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Chromosomal DNA fragmentation in apoptosis and necrosis induced by oxidative stress

Yoshihiro Higuchi*

Department of Molecular Pharmacology, Kanazawa University Graduate School of Medical Science, Kanazawa 920-8640, Japan

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Abstract

Chromosomal DNA dysfunction plays a role in mammalian cell death. Oxidative stress producing reactive oxygen species (ROS) induces chromatin dysfunction such as single- and double-strand DNA fragmentation leading to cell death through apoptosis or necrosis. More than 1 Mbp giant DNA, 200–800 or 50–300 kbp high molecular weight (HMW) DNA and internucleosomal DNA fragments are produced by oxidative stress and by some agents producing ROS during apoptosis or necrosis in several types of mammalian cells. Some nucleases involved in the chromosomal DNA fragmentation in apoptosis or necrosis are classified. ROS-mediated DNA fragmentation is caused and enhanced by polyunsaturated fatty acids (PUFAs) or their hydroperoxides through lipid peroxidation. A reduction of intracellular GSH levels induced by the inhibition of cystein transport or GSH biosynthesis leads to cell death through over production and accumulation of ROS in some types of mammalian cells. The ROS accumulation system has been used as a model of oxidative stress to discuss whether ROS-mediated DNA fragmentation associated with cell death is based on apoptosis or necrosis.

Keywords: Apoptosis; Endonucleases; Giant DNA fragmentation; GSH depletion; Necrosis; Oxidative stress

1. Introduction

Cellular genomes are continually subjected to endogenous and environmentally-induced structural alterations. Our environment contains a multitude of substances which are carcinogenic and which, in many cases, are thought to act *via* direct damage to DNA. Such damage can manifest itself as gross chromosomal abnormalities inducing cell death. Cell death arises solely as a consequence of pathological processes, but it is now recognized that the death of certain cells is a physiological phenomenon necessary for normal development, maintenance of tissue shape and cell renewal. Although the classification of cell death has proven difficult, two distinct patterns of cell death have been identified based on the morphology of dying cells, and on the DNA fragmentation or damage. These have been termed necrosis and apoptosis [1]. Mammalian cell death is induced through chromosomal DNA damage by ionizing radiation, ultraviolet (UV) radiation, anticancer drugs and various triggers of apoptosis.

ROS such as hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$) and superoxide anions (O_2^-) have been shown to damage chromosomal DNA and other cellular components, resulting in DNA degradation, protein denaturation, and lipid peroxidation. However, the mechanisms behind these cellular effects are rather complex, and are not yet fully understood. DNA damage induced by oxygen radicals occurs by oxidative nucleic acid base modification and scission of DNA strands. Most agents producing ROS induce cell death including apoptosis, by causing lipid peroxidation and DNA damage [2]. However, the implications of lipid peroxidation for ROS-induced DNA damage remain to be elucidated. There is a recent research review suggesting that amyloid β-peptide is heavily deposited in

* Tel.: +81-76-265-2186; fax: +81-76-234-4227.
E-mail address: higuchiy@med.kanazawa-u.ac.jp (Y. Higuchi).

Abbreviations: AIF, apoptosis-inducing factor; BSO, L-buthionine-(*S*,*R*)-sulfoximine; CAD, caspase-activated DNase; DFF, DNA fragmentation factor; GSH, reduced glutathione; GSSG, oxidized glutathione; L, lipid radical; LO, lipid alkoxyl radical; LOO, lipid peroxyl radical; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine; HMW, high molecular weight; PARP, poly(ADP-ribose) polymerase; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling.

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the brains of Alzheimer's disease patients, and free radical oxidative stress, particularly of neuronal lipids and proteins, is extensive [3].

Our purpose is to review the chromosomal DNA fragmentations such as giant DNA, HMW DNA, and internucleosomal DNA fragmentations and to reflect upon their significance in the cell death induced by oxidative stress.

2. Chromatin structure and pattern of chromosomal DNA fragmentation

A mammalian cell nucleus contains almost 50 cm of DNA requiring more than a 50,000-fold reduction in length to fit in the nucleus and nuclear matrix. The nuclear matrix is an important structural component in a variety of nuclear functions and nuclear morphology, including DNA organization, DNA replication, RNA synthesis and nuclear regulation. DNA loop domains of chromatin are attached to the nuclear matrix at their base and this organization is maintained throughout both interphase and metaphase. These loops are 50–150 kbp long and are equivalent in size to the replicon [4]. The haploid human genome contains 3000 megabase pairs (Mbp) of DNA with a mean chromosomal size of 130 Mbp.

Chromosomal DNA fragmentation is caused by two types of DNA breaks that are classified into single- and double-strand DNA breaks. Single-strand cleavage of DNA has been suggested to occur during apoptosis. At the level of the nuclear scaffold, single-strand DNA breaks were detected in HL-60 cells treated with camptothecin, a topoisomerase I inhibitor and inducer of apoptosis, but these were rapidly repaired after drug removal [5]. Internucleosomal DNA cleavage occurred after the repair of these single-strand cuts, suggesting that single-strand breaks at higher levels of DNA organization may not play an active role during apoptosis but can perhaps act as signals to induce the process. Clearly the role that single-stranded DNA breaks play during apoptosis requires additional studies. Double-strand DNA breaks are generally thought to have a greater biological consequence than single-strand DNA breaks because they can lead directly to chromosomal aberrations, and more frequently to the loss of genetic information [6]. Double-strand DNA breaks are 20 times less frequent than single-strand DNA breaks and are more difficult to measure at physiological doses. The application of gel electrophoresis to the measurement of double-strand DNA breaks has been described by some workers [7].

Chromosomal DNA fragments of more than 1 Mbp in size are double-strand DNA breaks and are classified as giant DNA fragments. DNA degradation accompanied by DNA fragmentation producing 1–2 Mbp and 200–800 kbp DNA fragments were observed during cell death in cells treated with some agents that can produce ROS [2,8], or under GSH depletion [9]. Chromosomal DNA fragments which are 200–800 and 50–300 kbp in size are called HMW DNA fragments. The 1–2 Mbp giant DNA and 200–800 kbp HMW fragmentations that may represent features of high-order chromatin structure such as minibands and loops of DNA [10], lead to apoptosis ascertained by internucleosomal DNA fragmentation [11]. However, little is known about the mechanism of giant DNA and HMW DNA fragmentation during apoptosis induced by ROS.

3. Reactive oxygen species (ROS)-mediated chromosomal DNA fragmentation

DNA damage caused by ROS *in vivo* or in cultured cell systems is classified into DNA cleavages such as single-strand breaks, and double-strand breaks and nucleotide base oxidative modifications [12]. We know a little about the *in vivo* action mechanism of ROS produced by anticancer drugs, ionizing radiations and ultraviolet (UV) ray on chromatin DNA in the nuclei of cells. Ionizing radiation such as X-rays and γ-rays are, in general, thought to produce hydroxyl radicals from water molecules in or around the target sites in the DNA, and these in turn attack DNA and break it down [13]. The reaction of intracellular ROS with DNA results in numerous forms of base damage, and 8-hydroxy-2′-deoxyguanosine (8-OH-dG) is one of most abundant and the most studied lesions generated [14]. In addition, the involvement of ROS in the induction of apoptosis has been suggested in several cell lines [11,15,16].

Using pulsed-field gel electrophoresis, some groups have reported the size distribution of radiation-induced DNA fragments in mammalian cells such as Chinese hamster ovary cells [17] and L-1210 mouse leukemia cells [18]. In L-1210 cells irradiated at 1–50 Gy, double-strand DNA break fragments, calculated from marker chromosomes to be in the range of 0.1–12.6 Mbp, have been demonstrated [18]. In another X-ray irradiation study on T-24 human bladder carcinoma cells, similar DNA fragments to the DNA fragments found in the X-ray irradiated L-1210 cells were observed in the 1–2 Mbp but not the 0.1–1 Mbp range [2]. Giant DNA and HMW DNA fragments ranging from100 kbp to 10 Mbp are distinctly produced by X-ray irradiation (Table 1). 1–2 Mbp giant and 200–800 kbp HMW DNA fragmentations prior to internucleosomal DNA fragmentation are caused by H_2O_2 [2,8]. All portions of the UV spectrum alone are capable of inducing the active oxygen-mediated formation of 8-OH-dG. UV radiation may generate ROS, which consequently induce DNA damage [19] including giant DNA fragmentation. However, the actual identities of ROS involved in UV radiation-induced oxidative DNA damage are still uncertain.

Some antibiotics that possess quinone moieties as part of their chemical structures are widely used as a drug to treat various human cancers. Most of these drugs produce ROS at various cellular sites *in vivo* [20–23]. Neocarzinostatin and bleomycin, both of which are anticancer drugs generating ROS *in vivo*, produce not only 1–2 Mbp and

Table 1
DNA fragmentation induced by oxidative stress and various agents in various cell types

Treatment	Cell type	Giant DNA (bp)	HMW DNA (bp)	Ladder DNA[a]	References
Ionizing radiation					
X-ray					
~50 Gy	L-1210 (mouse leukemia)	0.1–10 M		ND	[18]
1.5–12 Gy	EMT-6 (methotrexate-resistant)	3 M		ND	[76]
20–100 Gy	T-24 (human bladder carcinoma)	1–2 M	200–800 k	+	[2]
γ-Ray	HT-29 (colon adenocarcinoma)	~10 M, 2 M		ND	[25]
Ultraviolet C	T-24	1–2 M	100–800 k	+	[77]
Hydrogen peroxide					
>5 mM	T-24	1–2 M	200–800 k	−	[2]
1–5 mM	T-24	1–2 M	200–800 k	+	[2]
	U-937 (human myeloid leukemia)		50–500 k	+	[30]
1 mM	U-937	1–3 M	200–300 k	ND	[33]
GSH depletion					
Glutamate/BSO	C6 (rat glioma)	1–2 M	200–800 k, <50 k	+	[9,11]
Anticancer drugs					
BLM	Du145 (prostatic carcinoma)	>1 M	450–600 k, 30–50 k	+	[31]
	T-24	1–2 M	200–800 k	+	[2]
Neocarzinostatin	T-24	1–2 M		+	[2]
5-FdUrd	HT-29	~10 M, 2 M	200–800 k	−	[25]
Duocarmycins	HeLa (human uterine cervix carcinoma)	1–2 M	200–800 k	ND	[24]
Topoisomerase inhibitors					
VM-26	Thymocytes	800 k ~1 M,	200–600 k, <100 k	+	[32]
	U-937		50–100 k	+	[78]
mAMSA	Thymocytes	700 k ~1 M	30–80 k	+	[32]
	HeLa	1–2 M	900 k	ND	[24]
V-16 (etoposide)	Du145	>1 M	450–600 k, 30–50 k	+	[31]
	MCF-7 (breast adenocarcinoma)		50 k	+	[44]
	U-937	1–3 M	200–300 k	ND	[33]
TAS-103[b]	HL-60 (human leukemia)	1–2 M	~50 k	+	[34]

ND: not determined.
[a] Indicates internucleosomal DNA fragment and plus (+) is positive.
[b] TAS-103 is 6-[[2-(dimethylamino)ethyl]amino]-3-hydroxy-7*H*-indeno[2,1-*c*] quinolin-7-one dihydrochloride.

200–800 kbp DNA fragments but also apoptotic internucleosomal DNA fragments during cell death (Table 1). Although DNA-crosslinking agents and mitotic inhibitors do not induce DNA fragmentation [24], 5-fluoro-2-deoxyuridine (5-FdUrd), an inhibitor of DNA synthesis, causes both giant DNA and HMW DNA fragmentations [25]. Cellular DNA cleavage into HMW DNA fragments during apoptosis is highly reminiscent of topoisomerase II-mediated HMW DNA fragmentation in cells [26,27]. In fact, the pattern of HMW DNA fragmentation by topoisomerase II poison, and that produced in apoptotic cells induced by other stimuli, is found to be similar [28]. However, the relationship between topoisomerase II and the excision of chromosomal loops during apoptotic cell death is still unclear. A major function of topoisomerase II is to regulate the topological state of DNA replication and chromosome condensation and segregation through its delicate act of breaking/rejoining DNA strands [27]. Recently, several new mechanisms including DNA structural modifications, enzyme modifications, oxidative stress and acidic pH environment have also been shown to activate topoisomerase II-mediated DNA cleavage [29,30]. Topoisomerase II inhibitors such as VM-26, etoposide and TAS-103 also produced 1–3 Mbp or 800 kbp–1 Mbp giant DNA fragments together with 200–600 kbp and less than 100 kbp DNA fragments in thymocytes, Du145 human prostatic carcinoma cells, U937 human myeloid leukemia cells and HL-60 human leukemia cells [30–34]. Furthermore, although DNA damage caused by exogenously added ROS appears to activate apoptosis, it is important to elucidate the roles of ROS in the apoptosis. However, not only the mode of action of ROS but also the roles of such chromosomal giant DNA degradation remains to be elucidated.

4. Chromosomal DNA fragmentation in apoptosis or necrosis

Apoptosis and necrosis are two distinct forms of cell death that have profoundly different implications for the surrounding tissues. Apoptosis is characterized by chromatin condensation, activation of some caspases and fragmentation of DNA at internucleosomal linker sites giving rise to discrete bands of multiples of 180–200 bp [35]. This form of DNA degradation has been very widely observed in

apoptosis, although exceptions do exist. Different types of DNA fragmentation have been reported during apoptosis, in the presence or absence of the characteristic internucleosomal DNA cleavage (ladder-like) pattern. These enzymatic events encompass a vast array of chromosomal degradation states in the cell with the ultimate common consequence being cell death [36]. In contrast, necrosis is a passive process, typified by cell and organelle swelling with spillage of the intracellular contents into the extra cellular milieu. Necrosis is an uncontrolled event resulting from the loss of homeostasis and the cell contents which are dispersed, may then have adverse effects on neighboring tissues [37]. There have been some recent reports on apoptosis and necrosis caused under various conditions, including oxidative stress in some neuronal cells such as hippocampal neurons [38], cortical cell cultures [39], neonatal rat brain [40] and HT-22 hippocampus-derived cells [41].

One of the hallmarks of apoptosis is the digestion of genomic DNA by an endonuclease, generating a ladder of small fragments of double-stranded DNA. Single-strand nicks were found to be very frequent in the internucleosomal regions, but also to occur in the core particle-associated DNA. DNA fragmentation induced during apoptosis is not due to a double-strand cutting enzyme as previously postulated, but rather is the result of single-strand breaks. This ensures the dissociation of the DNA molecule at sites where cuts are found within close proximity [42]. There is a two-step process of DNA fragmentation in apoptosis: DNA is first cleaved into large fragments of 50–300 kbp that are subsequently cleaved into smaller oligonucleosomes in some, but not all cells. Significantly, only the first stage is considered essential for cell death since some cells, for example human MCF-7 breast carcinoma cells and human NT-2 neuronal cells, do not show this behavior but still display normal nuclear morphological apoptotic changes.

Some inducers of apoptosis such as etoposide and glucocorticoids have provided formations of 50–300 kbp HMW DNA fragments prior to internucleosomal DNA fragmentation [43] in apoptotic MCF-7 cells induced by etoposide [44], and in mouse L-929 cells induced by tumor necrosis factor (TNF-α) [26]. These DNA fragment formations have been observed in several human epithelial cells induced by serum deprivation [43], and in HeLa nuclei treated with apoptosis-inducing factor (AIF) [45]. Apoptosis has also been widely observed in some cells treated with anticancer drugs [46], and other cell death processes induced by some biological events such as depletion of nutrients [47]. However, little has been reported about the involvement of not only 1–2 Mbp giant DNA fragmentation but also HMW DNA fragmentation to 100–800 and 50–300 kbp fragments and their significance or roles in apoptosis. In some cases of apoptosis, ROS may be involved not only as inducers of DNA damage but also as specific second messengers in the signal transduction pathway, whereas in others they may be side effects of either the experimental system or changes in the cellular redox status as a result of ROS-independent apoptosis signaling pathways [48]. Therefore, it is still unclear whether endogenous ROS are really involved in DNA degradation leading to apoptosis.

5. Nucleases involved in DNA fragmentation

In apoptosis, internucleosomal DNA degradation in which some endonucleases are involved has been observed [49] and several studies on the enzyme activation process are in progress [50,51]. Cells may also detach parts of their cytoplasm, which sometimes includes highly condensed fragments of the karyorrhectic nucleus. The dying cells also activate catabolic enzymes that ensure digestion of critical cellular components from the inside. Such catabolic hydrolases include a class of specific protein-cleaving enzymes (caspases), as well as DNA-digesting enzymes (DNases), both of which participate directly or indirectly in nuclear pyknosis [51]. This DNase sensitivity is specific to the chromosomal regions (Fig. 1). The single-strand-specific nuclease, DNase I, is thought to be specific for some type of DNA structure. Recently, a new type of endonuclease involved in apoptosis has been reported. This nuclease is endonuclease G, a mitochondrion-specific nuclease that translocates to the nucleus during apoptosis. Endonuclease G cleaves chromatin DNA into nucleosomal fragments independently of caspases [52]. Sahara *et al.* [53] have suggested that caspase-3 cleaves Acinus, which is the precursor of a chromatin condensation factor. Another chromatin condensation factor, caspase-activated DNase (CAD) [54], for example, uses its DNase activity to cleave chromatin at the boundaries between nucleosomes. In this way, CAD generates stretches of DNA about 200 bp long or multiples thereof. A few proteins responsible for caspase-independent chromatin condensation have, in fact, been identified. AIF is a flavoprotein that is normally confined to the space between the outer and inner mitochondrial membranes [45]. AIF translocates from the mitochondrion to the nucleus, where it causes partial chromatin condensation in the periphery of the nucleus. AIF causes degradation of DNA into fragments greater than around 50 kbp in length. Another chromatin condensation factor, which translocates from the cytoplasm to the nucleus, is L-DNase II. When added to isolated nuclei, L-DNase II causes marked chromatin condensation and cleaves the chromatin into nucleosome-sized fragments. Yet other proteins that might contribute to chromatin condensation and internucleosomal DNA fragmentation are endonuclease-γ [55] and cathepsin B [54]. These proteins could be activated on their release from the lysosomes of apoptotic cells.

The TUNEL assay has been used to label the 3′ ends of nicked or fragmented DNA in apoptosis using [14-biotin]-dCTP and terminal deoxynucleotide transferase enzyme.

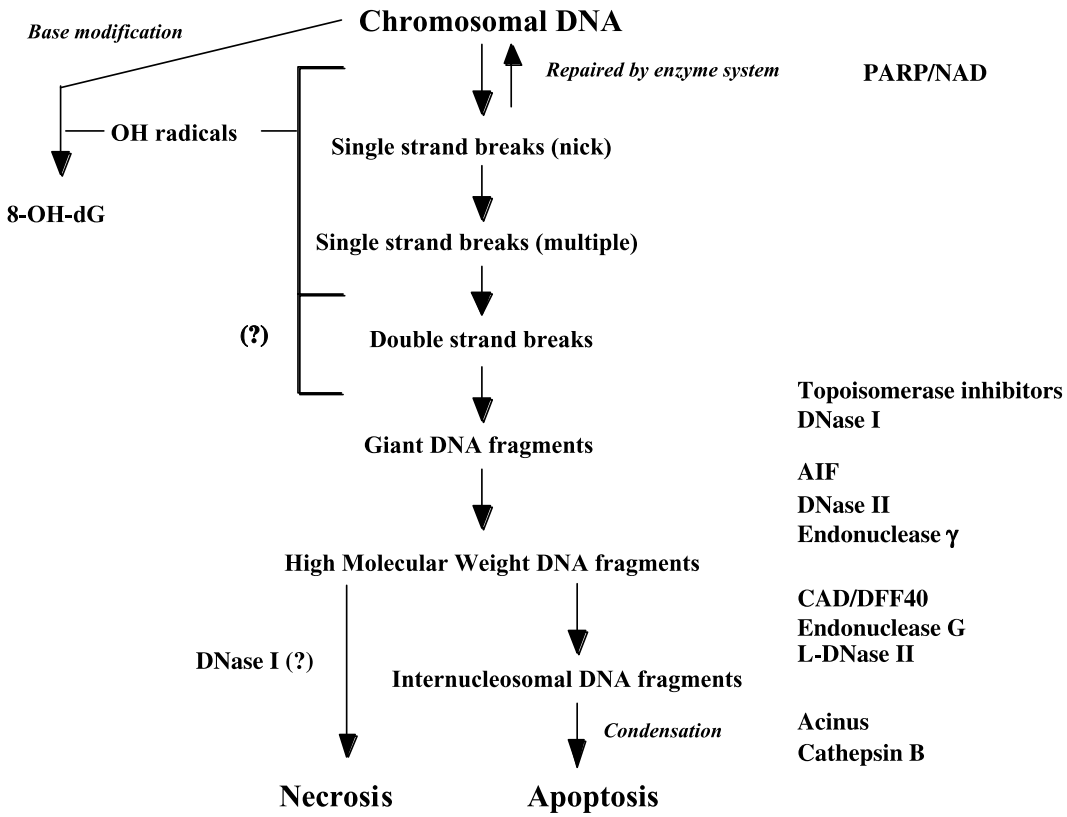

Fig. 1. Chromosomal DNA fragmentation induced by oxidative stress.

The labeled DNA is detected with horseradish peroxidase-conjugated streptavidin and diaminobenzidine. DNase I action can present a positive result in the TUNEL assay, and therefore, the TUNEL assay may be positive in both apoptosis and necrosis.

6. DNA fragmentation in apoptosis and necrosis by oxidative stress under GSH depletion

In addition to ROS such as H_2O_2, O_2^- and $^{\bullet}OH$, NO and lipid hydroperoxides are also considered to be important mediators of cytoxicity in a variety of situations, including the apoptosis of neuronal cells [15,48,56]. Glutamate neurotoxicity has been postulated to contribute to the neuronal injury and death that underlie many central nervous system disorders both acute, for example, hypoxia, ischemia and hypoglycemia and chronic, for example, Huntington's [57], Parkinson's and Alzheimer's diseases [58] and Down's syndrome [59]. Amyloid-peptide is heavily deposited in the brains of patients, and free radical oxidative stress, particularly of neuronal lipids and proteins, is extensive. Recent research suggests that these two observations may be linked by amyloid-peptide-induced oxidative stress in Alzheimer's disease brains. There is current knowledge on phospholipid peroxidation and protein oxidation in Alzheimer's disease brains, one potential cause of this oxidative stress, and the consequences of amyloid-peptide-induced lipid peroxidation and protein oxidation in Alzheimer's disease brains [3]. Besides the well-characterized receptor-mediated effects of excitatory amino acids such as glutamate, kainate and *N*-methyl-D-aspartate [40], it has also been proposed that high concentrations of exogenous glutamate inhibit the transport of cystine which is converted rapidly to cysteine followed by synthesis of glutathione (GSH) in cells. Consequently, intracellular GSH levels decrease *via* the depletion of intracellular cysteine [59,60] and thereby expose the cells to oxidative stress [61]. Most mammalian cells contain a high concentration of GSH (>4 mM) of which the majority is in the reduced form (>90%). Intracellular GSH depletion induced by BSO or glutamate causes apoptosis [9,11,61].

The GSH and GSH peroxidase system plays a major role in controlling cellular redox states and is the primary defense mechanism for peroxide removal from the brain protecting against the effects of ROS damage that may be involved in some neuropathological disorders [62]. 50–300 kbp HMW DNA fragments were also produced through rapid efflux of GSH during the apoptosis induced by anti-Fas/APO-1 antibodies [63]. In addition, not only 1–2 Mbp giant DNA and 100–800 kbp HMW DNA fragments but also internucleosomal DNA fragments were observed in C6 cells under glutamate or BSO-induced GSH depletion [9,11]. These DNA fragmentations were associated with intracellular accumulated ROS under

glutamate-induced GSH depletion [64]. However, the relationship between GSH depletion and active oxygen-induced DNA damage remains to be clarified. GSH depletion causes disturbance of cell membranes that releases phospholipase A_2 and phospholipase C [65]. Phospholipids contain mainly linoleic acid and arachidonic acid in position 2 which are mainly produced by phospholipase A_2. Polyunsaturated fatty acids with a homoconjugated *cis–cis*-pentadienyl system such as linoleic acid and arachidonic acid are substrates for lipoxygenases. Such polyunsaturated fatty acids enhance not only lipid peroxidation but also giant DNA fragmentation under both glutamate- and BSO-induced GSH depletion. The enhancements by these polyunsaturated fatty acids including linolenic acid and oleic acid are species-dependent [66]. Arachidonic acid is metabolized to some substances controlling cell survival and moreover is oxidized to its hydroperoxides not only by lipoxygenases or cyclooxygenases but also by a chemical reaction under aerobic conditions [67]. The lipoxygenase activity of lymphocytes and endogenous 15-hydroxyeicosaetraenoic acid (15-HETE), an arachidonate metabolite, are increased by X-ray irradiation of rats, stimulating internucleosomal DNA fragmentation [68]. 13-Hydroperoxy-octadecadienoic acid, a metabolite of linoleic acid and one of the lipid hydroperoxides, could cleave double-strand DNA at the position of guanosine nucleotides in pBR322, but neither linoleic acid nor 13-hydroxyoctadecadienoic acid, were effective in the cleavage [69]. In spite of these facts, the production of lipid hydroperoxides is considered by most researchers to be initiated nonenzymatically. The superoxide anion O_2^- is postulated to be able to escape the enzyme complex in which it is produced, and either it or further reaction products, $LOO^\bullet$, H_2O_2 and $^\bullet OH$, are suspected of attacking the double-allylic CH_2 groups of unsaturated fatty acids and initiating lipid peroxidation. Arachidonic acid converts apoptosis to necrosis representing the disappearance of internucleosomal DNA fragmentation under BSO-induced GSH depletion [70]. A decrease in GSH triggers the activation of neuronal 12-lipoxygenase leading to the production of peroxides, the influx of Ca^{2+} and ultimately to cell death [71]. In these cases, exogenous arachidonic acid can potentiate cell death by converting apoptosis to necrosis through lipid peroxidation and showing promotion of giant DNA fragmentation and reduction of internucleosomal DNA fragmentations [70]. Therefore, lipid metabolites, such as arachidonic acid-derived eicosanoids, may play a role in regulating cell survival [72]. We propose here, as has been suggested by many others, that lipid hydroperoxide production is a result of tissue injury. We suggest that lipid hydroperoxide formation in injured tissues is under the control of the GSH level.

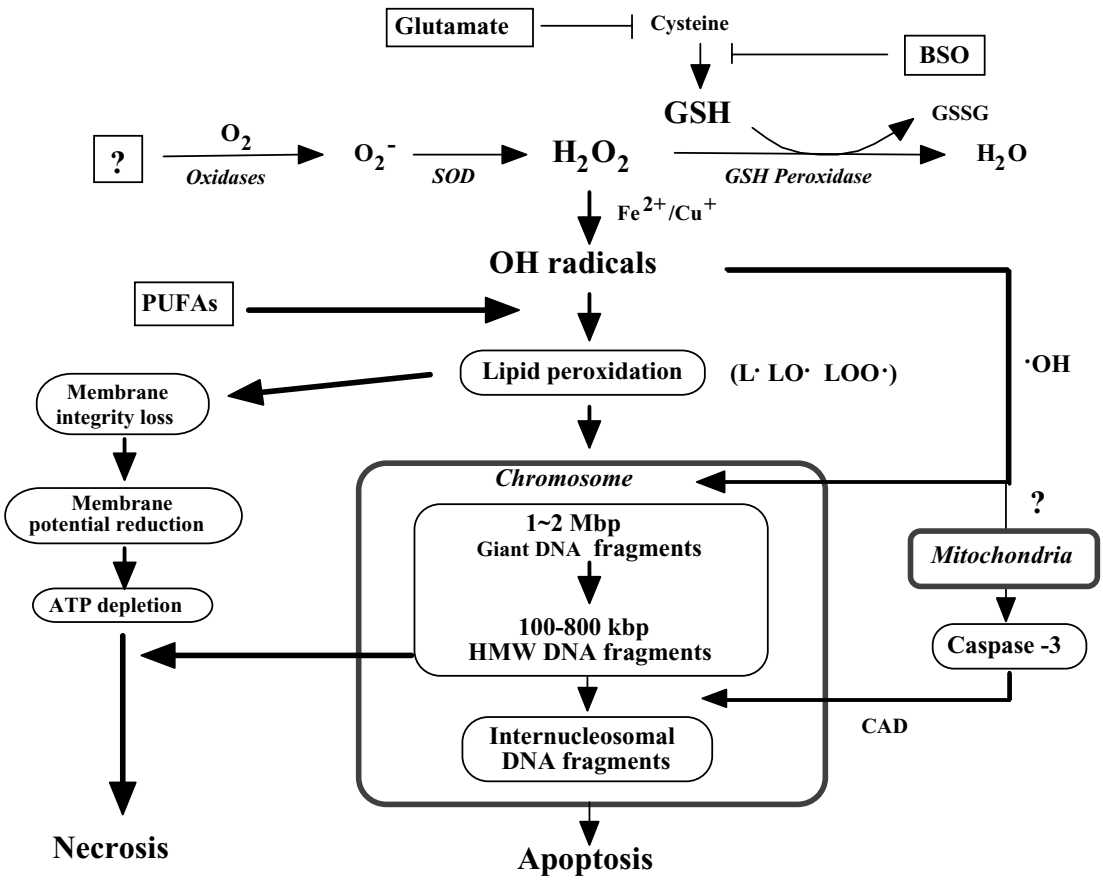

Fig. 2. Possible mechanism underlying the glutathione depletion-induced apoptotic or necrotic cell death in glioma cells.

7. Commentary remarks

We can consider that 1–2 Mbp giant DNA fragmentation under GSH depletion occurs by a few possible mechanisms (Fig. 2). First, lipid free radicals produced from PUFA directly attack chromatin DNA in nuclei. Second, lipid peroxidation in a cell leads to the loss of membrane integrity in cell membranes consisting of phospholipids and thereby may make suitable circumstances for other types of oxygen radicals, such as $^{\bullet}OH$ radicals, produced from hydrogen peroxide by Fenton's reaction or from O_2^- by the Haber–Weiss reaction, to attack chromatin DNA. If the first case of the direct action mechanism of lipid free radicals is preferred, lipid peroxidation producing lipid free radicals might proceed not only in plasma membranes but also in the nuclear membranes close to the chromosomes, and such radicals might attack preferential cleavage sites in the hinge domain of chromatin. $LO^{\bullet}$ or $LOO^{\bullet}$ radicals may cleave DNA strands leading to giant DNA fragmentation in chromatin. $LO^{\bullet}$ radicals are produced from lipid hydroperoxides by Fenton's reaction in the presence of iron or copper. However, it is also obscure whether PUFAs or their metabolites and derivatives act directly on the DNA in chromatin.

Intracellular iron and copper ions are known to be in close association with the chromatin in the nuclei. These metal ions may play an important role in the generation of hydroxyl radicals from H_2O_2 or O_2^- in or around the chromatin [73,74]. Hydroxyl radicals can cause single-strand DNA breaks associated with double-strand DNA breaks. Under the circumstance of membrane integrity loss induced by lipid peroxidation, hydroxyl radicals might attack and directly cleave chromosomal DNA into single-strand forms, consequently leading to double-strand DNA breaks. The location of specific DNA target site(s) attacked by $^{\bullet}OH$ radicals may exist but is still unclear. On the mechanism of giant DNA fragmentation, $^{\bullet}OH$ radical-mediated action is more possible and preferable to lipid radical-mediated action in providing 3′-OH-termini in single- or double-strand breakage. Lipid peroxidation induced under glutamate-induced GSH depletion promoted 8-OH-dG formation in chromatin DNA [75]. 8-OH-dG formation is thought to be caused by $^{\bullet}OH$ radicals but not by $LO^{\bullet}$ nor $LOO^{\bullet}$ [76], and therefore, lipid hydroperoxides made from PUFA induce a loss of membrane integrity and thereafter may change the environment to make $^{\bullet}OH$ radical attack on chromosomes easier.

References

[1] Wyllie AH, Kerr JF, Currie AR. Cell death; the significance of apoptosis. Int Rev Cytol 1980;68:251–306.

[2] Higuchi Y, Matsukawa S. Appearance of 1–2 Mbp giant DNA fragments as an early common response leading to cell death induced by various substances which cause oxidative stress. Free Radic Biol Med 1997;23:90–9.

[3] Butterfield A, Lauderback CM. Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid-peptide-associated free radical oxidative stress. Free Radic Biol Med 2002;32:1050–60.

[4] Pardoll DM, Vogelstein B, Coffey DS. A fixed site of DNA replication in eucaryotic cells. Cell 1980;19:527–36.

[5] Yoshida A, Ueda T, Wano Y, Nakamura T. DNA damage and cell killing by camptothecin and its derivative in human leukemia HL-60 cells. Jpn J Cancer Res 1993;84:566–73.

[6] Bryant PE. Enzymatic restriction of mammalian cell DNA using Pvu II and Bam HI; evidence for the double-strand break origin of chromosomal aberrations. Int J Radiat Biol 1984;46:57–65.

[7] Lobrich M, Ikepeme S, Kiefer J. DNA double-strand break measurement in mammalian cells by pulsed-field gel electrophoresis: an approach using restriction enzymes and gene probing. Int J Radiat Biol 1994;65:623–30.

[8] Matsukawa S, Higuchi Y. Oxidative damage and repair. New York: Pergamon Press; 1991.

[9] Higuchi Y, Matsukawa S. Active oxygen-mediated chromosomal 1–2 Mbp giant DNA fragmentation into internucleosomal DNA fragmentation in apoptosis of glioma cells induced by glutamate. Free Radic Biol Med 1998;24:418–26.

[10] Cohen GM, Sun X, Fearnhead H, MacFarlane M, Brown DG, Snowden RT, Dinsdale D. Formation of large molecular weight fragments of DNA is a key committed step of apoptosis in thymocytes. J Immunol 1994;153:507–16.

[11] Higuchi Y, Matsukawa S. Glutathione depletion induces chromosomal giant DNA and high molecular weight DNA fragmentation associated with apoptosis through lipid peroxidation and protein kinase C activation in C6 glioma cells. Arch Biochem Biophys 1999;363:33–42.

[12] Halliwell B, Aruoma OI. DNA damage by oxygen-derived species—its mechanism and measurement in mammalian systems. FEBS Lett 1991;281:9–19.

[13] Ward JF. DNA damage produced by ionizing radiation in mammalian cells. In: Cohn WE, Moldave K, editors. New York: Academic Press. Prog Nucleic Acid Res Mol Biol 1988:95–125.

[14] Kasai H, Crain P, Kunino Y, Nishimura S, Ootsuyama A, Tanooka H. Formation of 8-hydroxy guanosine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. Carcinogenesis 1986;7:1849–51.

[15] Slater A, Nobel S, Orrenius S. The role of intracellular oxidants in apoptosis. Biochim Biophys Acta 1995;1271:59–62.

[16] Clutton S. The importance of oxidative stress in apoptosis. Br Med Bull 1997;53:662–8.

[17] Wlodek DBJ, Olive JPL. Comparison between pulsed-field and constant gel electrophoresis for measurement of DNA double-strand breaks in irradiated Chinese hamster ovary cells. Int J Radiat Biol 1991;60:779–90.

[18] Erixon K, Cedervall B. Linear induction of DNA double-strand breakage with X-ray dose, as determined from DNA fragment size distribution. Radiat Res 1995;142:153–62.

[19] Peak MJ, Peak JG. Solar-ultraviolet-induced damage to DNA. Photodermatology 1989;6:1–15.

[20] Doroshow JH. Role of hydrogen peroxide and hydroxyl radical formation in the killing of Ehrlich tumor cells by anticancer quinones. Proc Natl Acad Sci USA 1986;83:4514–8.

[21] Teicher BA, Herman TS, Holder SA. Combined modality therapy with bleomycin, hyperthermia and radiation. Cancer Res 1988;48:6291–7.

[22] Chin D, Goldberg IH. Generation of superoxide free radical by neocarzinostatin and its possible role in DNA damage. Biochemistry 1986;25:1009–115.

[23] Kanofsky JR. Singlet oxygen production by bleomycin: a comparison with heme containing compounds. J Biol Chem 1986;261:13546–50.

[24] Okamoto A, Okabe M, Gomi K. Analysis of DNA fragmentation in human uterine cervix carcinoma HeLa S3 cells treated with duocarmycins or other antitumor agents by pulse field gel electrophoresis. Jpn J Cancer Res 1993;84:93–8.
[25] Dusenbury CE, Davis MA, Lawrence TS, Maybaum J. Induction of megabase DNA fragments by 5-fluorodeoxyuridine in human colorectal tumor (HT-29) cells. Mol Pharmacol 1990;39:285–9.
[26] Lagarkova MA, Iarovaia OV, Razin SV. Large-scale fragmentation of mammalian DNA in the course of apoptosis proceeds *via* excision of chromosomal DNA loops and their oligomers. J Biol Chem 1995; 270:20239–41.
[27] Wang JC. DNA topoisomerases. Annu Rev Biochem 1996;65:635–92.
[28] Bellamy CO. p53 and apoptosis. Br Med Bull 1997;53:522–38.
[29] Kingma PS, Osheroff N. Spontaneous DNA damage stimulates topoisomerase II-mediated DNA cleavage. J Biol Chem 1997;272: 7488–93.
[30] Li TK, Chen AY, Yu C, Mao Y, Wang H, Liu LF. Activation of topoisomerase II-mediated excision of chromosomal DNA loops during oxidative stress. Genes Dev 1999;13:1553–60.
[31] Rusnak JM, Calmels TPG, Hoyt DG, Kondo Y, Yalowich JC. Genesis of discrete higher order DNA fragments in apoptotic human prostatic carcinoma cells. J Pharm Exp Ther 1996;49:244–52.
[32] Filipski J, Leblanc J, Youdale T, Sikorska M, Walker PR. Periodicity of DNA folding in higher order chromatin structures. EMBO J 1990;9:1319–27.
[33] Mizutani H, Tada-Oikawa S, Hiraku Y, Oikawa S, kojima M, Kawanishi S. Mechanism of apoptosis induced by a new topoisomerase inhibitor through the generation of hydrogen peroxide. J Biol Chem 2002;277:30684–9.
[34] Sestili P, Cattabeni F, Cantoni O. Direct excision of 50 kbp pair DNA fragments from megabase-sized fragments produced during apoptotic cleavage of genomic DNA. FEBS Lett 1996;396:337–42.
[35] Carson DA, Ribeiro JM. Apoptosis and disease. Lancet 1993;341:1251–4.
[36] Bortner CD, Oldenburg NE, Cidlowski JA. The role of DNA fragmentation in apoptosis. Trends Cell Biol 1995;5:21–6.
[37] Schwartz LM, Smith SW, Jones ME, Osborne BA. Do all programmed cell deaths occur *via* apoptosis? Proc Natl Acad Sci USA 1993;90:980–4.
[38] Holtsberg FW, Steiner MR, Keller JN, Mark RJ, Mattson MP, Steiner SM. Lysophosphatidic acid induces necrosis and apoptosis in hippocampal neurons. J Neurochem 1998;70:66–76.
[39] Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, Lipton SA. Apoptosis and necrosis; two distinct events induced, respectively, by mild and intense insults with DMDA or nitric oxide/superoxide in cortical cultures. Proc Natl Acad Sci USA 1995;92:7162–6.
[40] Campagne ML, Lucassen PJ, Vermeulen JP, Balazs R. NMDA and kainate induce internucleosomal DNA cleavage associated with both apoptotic and necrotic cell death in the neonatal rat brain. Eur J Neurosci 1995;7:1627–40.
[41] Tan S, Wood M, Maher P. Oxidative stress induces a form of programmed cell death with characteristics of both apoptosis and necrosis in neuronal cells. J Neurochem 1998;71:95–105.
[42] Peitsch MC, Muller C, Tschopp J. DNA fragmentation during apoptosis is caused by frequent single-strand cuts. Nucleic Acids Res 1993;21:4206–9.
[43] Zhivotovsky B, Orrenius S. Involvement of Ca^{2+} in the formation of high molecular weight DNA fragments in thymocyte apoptosis. Biochem Biophys Res Commun 1994;202:120–7.
[44] Oberhammer F, Wilson JW, Dive C, Morris ID, Hickman JA, Wakeling E, Walker PR, Sikorska M. Apoptotic death in epithelial cells: cleavage of DNA to 300 and/or 50 kbp fragments prior to or in the absence of internucleosomal fragmentation. EMBO J 1993;12:3679–84.
[45] Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, Mangion J, Jacotot E, Costantini P, Loeffler M, Larochette N, Goodlett DR, Aebersold R, Siderovski DP, Penhinger JM, Kroemer G. Molecular characterization of mitochondrial apoptosis-inducing factor. Nature 1999;397:441–6.
[46] Kaufmann SH. Induction of endonuclolytic DNA cleavage in human acute myelogeneous leukemia cells by etoposide, camptothecin and other cytotoxic anticancer drugs. Cancer Res 1989;49:5870–5.
[47] Batistatou A, Greene LA. A urintricarboxylic acid rescues PC 12 cells and sympathetic neurons from cell death caused by nerve growth factor deprivation: correlation with suppression of endonuclease activity. J Cell Biol 1991;115:461–71.
[48] Jacobson MD. Reactive oxygen species and programmed cell death. Trends Biochem Sci 1996;21:83–6.
[49] Arends MJ, Murris RG, Wyllie AH. Apoptosis: the role of the endonucleases. Am J Pathol 1990;136:593–608.
[50] Walker PR, Leblanc J, Smith B, Pandey S, Sikorska M. Detection of DNA fragmentation and endonuclease in apoptosis. Methods 1999;17:329–38.
[51] Zamzami N, Kroemer G. Condensed matter in cell death. Nature 1999;401:127–8.
[52] Li LY, Wang X. Endonuclease G is an apoptotic DNase when released from mitochondria. Nature 2001;412:95–9.
[53] Sahara S, Aoto M, Eguchi Y, Imamoto N, Yoneda Y, Tsujimoto Y. Acinus is a caspase-3-activated protein required for apoptotic chromatin condensation. Nature 1999;401:168–73.
[54] Vancompernolle K, Van Herreweghe F, Pynaert G, Van de Craen M, De Vos K, Totty N, Sterling A, Fiers W, Vandenabeele P, Grooten J. Atractyloside-induced release of cathepsin B, a protease with caspase-processing activity. FEBS Lett 1998;438:150–8.
[55] Shiokawa D, Ohyama H, Yamada T, Tanuma S. Purification and properties of DNase gamma from apoptotic rat thymocytes. Biochem J 1997;326:675–81.
[56] Greenlund LJS, Dockworth TL, Johnson EM. Superoxide dismutase delays neuronal apoptosis: a role for reactive oxygen species in programmed neuronal death. Neuron 1995;14:303–15.
[57] Portera-Cailliau C, Hedreen JC, Price DL, Koliatsos VE. Evidence for apoptotic cell death in Huntington disease and excitotoxic animal models. J Neurosci 1995;15:3775–87.
[58] Loo DT, Agata C, Pike CJ, Whittemore ER, Wulencewics AJ, Cotman CW. Apoptosis is induced by β-amyloid in cultured central nervous system neurons. Proc Natl Acad Sci USA 1993;90:7951–5.
[59] Busciglio J, Yankner BA. Apoptosis and increased generation of ROS in Down's syndrome neurons *in vitro*. Nature 1995;378:776–9.
[60] Kato S, Negishi K, Mawatari K, Kuo CH. A mechanism for glutamate toxicity in the C6 glioma cells involving inhibition of cystein uptake leading to glutathione depletion. Neuroscience 1992; 48:903–14.
[61] Pereira CM, Oliveira CR. Glutamate toxicity on a PC12 cell line involves glutathione (GSH) depletion and oxidative stress. Free Radic Biol Med 1997;23:637–47.
[62] Simonian NA, Coyle JT. Oxidative stress in neurodegenerative diseases. Ann Rev Pharmacol Toxicol 1996;36:83–106.
[63] Dobbelsteen DJ, Nobel SI, Schlegel J, Cotgreave IA, Orrenius S, Slater AFG. Rapid and specific efflux of reduced glutathione during apoptosis induced by anti-Fas/APO-1 antibody. J Biol Chem 1996;271:15420–7.
[64] Walker PR, Weaver VM, Lach B, LeBlanc J, Sikorska M. Endonuclease activity associated with high molecular weight and internucleosomal DNA fragmentation in apoptosis. Exp Cell Res 1994;213: 100–6.
[65] Beetens JR, Herman AG. Ascorbic acid and prostaglandin formation. Int J Vitam Nutr Res Suppl 1983;24:131–44.
[66] Higuchi Y. Polyunsaturated fatty acids promote 8-hydroxy-2-deoxyguanosine formation through lipid peroxidation under the glutamate-induced GSH depletion in rat glioma cells. Arch Biochem Biophys 2001;392:65–70.
[67] Spiteller G. Enzymic lipid peroxidation: a consequence of cell injury. Free Radic Biol Med 1996;21:1003–9.

[68] Matyshevskaia OP, Pastukh VN, Solodushko VA. Inhibition of lipoxygenase activity reduces radiation-induced DNA fragmentation in lymphocytes. Radiat Biol Radioecol 1999;39:282–6.
[69] Inoue S. Site-specific cleavage of double-strand DNA by hydroperoxide of linoleic acid. FEBS Lett 1984;172:231–4.
[70] Higuchi Y, Yoshimoto T. Archidonic acid converts the glutathione depletion-induced apoptosis to necrosis by promoting lipid peroxidation in glioma cells. Arch Biochem Biophys 2002;400: 133–40.
[71] Li Y, Maher P, Schubert D. A role for 12-lipoxygenase in nerve cell death caused by glutathione depletion. Neuron 1997;19:453–63.
[72] Tang D, Chen YQ, Honn KV. Arachidonate lipoxygenases as essential regulations of cell survival and apoptosis. Proc Natl Acad Sci USA 1996;93:5241–6.
[73] Mello Filho AC, Meneghini R. *In vivo* formation of single-strand breaks in DNS by hydrogen peroxide is mediated by the Haber–Weiss reaction. Biochim Biophys Acta 1984;781:56–63.
[74] Mello Filho AC, Hoffman ME, Meneghini R. Cell killing and DNA damage by hydrogen peroxide are mediated by intracellular iron. Biochem J 1984;218:273–5.
[75] Kasai H, Crain P, Kunino Y, Nishimura S, Ootsuyama A, Tanooka H. Formation of 8-hydroxy guanosine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. Carcinogenesis 1986;357:1849–51.
[76] Nevaldine B, Longo JA, King GA, Vilenchik M, Sagerman RH, Hahn PJ. Induction and repair of DNA double-strand breaks. Radiat Res 1993;133:370–4.
[77] Higuchi Y, Mizukami Y, Yoshimoto T. Ultraviolet irradiation-induced chromosomal giant DNA fragmentation leads to apoptosis providing internucleosomal DNA fragments in glioma cells. Ann NY Acad Sci 2003, in press.
[78] Bicknell GR, Cohen GM. Cleavage of DNA to large kilobase pair fragments occurs in some forms of necrosis as well as apoptosis. Biochem Biophys Res Commun 1995;207:40–7.