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Special Award Review Article

Oxidative Stress, Radiation-Adaptive Responses, and Aging

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Oxidative stress/Redox regulation/Radiation-adaptive response/Aging

Organisms living in an aerobic environment were forced to evolve effective cellular strategies to detoxify reactive oxygen species. Besides diverse antioxidant enzymes and compounds, DNA repair enzymes, and disassembly systems, which remove damaged proteins, regulation systems that control transcription, translation, and activation have also been developed. The adaptive responses, especially those to radiation, are defensive regulation mechanisms by which oxidative stress (conditioning irradiation) elicits a response against damage because of subsequent stress (challenging irradiation). Although many researchers have investigated these molecular mechanisms, they remain obscure because of their complex signaling pathways and the involvement of various proteins. This article reviews the factors concerned with radiation-adaptive response, the signaling pathways activated by conditioning irradiation, and the effects of aging on radiation-adaptive response. The proteomics approach is also introduced, which is a useful method for studying stress response in cells.

INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are constantly produced in various cells and tissues. Their concentrations are determined by the balance between their rates of production and the rates of clearance by antioxidant enzymes and compounds (Fig. 1). Living cells and tissues possess several mechanisms used for maintenance of the redox balance even after temporary exposure to increased concentrations of ROS or RNS, so-called redox homeostasis, which is maintained by antioxidant defense systems and their associated regulation pathways[1].

However, when ROS is vigorously and persistently produced, the antioxidant response may not be sufficient to reset the system to its original level of redox homeostasis, thereby very likely resulting in a disregulated redox balance. Elevated ROS damages various proteins, membranes, and nucleic acids and consequently gives rise to apoptotic cell death. Uncontrolled ROS production is usually responsible for environmental, inflammatory, and mitochondrial oxidative stress. However, if the initial increase in ROS is relatively small, the antioxidant response may be sufficient to compensate for resetting the original balance. Subsequently, various redox-sensitive signaling pathways are activated, which in turn activate repair and defense systems (Fig. 1). Thus physiological redox regulation can be attributed to a temporary increase in ROS and a temporary shift in the redox state toward more oxidative conditions. The biological response to slight or controlled oxidative stress is distinct from that to vigorous and deleterious oxidative stress and cannot be extrapolated or estimated from the response based on uncontrolled oxidative stress. Studies on the biological response to low dose of oxidative stress are important for the elucidation of the mechanisms responsible for redox regulation and the maintenance of redox homeostasis.

Ionizing radiation is the oxidative stress most widely studied for its low-dose effects. Low-dose radiation has been reported to induce hormesis[2–3], which is a beneficial stimulant effect of chronic low-dose radiation and radiation-adaptive response, which is a radioprotective effect of low-dose preirradiation followed by subsequent high-dose challenging irradiation[4]. It is difficult to estimate the stimulating effects of chronic low-dose irradiation on growth, longevity, immune response, and repair of genetic damage[5–7]. However, radiation-adaptive response is distinct from hormesis with regard to the requirement for an optimum preirradiation dose range and an optimum interval between preirradiation and challenging irradiation. Radiation-adaptive response is a biological defensive response that is induced by single low-dose irradiation under certain conditions. Recently, Rothkamm and Lobrich reported on γ-H2AX foci formation due to exposure to 1 mGy[8], indicating that DNA double-strand breaks (DSBs) are formed by irradiation as low as 1 mGy and that DNA-repair proteins are recruited to DSB sites. This phenomenon indicates the occurrence of a biological

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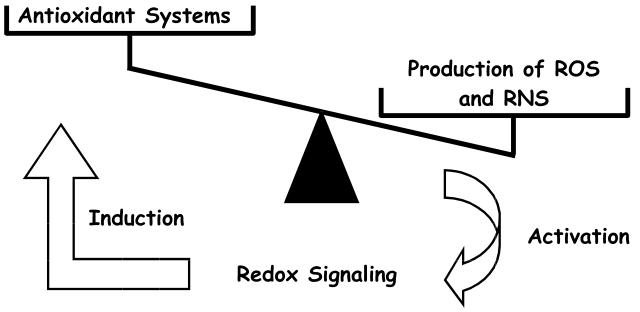


Fig. 1. Regulation of redox balance in biological systems. Redox balance between ROS/RNS production and antioxidant systems is maintained in biological systems. The imbalance to the oxidative state triggers redox-sensitive signaling pathways, leading to the induction of antioxidant systems and the maintenance of redox homeostasis.

response to slight or controlled oxidative stress, the first step in a radiation-adaptive response.

Organisms generally undergo qualitative changes with aging and their biological functions gradually degenerate. Biological responses to various oxidative stresses also decline with aging. Eventually, the activation of signaling pathways because of oxidative stress and the induction of stress-responsive molecules is diminished[9]. The free radical theory of aging implies that the age-related degenerative process is to a large extent the consequence of free radical damage. It is unknown whether the disfunction of redox regulation with aging causes the accumulation of free radical damage, or if free radical damage contributes to the disfunction of redox regulation. However, numerous cellular processes, including redox regulation and stress response, change with aging, and the effect of aging must be considered in all studies on biological phenomena. This review focuses on the mechanisms responsible for radiation-adaptive response and its variations as a result of aging.

RADIATION-ADAPTIVE RESPONSE

The adaptive response induced by low-dose genotoxic stress, which exerts protective effects against a subsequent larger dose, has been observed in several biological systems ranging from prokaryotes to eukaryotes. Early studies on prokaryotes showed that *Escherichia coli* treated with low fractionated doses of alkylating agents becomes refractory to lethal or mutagenic effects induced by higher doses[10]. The radioprotective effect elicited in response to primary irradiation has also been described for green alga *Chlamydomonas reinhardtii*[11], spores of the fern *Osmunda regalis*[12], the yeast *Saccharomyces cerevisiae*[13,14], and the bacterium *Vibrio cholerae*[15]. In the nematode *Caenorhabditis elegans*, it was also reported that pre-exposure to high oxygen concentrations brings about a protective effect against the lethality of subsequent X-irradiation[16]. Olivieri *et al*. reported on adaptive response in mammalian cells[17] and found that the incorporation of low concentrations of radioactive thymidine reduces chromatid aberrations as a result of subsequent higher doses of X-irradiation in human lymphocytes. Since then, various adaptive responses have been observed for diverse end points, such as cell survival, chromosomal aberration, micronucleus frequency, gene mutations, apoptosis, and cell growth[18–28]. Furthermore, for whole body irradiation, preirradiation at a low dose induces a radioprotective effect to subsequent challenging irradiation[29,30].

However, the molecular mechanisms that drive the adaptive response under these diverse conditions remain obscure. As shown in Fig. 2, primary irradiation may cause a redox imbalance. This causes the activation of signaling pathways, which induces various defense systems and results in an acquirement of radioresistance to high-dose challenging irradiation. Intervals between primary and challenging irradiation are necessary for these process to induce various defense systems. In yeast, the adaptive response is mainly due to the enhancement of cellular repair capacity, which is sufficient to repair any form of DNA damage[13,14]. In *Drosophila* oocytes, DNA repair systems play a role in the radiation-adaptive response[31]. Therefore, one possibility is that the adaptive response is a consequence of the induction of DNA repair mechanisms because of low-dose preirradiation. However, other systems including the antioxidant defense system and stress-response proteins are also candidates for primary factors responsible for radioprotective effects. In the next chapter, the factors and molecules involved in the adaptive response will be addressed.

IMPORTANT FACTORS IN RADIATION-ADAPTIVE RESPONSE

Diverse factors involved in radiation-adaptive response have been reported as a result of the use of various experimental models. These factors are classified as follows: [1] DNA repair systems, [2] cell cycle regulation systems, [3] antioxidant defense systems, [4] molecular chaperone or stress-responsive proteins, and [5] intercellular communication systems. Table 1 summarizes the possible involvement of these factors in radiation-adaptive response. However, it should be noted that the mechanisms that produce radiation-adaptive response might be dissimilar for the experimental conditions including the biological systems and observed end points.

DNA repair systems

The most important factors of the adaptive response are DNA repair systems[32–39]. ADP-ribose polymer synthesis is involved in the repair of DNA damage induced by genotoxic compounds such as alkylating agents[40,41]. Therefore, poly (ADP-ribosyl)ation may play a role in radiation-adaptive response. In human lymphocytes, Wolff and co-workers[34]

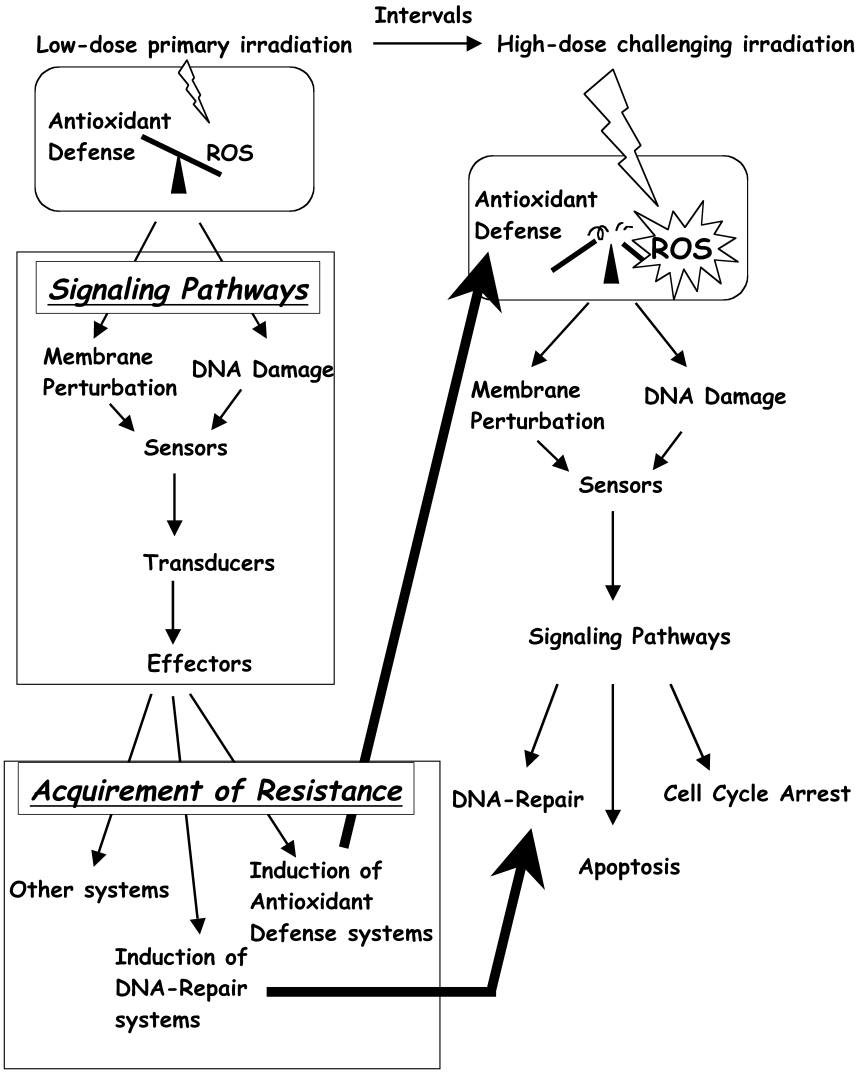


Fig. 2. Mechanisms responsible for radiation adaptive response. Low-dose primary irradiation causes a regulatory imbalance in redox homeostasis and triggers redox-sensitive signaling pathways, resulting in the induction of biological defense systems, including DNA repair and antioxidant defense systems, and the acquirement of resistance to subsequent high-dose challenging irradiation. Although subsequent challenging irradiation gives rise to various types of cellular damages, the induction of radioresistance by low-dose preirradiation reduces genotoxic or epigenotoxic damage because of challenging irradiation.

suggested that poly (ADP-ribosyl) ation is involved in radiation adaptive-response to chromosomal aberrations. Ikushima[35] reported that radiation-adaptive response observed in Chinese hamster V79 cells, which incorporated radioactive thymidine (^{3}H-adapted cells), is inhibited by the poly (ADP-ribose) polymerase (PARP) inhibitor, 3-aminobenzamide (3-AB). Kleczkowska and Althaus examined the response of the poly(ADP-ribosyl)ation system in low-dose adaptation. Poly (ADP-ribose) formation is enhanced in low-dose adapted cells and therefore suggests that PARP is part of a pathway that protects cells from downstream events resulting from DNA damage[38,39].

Cell cycle regulation systems

Zhou *et al*. examined changes in gene expression associated with radioadaptation in human lymphoblasts by using differential display and the rapid amplification of cDNA ends (RACE) method[42]. They found that in adapted cells, the down regulation of the CDC16 gene occurs more rapidly after challenging irradiation than in nonadapted cells. Since the CDC16 protein belongs to the anaphase-promoting complex (APC), which plays a role in cell progression through mitosis, radio-adapted cells are arrested earlier after the challenging high dose than nonadapted cells are. This phenomenon allows the repair of DNA damage more quickly in adapted cells, resulting in a resistance to subsequent irradiation.

Table 1. Possible factors involved in radiation adaptive response

Factors	Cell	Dose	Endpoints	Conditions	Ref.
DNA Repair					
Poly (ADP-ribose) polymerase	Human lymphocytes	0.1μCi/ml [^{3}H]dThd and 1.5 Gy X-ray	Chromosomal aberrations	The addition of 3-aminobenzamide, an inhibitor of PARP, abolished the adaptive response.	34
Poly (ADP-ribose) polymerase	Chinese hamster V79 cells	0.74 kBq/ml [^{3}H]dThd and 1Gy γ-ray	Micronuclei	The addition of 3-aminobenzamide, an inhibitor of PARP, abolished the adaptive response.	35
Poly (ADP-ribose) polymerase	Human hepatoma and Human lymphoblastoid	0.01 Gy and 0.5 Gy γ-ray with 1, 3, 48, or 72 h intervals	Chromatid breaks	The addition of 3-aminobenzamide, an inhibitor of PARP, abolished the adaptive response.	36, 37
Poly (ADP-ribose) polymerase	Human ovarian carcinoma and myeloma	0.01 Gy and 6 Gy γ-ray with 3 h intervals	Apoptosis	The addition of 3-aminobenzamide, an inhibitor of PARP, abolished the protective effects.	20
Cell Cycle Regulation					
CDC16	Human lymphoblastoid	0.02 Gy and 4 Gy γ-ray with 6h interval	CDC16 gene expression	The expression of CDC16 gene was repressed in adapted cells.	42
Antioxidant Defense					
MnSOD	Human lymphoblastoid	0.02 Gy and 3 Gy γ-ray with 6 h intervals	Enzyme activity	One and 3 hr after challenge doses, the activity was elevated in adapted cells compared with non-adapted cells.	43
Catalase	Human lymphoblastoid	0.02 Gy and 3 Gy γ-ray with 6 h intervals	Enzyme activity	Three hr after challenge doses, the activity was elevated in adapted cells compared with non-adapted cells.	
Gpx	Human lymphoblastoid	0.02 Gy and 3 Gy γ-ray with 6 h intervals	Enzyme activity	Three hr after challenge doses, the activity was elevated in adapted cells compared with non-adapted cells.	43
GST	Human lymphoblastoid	0.02 Gy and 3 Gy γ-ray with 6 h intervals	Enzyme activity	One hr after challenge doses, the activity was elevated in adapted cells compared with non-adapted cells.	43
MnSOD	Human breast adenocarcinoma	Fractionated irradiation at 60 Gy γ-ray (2 Gy per fraction, five times per week for 6 weeks)	Cell survival, MnSOD expression, and MnSOD activity	Cells following fractionated radiation were resistant to γ-irradiation, and the expression and the activity of MnSOD were increased.	48
Molecular Chaperone					
HSP70	Fibrosarcoma cells, Mouse NIH 3T3 cells	0.01 Gy and 4 Gy γ-ray with 4 or 7 h intervals	Clonogenic cell survival	Inducible Hsp70 transfected cells acquired the adaptive response.	50
HSP70	Fibrosarcoma cells, L929 cells	0.01 Gy and 2 Gy γ-ray with 4 h intervals	Clonogenic cell survival Apoptosis	Inducible Hsp70 transfected cells acquired the adaptive response.	51
HSP70	Splenocytes from C57BL6 mice	0.01 Gy and 2 Gy γ-ray with 4 h intercals	Apoptosis	Splenocytes from Hsp70 transgenic mice acquired the adaptive response.	52
HSP25	Fibrosarcoma cells, L929 cells	0.01 Gy and 2 Gy γ-ray with 4 h intervals	Clonogenic cell survival Apoptosis	Inducible Hsp25 transfected cells acquired the adaptive response.	51
PBP74/mortalin/GRP75	Human colorectal carcinoma and human breast adenocarcinoma	0.25 Gy and 4.0 Gy γ-ray with 4.5 h intervals	Clonogenic cell survival	The adaptive response was enhanced in cells transfected with the PBP74 construct and repressed in cells transfected with the anti-PBP plasmid.	53

Antioxidant defense system

Since ionizing radiation is one of oxidative stress, it is possible that the radioprotective response depends not only on DNA repair and cell cycle regulation, but also on antioxidant defense systems. Bravard *et al.*[43] reported on the activities of antioxidant enzymes after low-dose and/or subsequent high-dose irradiation. They examined the activities of antioxidant enzymes, such as Cu-Zn SOD (SOD1), MnSOD (SOD2), catalase, glutathione peroxidase (GPx), glutathione-S-transferase (GST), glutathione reductase (GR), and glucose-6-phosphate dehydrogenase (G6PD), in adapted and nonadapted AHH-1 lymphoblasts cells and found that 1 or 3 h after challenge dose the activities of SOD2, GST, GPx, and catalase were slightly more increased in adapted cells than in nonadapted cells. The increased activity of some antioxidant enzymes after challenge dose results in the rapid scavenging of ROS and consequently less cell damage. However, as described in their report, moderate alterations

of these antioxidant defenses only partly contribute to the protective mechanism underlying the radioadaptation of AHH-1 lymphoblasts. We examined the activities of catalase, GPx, GR, and glutathione content after low-dose and subsequent high-dose X-irradiation in rat glial cells. The activities of catalase, GPx, GR and glutathione content were not significantly induced (Fig. 8). Therefore antioxidant defenses only partly contribute to the radiation adaptive mechanism in glial and AHH-1 cells.

An endogenous antioxidant content is increased by low-dose radiation. The expression of the thioredoxin gene, which produces a key protein that is involved in regulating the cellular redox reaction, increases with low-dose γ-radiation (0.25Gy) in human lymphocytes[44]. Furthermore, glutathione (GSH) content in the liver of mice (C57BL/6, 8 weeks) is also elevated by whole body irradiation with 50 cGy[45], and intracellular GSH in the mouse macrophage-like cell line RAW 264.7 cell is increased by 50 cGy γ-radiation[46–47]. However, the mechanisms responsible for GSH elevation are different between the liver and macrophage-like cell line, because the expression of mRNA of γ-glutamylcysteine synthetase (γ-GCS), the rate-limiting enzyme for *de novo* glutathione synthesis, increases in RAW 264.7 cells, but not in the liver of mice. Although an irradiation dose of 50 cGy is higher than the conditioning dose required for a radiation-adaptive response in other experimental models, an elevation of the expression of intracellular antioxidants such as TRX and GSH results in a radioprotective effect and might contribute to radiation-adaptive response.

Redox alteration via the induction of MnSOD might play a role in the adaptive response rather than in antioxidant activity. Guo *et al*.[48] examined radioresistance and gene expression in human carcinoma cells following fractionated ionizing radiation and proposed that the induction of MnSOD after fractionated ionizing radiation causes redox alterations that result in the upregulation of stress-responsive genes and radiation adaptive responses.

Molecular chaperone or stress-responsive proteins

Stress events induce the expression of heat-shock proteins (HSPs), which can be divided into two categories: those that are constitutively expressed and those that are stress induced. Constitutive HSPs are involved in processes that control the quality of cellular proteins, including the folding of nascent protein, the targeting of proteins to lysosomes, and other functions performed by chaperones. Inducible HSPs are produced in response to stresses such as heat, heavy metals, oxidative stress and radiation, and they also function as cytoprotectants. HSP-transfected cells acquire an adaptive response to low-dose irradiation[49–52], and this response is repressed in cells transfected with the anti-PBP74 (a member of the HSP 70 family) plasmid[53]. These results suggest that some members of the HSP family are involved in radiation adaptive response. Park *et al*. suggested that radioresistance attributed to HSP70 is associated with elevated levels of PKC activity[50].

Intercellular communication systems

Gap-junctions are clusters of small aqueous channels that mediate the bystander effect by allowing the direct intercellular exchange of ions, small metabolites, and second messengers between irradiated and nonirradiated cells. Ishii & Watanabe[54] suggested that gap-junctional intercellular communication plays a role in the radioadaptive response as well as the bystander effect, based on their experiments using human embryonic (HE) cells cultured in Ca^{2+} ion- or 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA)-containing medium, which regulates or inhibits gap-junctional intercellular communication. Azzam *et al*.[55] found by using gene expression profiles that Connexin43 (cx43), which plays a role in gap-junctional intercellular communication, is upregulated by exposure to a low-dose γ-rays (0.5 Gy). The direct participation of cx43 in radiationadaptive response remains unclear. However, it appears that intercellular communication also plays a role in cellular responses to low-dose irradiation.

SIGNALING PATHWAYS OF RADIATION-ADAPTIVE RESPONSE

Cell responses to ionizing radiation are mediated by various signaling pathways[56,57]. For the adaptive response to occur, preirradiation is recognized by cellular sensing systems and transduced to response networks, which allows effector molecules to moderate the harmful damage induced by subsequent irradiation (Fig. 2). Among the signaling factors involved in cell response to radiation, protein kinase C (PKC), mitogen-activated protein kinase (MAPK), p53 tumor suppressor protein, nuclear factor κB (NF-κB), ataxia-telansiectasia mutated (ATM), and DNA-dependent protein kinase (DNAPK) will be the focus of this chapter.

PKC and MAPK

Members of the protein kinase C (PKC) family and mitogen-activated protein kinases (MAPKs) play roles in signaling pathways and cell responses to ionizing radiation[58,59]. Even low-dose irradiation (0.25–2.0 Gy) activates PKC, resulting in the enhancement of immediately early gene expression, such as proto-oncogenes c-fos, c-jun, c-myc, and c-Ha-ras in human lymphoblastoid 244B cells[60]. Woloschak *et al*.[61] examined the accumulation of PKC mRNA after γ-irradiation and reported that the expression of PKC-β mRNA is increased for doses ranging from 6 to 200 cGy in Syrian hamster embryo cells. Furthermore, PKC contains unique structural features that are susceptible to oxidative modification, and its activity is regulated by redox modifications.[62] Therefore PKC might be directly activated by oxidative

stress via redox reactions. For members of the MAPK family, Suzuki *et al*.[63] showed that low-dose ionizing radiation (between 2 and 5 cGy of X-irradiation) activates extracellular signal-regulated kinase (ERK1/2) via the activation of epidermal growth factor (EGF) receptor and mitogen-activated protein / ERK kinase (MEK 1).

From the experiments using PKC inhibitors such as calphostin C and 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7), the involvement of PKC in the adaptive response has been reported by some investigators[64,65]. However, the manners in which PKC and MAPKs are activated by low-dose irradiation, which eventually affords the cells radioprotection against subsequent challenging irradiation, is still being studied. Shimizu *et al*.[66] proposed a coordinated regulation mechanism involving PKCα, p38MAPK, and phospholipase C (PLC-δ1) in radiation-adaptive response by the use of cultured mouse cells. They determined the existence of a circular damage-sensing pathway as follows: Low-dose preirradiation activates PKCα followed by p38 MAPK. p38 MAPK physically associates with PLC-δ1, which produces diacylglycerol, an activator of PKC. The activity of PKCα is then regulated by PLC-δ1. Since PKC is responsible for the activation of DNA repair systems including DNA polymerase α[67] or DNA topoisomerase I[68], the induction of DNA repair systems mediated by the activation of the PKC pathway may be involved in radiation-adaptive response. Furthermore, since the enhancement of PKC activity con-

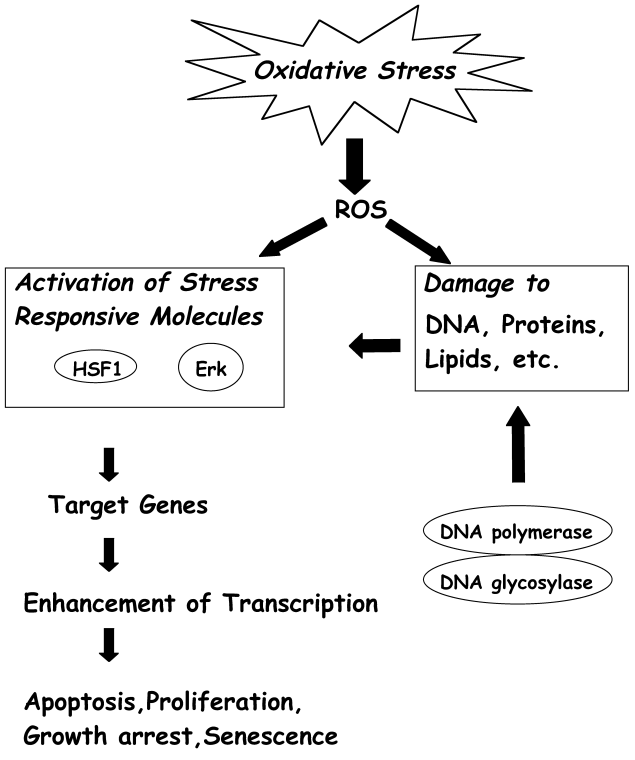


Fig. 3. Oxidative stress-responsive factors that are altered with age. ROS derived from oxidative stress activates the stress-responsive molecules, and/or causes damage to various cell components such as DNA, proteins, and lipids. Each activated stress-responsive molecule enhances the transcription of its target gene and regulates the apoptosis, proliferation, growth arrest, and senescence. Damaged components are repaired by various repair-molecules or are decomposed. The circled factors represent altered activities as a result of aging.

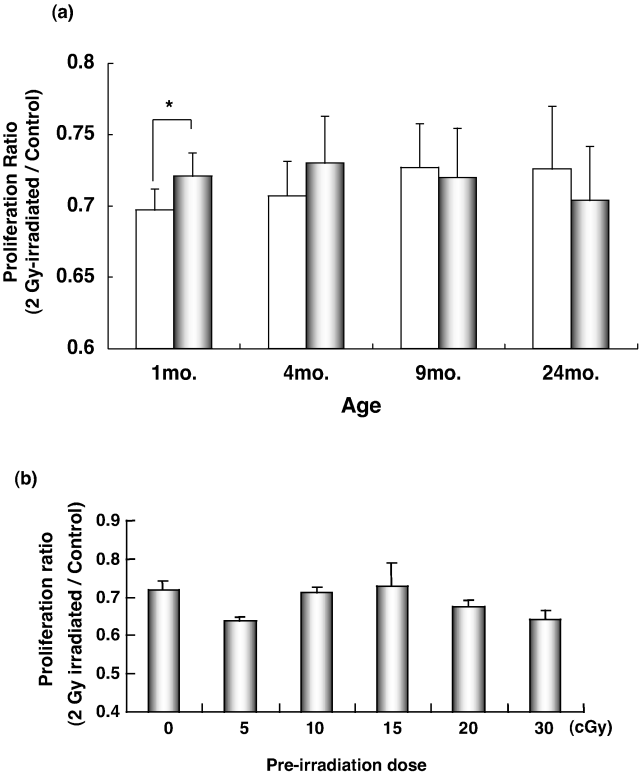


Fig. 4. Aging suppresses adaptive response of growth in rat glial cells. (a)Effects of aging on adaptive response induced by low-dose hydrogen peroxide. Glial cells from the hippocampus of Wistar rats aged 1, 4, 9, and 24 months were cultured. The cells were divided into four groups: no-irradiation without pretreatment, no-irradiation with 1 μM H_2O_2 pretreatment, 2 Gy-irradiation without pretreatment, and 2Gy-irradiation with 1 μM H_2O_2 pretreatment. Hydrogen peroxide diluted in serum-free medium was added to the cultures for 30 min at 1 μM, and 3 hr after washing with PBS they were irradiated X-ray at 2 Gy (0.34 Gy/min). Non-pre-treatment cells were cultured in serum-free medium for 30 min, and nonirradiated cells were sham irradiated. They were taken out of the CO_2 incubator for the same length of time as the 2 Gy-irradiated cells. Two days later, the cells were counted after trypsinization, and proliferation ratios were determined from the ratio of 2Gy-irradiated cells to nonirradiated cells with or without pretreatment. The open columns represent the proliferation ratios of non-pre-treatment cells, and the shadowed columns represent those of 1 μM H_2O_2 pretreatment cells.. (b) The dose dependence of preirradiation on the proliferation ratio of aged cells. The cells from aged rats (24 months old) were cultured. The experiments were performed under the same conditions as described in (a), except for preirradiation at each dose in place of 1 μM H_2O_2 pretreatment.

tributes to the radioresistance induced by HSP70[50] as described in the chapter entitled *Molecular chaperone or stress-responsive proteins*, it is possible that the activation of the PKC pathway is involved in radiation-adaptive response through cytoprotective stress-response proteins such as HSP70.

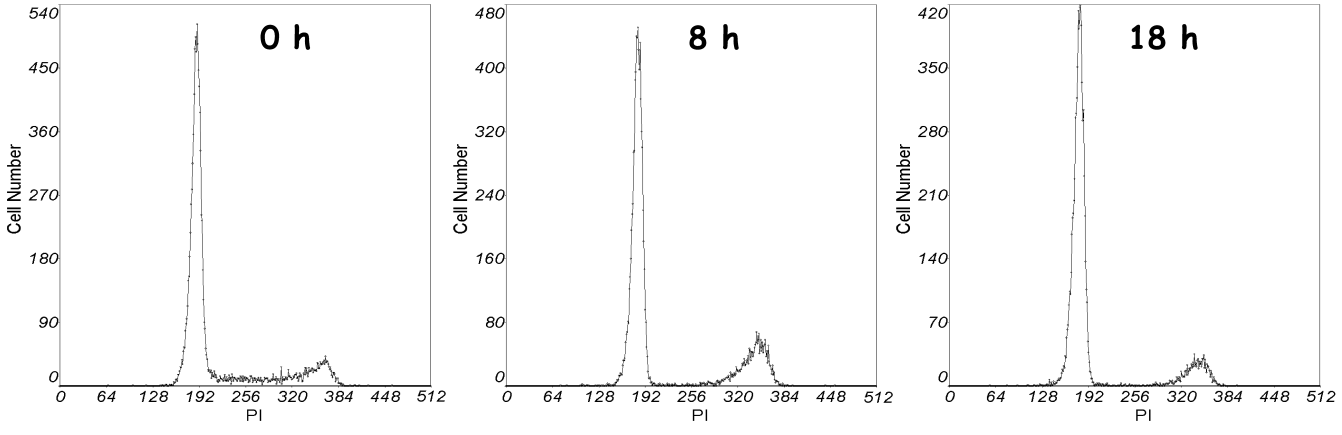
Furthermore, Lee *et al*.[69] found that low-dose preirradiation (0.01 Gy γ-ray) inhibits the translocation of PKCδ in primary mouse epidermal keratinocytes (PK). It appears that PKC isozymes involved in radiation adaptive response vary in each cell type and for each condition.

p53

p53 is the most important factor in the signaling pathway of radiation-adaptive response[70,71]. Radiation-adaptive response at various end points, such as micronuclei, apoptosis, and chromosome aberrations, is suppressed in p53 mutant cells[72–75]. For radiation-adaptive response induced by whole-body irradiation, knockout p53(–/–) mice show no radioprotective effects of preirradiation as seen with the use of the endogenous spleen colonies and the recovery of peripheral blood-cell counts[76]. Sasaki *et al*.[75] studied radiation-adaptive response in cultured mice and human cells with different genetic backgrounds relevant to the DNA damage response pathway and found that p53 protein plays a role in the adaptive response. Since p53 physically associates with p38 MAPK and is directly phosphorylated[77,78], they proposed that the signals are translated to p53 via a coordinated regulation mechanism involving a PKC-p38 MAPK-PLC pathway, as described in the previous chapter.

ATM and DNAPK

It is described above that p53 plays a role in radiation adaptive response, as seen in the use pf diverse experimental models. For signaling pathways upstream of p53, there are several activating protein kinases including ATM, ATR, DNAPK, and Chk1/Chk2[79]. Among them, the involvement of ATM and DNAPK in radiation-adaptive response has been studied. Seong *et al*.[37] discovered that lymphoblastoide derived from AT homozygotes and heterozygotes exhibit



Condition	Cell Cycle	0h	3h	8h	18h	24h
0 Gy + 5 Gy	G1	76.1 ± 1.98	71.6 ± 2.94	78.2 ± 3.42	88.4 ± 1.75	86.9 ± 2.35
	S	15.4 ± 1.75	18.4 ± 2.60	7.4 ± 4.29	1.7 ± 0.24	2.4 ± 0.35
	G2/M	8.5 ± 0.34	10.1 ± 0.47	14.5 ± 2.62	10.0 ± 1.59	8.2 ± 2.01
0.1 Gy + 5 Gy	G1	75.0 ± 2.11*	73.2 ± 1.84	78.3 ± 2.80	89.9 ± 1.60	89.4 ± 2.16
	S	15.9 ± 1.41	16.1 ± 1.39	6.2 ± 3.01	2.0 ± 0.52	2.5 ± 0.62
	G2/M	9.1 ± 0.71	10.8 ± 0.91	15.5 ± 1.80	8.1 ± 1.16*	8.2 ± 1.76

The data represents the percentage of cells in each phase determined by Muticycle software as the mean ± standard error.
* $p < 0.05$, significantly different from the cell cycle distribution of non pre-irradiation at the same time after 5 Gy irradiation.

Fig. 5. Cell cycle distribution after 5 Gy irradiation of glial cells. Above: Representative single-parameter histograms of propidium iodide (PI) fluorescence (DNA content). Glial cells cultured from young rats (1 month old) were irradiated at 5 Gy, and fixed and stained by PI each time after irradiation. Analysis of DNA content was carried out with a flow cytometer (EPICS ALTRA, Beckman Coulter, Co.). The radiation-induced loss of S-phase cells and cell cycle arrest are shown 8 and 18 h after irradiation. Below: The effects of preirradiation on cell cycle distribution after 5 Gy-irradiation. The cells were exposed to 5Gy-irradiation 3 hr after 0.1 Gy-pre-irradiation (0.1 Gy + 5 Gy in table) or sham irradiation (0 Gy + 5 Gy in table). For each indicated time after 5Gy-irradiation, the cells were fixed and stained by PI.

radiation-adaptive response to chromatid breaks, and this also occurs for lymphoblasticide derived from a normal individual. Sasaki *et al*.[75] reported results by using cultured fibroblasts with mutated ATM genes. These reports suggest that ATM is not responsible for the adaptive response. In contrast, Nemethova *et al*.[80] examined the adaptive response in the lymphocytes of AT patients and found that it was absent in cells from AT homozygotes after low-dose γ-rays exposure. They described that the adaptive ability of the lymphocytes varied among AT donors.

DNAPK does not play a role in adaptive responses responsible for cell survival, chromosome aberrations, and apoptosis.[75,81,82] However, we examined the effects of low-dose preirradiation on the cell growth of cultured glial cells[83] and showed that glial cells cultured from scid mice, which has no detectable DNAPK activity, do not exhibit radiation-adaptive response. These inconsistent results between our experimental system and others might be due to the difference between genetic and epigenetic endpoints in which the adaptive responses were observed. Furthermore, Takahashi *et al*.[84] examined the induction of apoptosis in the spleens of scid mice by acute irradiation and found that the adaptive response is not observed in the scid mice, which is different from wild-type mice. They noted that immune responses might participate in the radiation-adaptive response in *in vivo* studies.

NF-κB

Nuclear factor κB (NF-κB) is an oxidative stress-responsive transcription factor, which is involved in the transcriptional regulation of several genes[85]. Meltz and co-workers[86,87] reported that low-dose irradiation (0.1–2.0 Gy) induces the expression of NF-κB in human lymphoblastoid 244B cells and that the activation of NF-κB is inhibited by antioxidants such as N-acetyl-L-cysteine. Although it is unknown whether the activation of NF-κB by low-dose irradiation is directly involved in the adaptive response, as Guo *et al*. suggested[48], redox alteration resulting from NF-κB activation and the subsequent induction of MnSOD might play a role in radioresistance exhibited by low-dose-irradiated cells.

AGE-RELATED DECLINE IN THE CELL RESPONSE TO OXIDATIVE STRESS

Figure 3 shows the major influence of oxidative stress on biological systems and the factors whose activities are altered with aging. Elevated ROS activates stress-responsive signaling pathways and damage various biological components, including DNA, proteins, and lipids. Several of the pathways activated by acute oxidative stress show diminished activity as a function of aging[9].

The induction of heat-shock factor (HSF) and HSP, especially HSP70, decreases with age[88]. Since HSP plays a role in cellular defense mechanisms against heat, oxidative stress and other types of stress, the decrease in this system could seriously reduce the capacity of an organism to respond to changes in its environment. In contrast, however, complementary DNA microarray analysis has provided evidence that the basal expression of heat-shock proteins actually increases with aging[89,90]. In the aged rat brain[91] or human fibroblasts[92], it also results in an increase in the basal level of HSP70. This elevated expression occurs as a response to the age-associated accumulation of proteins damaged by oxidation. Age-associated elevations in the expression of heat-shock proteins have also been observed in *Drosophila*[93].

The efficiency of signal transduction pathways activated by oxidative stress declines with aging.[94,95] The expressions of extracellular signal-regulated kinase (ERK) and Akt kinase, known as protein kinase B, are reduced in aged hepatocytes, indicating that old hepatocytes are more susceptible to ROS-induced apoptosis than young ones are.[96–98]

The capacity of DNA repair systems also decreases with aging, as seen by the decline in the activity of the 8-oxoguanine repair-specific enzyme, 8-oxoguanine-DNA glycosylase (Ogg 1)[99]. The induction of DNA polymerase β and γ as a result of γ-irradiation was decreased with aging[100–101]. Thus

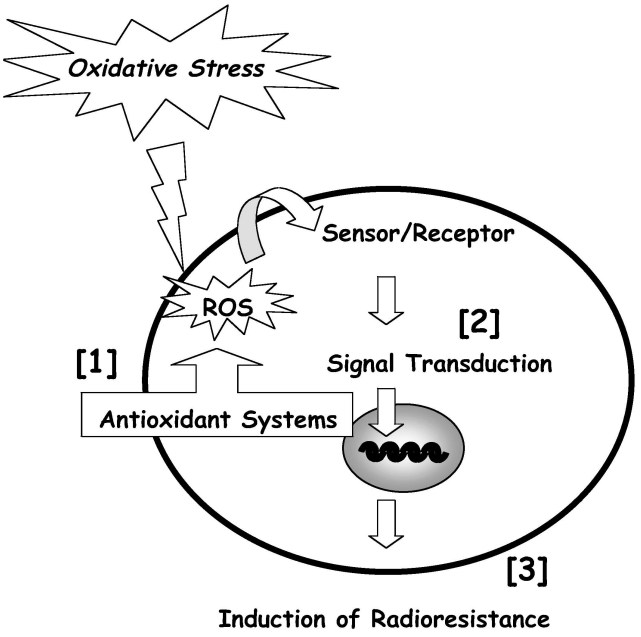


Fig. 6. Hypothesis of aging mechanisms that affect adaptive response to oxidative stress. Oxidative stress generates ROS in biological systems, which are quenched by endogenous antioxidant systems, including antioxidant enzymes and substances [1]. However, ROS rescued from antioxidant systems causes redox imbalance and activates redox-sensitive signaling pathways [2], leading to cellular responses and the acquirement of resistances including diverse protein synthesis [3]. Since aging suppresses radiation adaptive response, it prevents any steps among these processes ([1], [2], and/or [3]) from occurring.

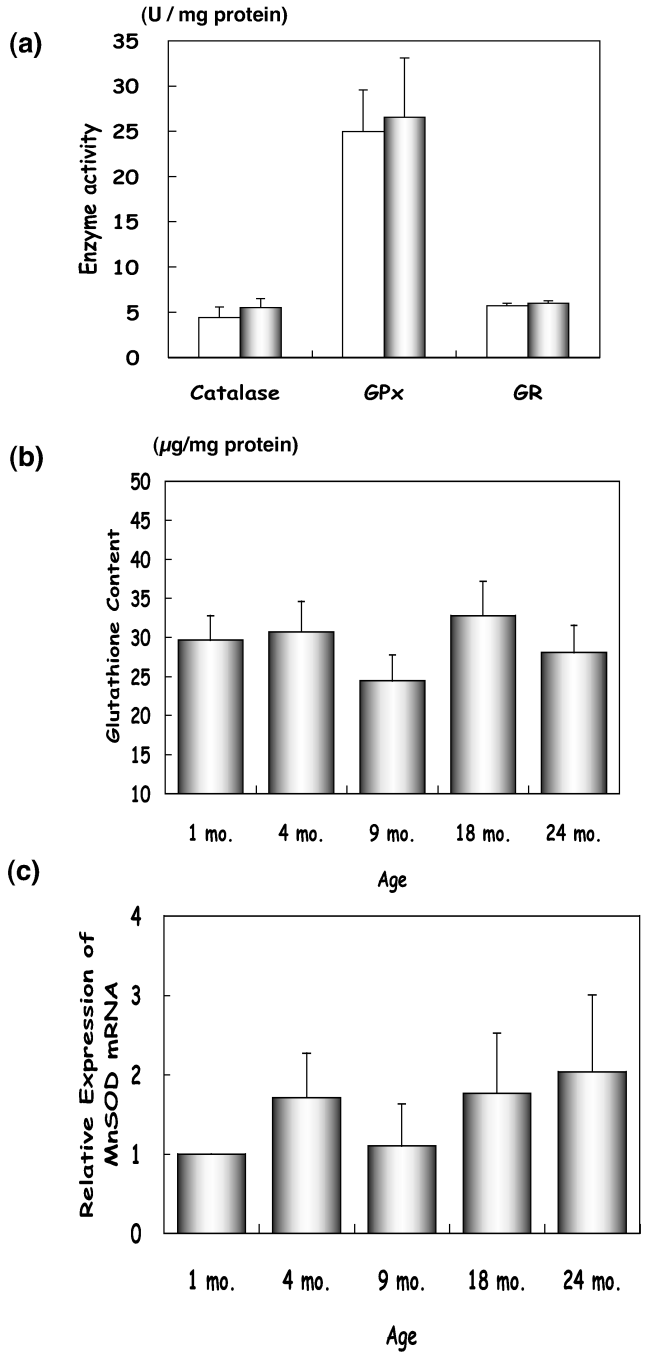


Fig. 7. Effects of aging on antioxidant systems in glial cells. (a) The effects of aging on the activities of catalase, glutathione peroxidase (GPx), and glutathione reductase (GR). Catalase activity was measured by following H_2O_2 breakdown at 240 nm, and GPx activity was determined by following NADPH oxidation, using t-butyl-hydroperoxide as a substrate. GR activity was determined by measuring NADPH oxidation in the presence of oxidized glutathione, and protein content was determined by using a Bio-Rad kit based on Bradford's method with bovine γ-globulin as a standard. The white columns represent the activities in cells from young rats (1 mo.), and the shadow columns represent the activities in cells from aged rats (24 mo.). b) The effects of aging on reduced glutathione content in glial cells. Cells were cultured from variously aged rats (1, 4, 9, 18, and 24 months old). The reduced form of glutathione was extracted from cells with 4% monochloroacetic acid and determined by reversed-phase HPLC equipped with an electrochemical detector (0.6 V, LC-4C, BAS Co.). (c) MnSOD mRNA was determined by Northern blot analysis. Nylon filters containing total RNA extracted from variously aged cells were hybridized with radioactively labeled oligonucleotides. Filters were placed against an imaging plate (BAS-IP, Fuji Film Co.) for several days, and gene expression levels were estimated by BAS-2500 (Fuji Film, Co.). The gene expression level of glyceraldehyde-3-phospate dehydrogenase (GAPDH) was used as an internal standard.

oxidative damage and DNA mutations accumulate in aged tissues.

For radiation adaptive response, aging represses the extent of adaptation. Venkat *et al.*[102] examined the effect of low-dose irradiation on the frequency of micronuclei in human lymphocytes in younger (25–30), middle-aged (31–40) and older (41–57) people. They reported that adaptive response depends on the age of the donor and decreases with increasing age. Gadhia[103] also found that aging abolishes the adaptive response by examining chromatid and isochromatid breaks in human peripheral blood lymphocytes. The variability of adaptive response is likely related to the physiological state of the cell at the time of irradiation, and aging might affect this state. In the next chapter, our experiments that examined the adaptive response by observing the growth of rat glial cells will be addressed, and we will elaborate on the effect of aging on the adaptive response.

EFFECTS OF AGING ON ADAPTIVE RESPONSE IN GLIAL CELLS

We aimed to clarify the effects of aging and its associated mechanisms on radiation-adaptive response by using glial cells cultured from rats of various ages. In the central nervous system, oxidative metabolism and the generation of ROS are important because of [1] the high metabolic demand for oxygen by neuronal mitochondria, [2] many oxidizable polyunsaturated fatty acids present in membrane lipids, and [3] non-protein-bound irons in the cerebrospinal fluid, which produce highly reactive hydroxyl radicals by the Haber-Weiss reaction[104]. Oxidized proteins are found in the brain affected by neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotropic lateral sclerosis, suggesting that ROS generation in the central nervous system is associated with neuropathological consequences[105]. Glial cells, especially astrocytes, are more resistant to oxidative stress than neurons and support the survival of neuronal cells by releasing various antioxidants and nutritional factors[106–110]. Therefore astrocytes respond to various environmental stresses, including ionizing radiation. Fur-

thermore, they can be cultured from aged animals distinct from neurons and are suitable for studying the aging process. Thus we examined the adaptive response to low-dose irradiation and low-dose hydrogen peroxide by observing cell growth and the effects of aging on the adaptive response by using astrocytes cultured from young and aged rats.

Figure 4a shows the adaptive response induced by low-dose hydrogen peroxide in astrocytes cultured from variously aged rats. The conditioning concentration and challenging irradiation were 1 μM H_2O_2 and 2 Gy of X-rays, respectively, separated by a 3 hr interval. Radiation adaptive response induced by 0.1 Gy preirradiation and 2 Gy-challenging irradiation was previously reported[83]. In glial cells from young rats, adaptive response is induced by a low dose of H_2O_2 or low-dose preirradiation, and aging suppresses the adaptive response. Since it is possible that in cells from aged rats the dose of preirradiation required to induce adaptive response is different from that of young rats, we varied the preirradiation dose for the aged cells. However, no radiation adaptive response was observed by any preirradiation dose from 0.05 to 0.3 Gy for these cells (Fig. 4b). From experiments using 5-bromo-2'-deoxyuridine (BrdU) incorporation as an indicator of cell proliferation, radiation adaptive response was also found to be increasingly suppressed with age[83].

Next, the effects of preirradiation on cell cycle arrest because of a challenging dose were examined by cell cycle analysis with a flow cytometer. Conditioning irradiation at 0.1 Gy did not significantly affect the cell cycle distribution after 5 Gy-irradiation (Fig. 5). It was suggested that preirradiation did not inhibit cell cycle arrest because of the challenging dose in glial cells and that the alteration of checkpoint regulation by preirradiation did not contribute to the result shown in Fig 4.

To determine why radiation adaptive response is suppressed with age, three hypotheses on the mechanisms responsible for aging are given as follows (Fig. 6); [1] antioxidant defense systems are already induced in aged cells by environmental and chronic oxidative stress, resulting in a nonrecognition of low-dose irradiation as a redox imbalance; [2] the signal transduction pathways responsive to low-dose irradiation are disturbed in aged cells; [3] the induction or activation of proteins involved in radioresistance are decreased in aged cells. First we examined the activities of antioxidant defense systems in young and aged cells. Shown in Fig. 7 are the effects of aging on the activities of antioxidant defense systems, including antioxidant enzymes such as catalase, glutathione peroxidase (GPx), and glutathione reductase (GR) (Fig. 7a); the content of reduced glutathione in cells (Fig. 7b); and the relative expression of MnSOD mRNA (Fig. 7c). These data show that the activities of antioxidant enzymes, the contents of reduced glutathione, and MnSOD expression are not significantly changed with aging. Therefore aging does not enhance antioxidant defense systems in cell culture under the present conditions. Furthermore, we examined the effects of low-dose preirradiation and subsequent high-dose challenging irradiation on the activities of antioxidant enzymes (Fig. 8). In cells from young rats, the activities of catalase, GPx, and GR tended to

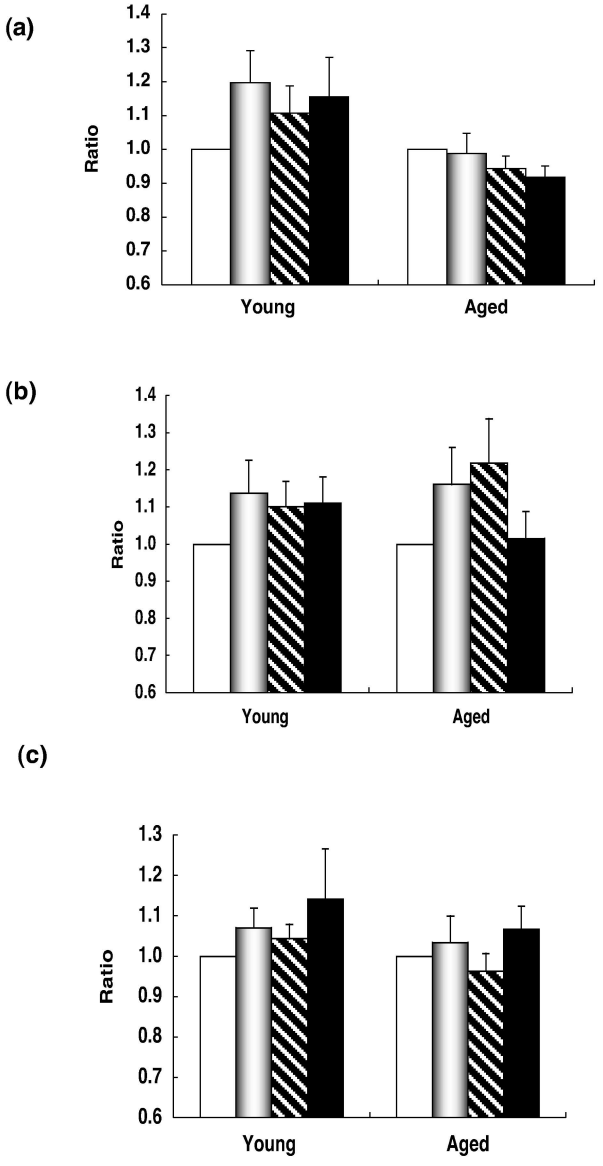


Fig. 8. Radiation-induced alterations in antioxidant enzyme activities in young and aged rat glial cells. Cells were cultured from young (1 month old) and aged (24 months old) rats, and each cell culture was divided into four groups: no-irradiation without preirradiation, no-irradiation with 0.1 Gy preirradiation, 2 Gy-irradiation without preirradiation, and 2Gy-irradiation with 0.1 Gy preirradiation. The interval between preirradiation and subsequent challenging irradiation was 3 hr. (a) Catalase activity, (b) GPx activity, and (c) GR activity were determined as described in the legend of Fig. 7. White columns; no-irradiation without preirradiation cells, shadowed columns; 2 Gy-irradiation without preirradiation, stripe columns; no-irradiation with 0.1 Gy preirradiation, black columns; 2Gy-irradiation with 0.1 Gy preirradiation.

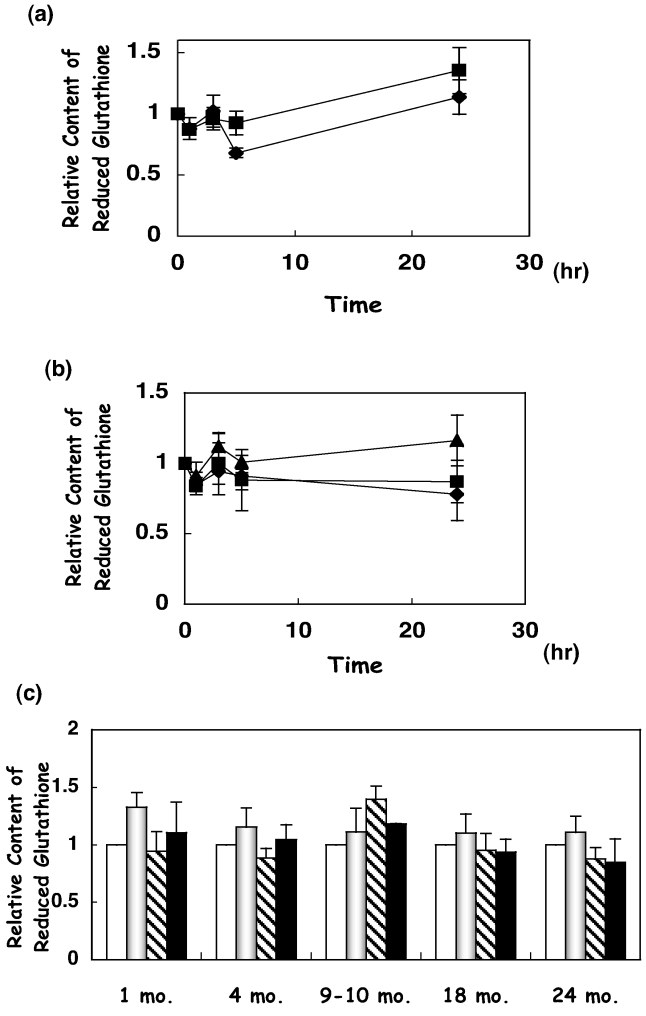


Fig. 9. Glutathione content after low-dose H_2O_2 or subsequent high-dose irradiation in glial cells from various aged rats. Cells were cultured from variously aged rats (1, 4, 9, 18, and 24 months old). The amount of the reduced form of glutathione was determined as described in the legend of Fig. 7 (a) and (b). Cells were treatment with 1 μM H_2O_2 and harvested each time after treatment. (a) Cells from younger rats. diamonds; 1 mo. squares; 4 mo. (b) Cells from elder rats. diamonds; 9 mo. squares; 18 mo. triangles; 24 mo. (c) The effects of H_2O_2 pretreatment on reduced glutathione content after 2 Gy-irradiation. Each cell culture was divided into four groups in the same manner described in the legend of Fig. 4 (a). The cells were harvested 24 hr after 2 Gy-irradiation, and the amount of the reduced glutathione was determined. White columns; no-irradiation without pretreatment, shadowed columns; 2 Gy-irradiation without pretreatment, stripe columns; no-irradiation with 1 μM H_2O_2 pretreatment, black columns; 2Gy-irradiation with 1 μM H_2O_2 pretreatment.

increase by exposure to low and/or high dose irradiation, but not significantly. In cells from aged rats, the activities of catalase and GR were not changed, and that of GPx tended to increase from radiation, but not significantly. These results suggest that irradiation does not significantly change the activities of antioxidant enzymes in young and aged cells. Next, the effect of irradiation on the reduced glutathione content was examined. Figs. 9 (a) and (b) show the alteration in reduced glutathione content after low-dose irradiation in cells from younger rats (a) and older rats (b), respectively. It was found that the GSH content of cells from 1 or 4 months rats increased 24 h after low-dose irradiation but not significantly, but that of cells from aged rats was not increased by low-dose irradiation. Figure 9 (c) shows the reduced glutathione content after low-dose and the subsequent high-dose irradiation. It has been suggested that glutathione content after irradiation is not significantly changed in younger and older rat cells. Although Bravad *et al*.[48] and Kojima *et al*.[45–47] reported on the significant enhancement of antioxidant activities or glutathione content, as described in the chapter on *antioxidant systems*, the antioxidant defense systems in rat glial cells were not significantly induced under the present conditions. Therefore we concluded that the decrease in the response to low-dose radiation with aging in rat glial cells was not explained by hypothesis [1], that the antioxidant defense systems are already induced in aged cells by environmental and chronic oxidative stress. Thus the primary factor responsible for the decrease in the cell response to low-dose radiation might be explained by hypothesis [2] or [3] for glial cells.

PERSPECTIVE

Cellular responses to ionizing radiation are mediated by genes, which control complex pathways. DNA arrays provide a means for evaluating the relative expression of thousands of genes in a single hybridization experiment and are suitable for a comprehensive search for genes involved in the stress response. Using DNA arrays, it was clarified that high-dose ionizing radiation induces diverse gene groups such as cell cycle regulation, DNA repair, signal transduction, apoptosis induction, and the damage response/maintenance of genetic stability in various types of cells[111–116]. For low-dose irradiation, Yin *et al*. reported on changes in gene expression in the mouse brain after 0.1 Gy γ-irradiation[117]. They found that low-dose radiation also modulates the expression of genes involved in the stress response, cell cycle regulation, and DNA synthesis/repair. It was suggested that 0.1 Gy-irradiation causes changes in gene expression involved in protective and reparative functions and qualitatively different biological response compared to 2 Gy-irradiation.

Genomic and transcriptomic profiles have been determined for a large number of potential biomarkers, but the functional components of a biological system are proteins and not genes. Even if one gene is mutated, its mRNA copy does not necessarily reflect the functional protein molecule through transcription and translation. Therefore focusing on proteins has certain advantages compared to focusing on mRNA. Proteomics, the large-scale analysis of proteins, will

greatly contribute to our understanding of gene function in the post-genomic era[118–119]. Proteomics can be divided into three main areas: [1] protein characterization for large-scale identification and to identify posttranslational modifications; [2] "differential display" for the comparison of protein levels in a range of diseases or stresses; and [3] studies of protein-protein interactions. Although several technical challenges in proteomics remain, proteomic analysis offers great potential for studies on cellular protein alterations in various disease, aging, or oxidative stress[120–123]. Furthermore, one unique feature of proteomics is the ability to analyze the posttranslational modification of proteins. Phosphorylation and glycosylation as well as other modifications are important for protein function because they determine the activity, stability, localization, and turnover. These modifications cannot be analyzed by genomic sequencing or mRNA expression data.

Szkanderova *et al*.[124] reported only on radiation effects on the protein expression profile of L929 cells using a 2-DE differential display. They showed that X-rays at 6 Gy induce the synthesis of diverse proteins that participate in protective and reparative cell responses or in the induction of apoptosis and oncogenesis. It should be confirmed whether these protein candidates whose expression patterns are influenced by radiation are intrinsically important in radiation biology. However, it is expected that the proteomics approach will soon be a useful method for unraveling the molecular mechanisms involved in cell response to ionizing radiation.

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