549 cells were smaller than those of control cells (0.33±0.24 vs. 0.49±0.30) ($p < 0.05$). Areas under SOCI response were decreased by γ-ray irradiation from 221±116 to 164±95, with statistical significance ($p < 0.05$). The increment of areas under SOCI response in RKO cells by γ-ray irradiation was significantly different from that in A549 cells, which was decreased by γ-ray irradiation, as shown in Fig. 2E ($p < 0.0001$).

ATP-induced Ca^{2+} release from the ER

Ca^{2+} release from the ER was measured by applying ATP extracellularly which activates IP_3 receptor channels in the ER (Fig. 3 and Table 4). When the Tyrode solution contain-

Table 3. Comparison of SOCI-induced $R_{340/380}$ changes in RKO and A549 cells

	Cell type	Non-irradiated control	Gamma-irradiated
Basal level	RKO	0.76±0.09 (n=22)	0.74±0.09 (n=20)
	A549	0.77±0.06 (n=35)	0.77±0.12 (n=35)
Peak value	RKO	1.14±0.14	1.60±0.44
	A549	1.26±0.30	1.10±0.24
Changes in $R_{340/380}$	RKO	0.38±0.14	0.86±0.44***
	A549	0.49±0.05	0.33±0.24*

*$p<0.05$, ***$p<0.0001$.

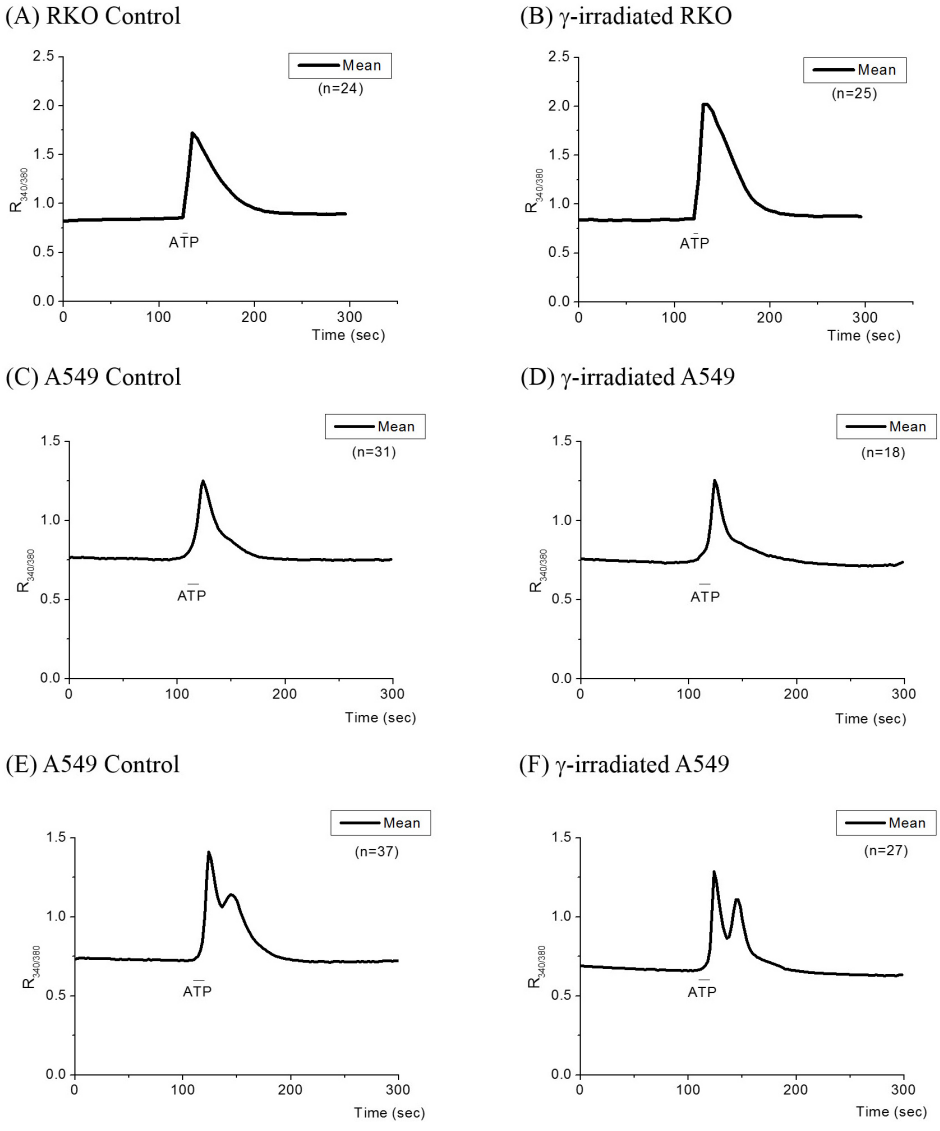

Fig. 3. Effects of γ-irradiation on ATP-induced $[Ca^{2+}]_i$ changes in RKO and A549 cells. (A) Application of 100 μM ATP induced transient changes in $R_{340/380}$ (ATP-induced Ca^{2+} responses) in RKO control cells. (B) In γ-ray irradiated RKO cells, transient changes in $R_{340/380}$ were enhanced, compared to control cells (1.18±0.07 vs. 0.89±0.06; $p<0.0001$). (C) In A549 control cells, transient changes in $R_{340/380}$ were elicited by application of 100 μM ATP. (D) In γ-ray irradiated A549 cells, transient changes in $R_{340/380}$ were not significantly different from those in control cells. (E) In more than 60% of A549 cells measured, multiple transient changes in $R_{340/380}$ were observed with a single application of ATP. (F) The frequency of multiple transient changes was increased in γ-ray irradiated A549 cells (See Table 5). Tracings in (A) to (F) represent the average values of $R_{340/380}$.

ing 100 μM ATP was applied for 10 sec, $R_{340/380}$ in RKO cells increased transiently, 0.83±0.05 to 1.72±0.29, and returned slowly to the basal level (Fig. 3A). In γ-ray irradiated RKO cells, $R_{340/380}$ increased from 0.84±0.05 to 2.02±0.35. The difference between the peak and basal values for $R_{340/380}$ in the control cells (n=24) was 0.89±0.29 and that in the γ-ray irradiated cells (n=25) was 1.18±0.35 ($p<0.0001$; Table 4).

When A549 cells were superfused with the Tyrode solution containing 100 μM ATP for 10 sec, $R_{340/380}$ increased transiently and returned to the basal level eliciting a single Ca^{2+} transient, as shown in Fig. 3C, in 50 cells out of 154 cells (32%) measured (Table 4). In other A549 cells, multiple Ca^{2+} transients were observed (Fig. 3E and Table 4).

Table 4. Comparison of ATP-induced $R_{340/380}$ changes in RKO and A549 cells

	Cell type	Non-irradiated control	Gamma-irradiated
Basal level	RKO	0.83±0.05 (n=24)	0.84±0.05 (n=25)
	A549	0.75±0.11 (n=31)	0.74±0.13 (n=18)
Peak value	RKO	1.72±0.29	2.02±0.35
	A549	1.25±0.17	1.25±0.17
Changes in $R_{340/380}$	RKO	0.89±0.29	1.18±0.35***
	A549	0.49±0.17	0.51±0.13

***$p < 0.0001$.

Table 5. Changes in $R_{340/380}$ induced by ATP in A549 cells

		Control (n=154)	γ-ray (48 hr) (n=167)
Events with 1 peak	Number of event	50	19
	Basal level	0.75±0.14	0.74±0.10
	Peak value	1.25±0.21	1.25±0.13
	Changes in $R_{340/380}$	0.49±0.21	0.51±0.10
2 peaks	Number of event	44	28
	Basal level	0.73±0.07	0.66±0.05
	Peak value	1.41±0.20	1.28±0.16
	Changes in $R_{340/380}$	0.68±0.20	0.63±0.16
3 peaks	Number of event	32	30
	Basal level	0.73±0.06	0.67±0.05
	Peak value	1.48±0.17	1.31±0.22
	Changes in $R_{340/380}$	0.75±0.17	0.64±0.22
Multi peaks	Number of event	28	90
	Basal level	0.71±0.05	0.69±0.09
	Peak value	1.41±0.16	1.33±0.47
	Changes in $R_{340/380}$	0.69±0.16	0.65±0.38

The amplitudes of Ca^{2+} transients, the differences between the peak and basal values of $R_{340/380}$, were not significantly different between control cells and γ-ray irradiated cells (Table 4). However, the frequency of multiple transients was increased in γ-ray irradiated cells (Table 5).

DISCUSSION

Surge of intracellular Ca^{2+} causing cell death can arise from a variety of sources. $[Ca^{2+}]_i$ can be increased by the entry of extracellular Ca^{2+} via SOCI or the release of stored Ca^{2+} form the ER via IP_3 receptors and ryanodine receptors in ER membranes [14-17]. Ca^{2+} influx via reverse mode of NCX also contribute to $[Ca^{2+}]_i$ increase [17,27,28].

By γ-irradiation, RKO cells begin to exit from G2/M arrest to apoptosis by 24 hrs after irradiation. Only small fractions of cells remain in G2/M phase by 48 hrs, implying that the post-mitotic apoptosis occurs by 48 hrs after irradiation [29]. During this time span, basal level of $[Ca^{2+}]_i$ in RKO cells remained relatively unchanged (Table 1) although irradiation elicited enlargement of viable cells (data no shown). Thus experiments were done with cells incubated for 48 hrs after γ-ray irradiation.

The change in $[Ca^{2+}]_i$ via reverse mode of NCX can be measured by blocking other cellular Ca^{2+} pathways as previously reported [26,27]. Irradiation does not seem to influence NCX ability to import Ca^{2+} into the cytosol of both RKO and A549 cells (Fig. 1). The forward mode of NCX plays a major role in clearing Ca^{2+} out of cytosol and can be measured by the decline of $[Ca^{2+}]_i$ as shown in Fig. 1. Interestingly, irradiation tends to speed up the pumping activity of NCX forward mode in both cells (Table 2). It is not clear that irradiation-induced pumping activity has any physiological role in cellular metabolism. Meanwhile, to understand the cause for additional increase in $[Ca^{2+}]_i$ via NCX over the plateau region, more information on the irradiation-induced changes in membrane fluidity is needed, since an enlargement of cells by irradiation was observed (data not shown).

Depletion or depression of Ca^{2+} content from ER can signal long-term cellular responses such as gene expression and programmed cell death or apoptosis [30,31] and provides a signal that activates Ca^{2+} entry through the SOCI channels [32,33]. Enhancement of Ca^{2+} entry via SOCI in RKO cells by irradiation, as shown in Fig. 2, may contribute to promotion of cell death. The enhancement of the SOCI activity may be a consequence of other cellular changes induced by irradiation, such as emptying of the ER following the increased Ca^{2+} release by irradiation (Fig. 3B). Irradiation may induce direct effects on SOCI-modulating proteins such as STIM and synergistic interaction of SOCI with other cellular components as reported in studies of irradiation-induced BAX interaction with SOCI [34,35].

The data of Fig. 2 provide indirect information on the ER content of Ca^{2+}. Pre-emptying the ER to induce Ca^{2+} influx via SOCI can estimate the size of releasable Ca^{2+} pool. The results of Fig. 2 and Table 3 imply that the Ca^{2+} content in the ER of RKO cells is much greater than that of A549 cells. The amounts of Ca^{2+} released from the ER

by ATP also feature the same character: RKO cells release greater amount of Ca^{2+} than A549 cells do (Table 4). Interestingly, γ-irradiation on A549 cells elicited decrements of ER Ca^{2+} content and Ca^{2+} influx via SOCI, while γ-irradiation on RKO cells resulted in enhancements of Ca^{2+} influx via SOCI (Fig. 2). These results, along with enhanced Ca^{2+} release from the ER by ATP in RKO cells as shown in Fig. 3, can provide possible explanation for distinct difference in cell death between RKO and A549 cells. Assuming that Ca^{2+} flux from the ER promotes cell death [20], enhanced Ca^{2+} release from the ER in RKO cells by γ-irradiation may explain radio-sensitivity of RKO cells. Unchanged Ca^{2+} release from the ER may be one of possible mechanisms for radiation resistivity of A549 cells. These observations are well supported by other reports stating that Ca^{2+} released from the reduction in ER is not sufficient to produce apoptosis [10,24].

Not surprisingly, it was found that the activity of Ca^{2+} transporters of A549 cells investigated in this study was not as much affected by γ-irradiation as that of radiosensitive RKO cells. However, γ-irradiation increased the incidence of multiple Ca^{2+} peaks in A549 cells which suggests that Ca^{2+}-induced Ca^{2+} release mechanism was activated by γ-irradiation (Table 5). To clarify the involvement of this Ca^{2+}-induced Ca^{2+} release mechanism in radiation-induced Ca^{2+} deregulation, further study with immunochemical and molecular biological methods will be needed [36]. However, the resting values of $[Ca^{2+}]_i$ were not increased by multiple Ca^{2+} transients (Table 4). The results of Table 2 and 3 also support the theme that γ-irradiation does not affect intracellular Ca^{2+} metabolism of A549 cells and these cells may not employ the Ca^{2+}-activated cellular process of cell death. In conclusion, these results suggest that γ-irradiation enhances the cellular Ca^{2+} metabolism in radiation-sensitive RKO cells and elicits programmed cell death. The results of this study may provide further understanding of the role of Ca^{2+} in promoting cell death and the opportunities for therapeutic intervention of cancer.

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