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Role of oxidatively induced DNA lesions in human pathogenesis

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Abstract

Genome stability is essential for maintaining cellular and organismal homeostasis, but it is subject to many threats. One ubiquitous threat is from a class of compounds known as reactive oxygen species (ROS), which can indiscriminately react with many cellular biomolecules including proteins, lipids, and DNA to produce a variety of oxidative lesions. These DNA oxidation products are a direct risk to genome stability, and of particular importance are oxidative clustered DNA lesions (OCDLs), defined as two or more oxidative lesions present within 10 bp of each other. ROS can be produced by exposure of cells to exogenous environmental agents including ionizing radiation, light, chemicals, and metals. In addition, they are produced by cellular metabolism including mitochondrial ATP generation. However, ROS also serve a variety of critical cellular functions and optimal ROS levels are maintained by multiple cellular antioxidant defenses. Oxidative DNA lesions can be efficiently repaired by base excision repair or nucleotide excision repair. If ROS levels increase beyond the capacity of its antioxidant defenses, the cell's DNA repair capacity can become overwhelmed, leading to the accumulation of oxidative DNA damage products including OCDLs, which are more difficult to repair than individual isolated DNA damage products. Here we focus on the induction and repair of OCDLs and other oxidatively induced DNA lesions. If unrepaired, these lesions can lead to the formation of mutations, DNA DSBs, and chromosome abnormalities. We discuss the roles of these lesions in human pathologies including aging and cancer, and in bystander effects.

Keywords

Reactive oxygen species; DNA damage; DNA double-strand breaks; Aging; Cancer; Bystander effect

1. Induction and processing of oxidative DNA lesions in human cells and tissues

Elevated ROS levels can create oxidative stress in a cell and chronic exposure to this stress can result in permanent changes in the genome [1,2]. It is generally accepted that the accumulation of oxidative DNA lesions may promote mutagenesis, human pathogenesis and

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loss of homeostasis. These oxidative lesions can be induced not only by ROS generated by exposure to exogenous agents including ionizing or non-ionizing radiation (IR), drugs, and other chemicals such as metals [3–7] but also from endogenous sources including oxygen metabolism, apoptosis, and inflammatory responses involving the immune system [2,8–13] (Fig. 1).

Among these oxidative DNA lesions are abasic sites, single strand DNA breaks (SSBs), sugar moiety modifications, and deaminated and adducted bases [2,14,15]. Various studies have estimated that anywhere from 0.1 to 100 oxidative DNA lesions per Mbp may exist in normal cells and tissues [16–19]. One of the more common oxidative DNA lesions, 8-oxo-2′-deoxyguanosine (8-oxo-dG), is estimated to be present at approximately 1 per Mbp [20–22]. When two or more oxidative DNA lesions are present within 10 base pairs of each other, it is considered an oxidative clustered DNA lesion (OCDL). These OCDLs have been variously estimated to be present at levels between 0.02 and 0.8 clusters per Mbp in normal human primary cells as well as in tumor cells [23–25].

While individual DNA lesions are generally repaired efficiently, some OCDLs may be more difficult to resolve [26–31]. In some circumstances oxidative lesions can lead to DNA double-strand break (DSB) formation (Fig. 2) [32]. A DSB can arise when two SSBs form close to each other on opposite strands, when topoisomerases (Topos) cleave next to a SSB on the opposite strand, and when ROS-induced DNA damage interferes with either DNA replication or transcription [33–35]. In addition, DSBs can be generated during attempted simultaneous repair of two lesions in a cluster or when excision of a modified base takes place near an unrepaired SSB on the opposite strand [36]. DSBs are the most serious type of DNA damage because a small number of these lesions are sufficient to induce gene mutations, chromosomal aberrations, and cell transformation [37].

Oxidative base lesions and abasic sites are predominantly repaired by base excision repair (BER) and to a lesser extent nucleotide excision repair [38,39]. The core BER pathways (Fig. 1) are initiated by a DNA glycosylase (mainly hOGG1 or hNTH1 in human cells) that recognizes and hydrolytically cleaves and removes the altered base, giving rise to an abasic site. The abasic site is then processed by an AP endonuclease (APE1), which incises the DNA strand 5′ to the baseless sugar. Then, DNA polymerase β catalyzes the β-elimination of the 5′-deoxyriboso-phosphate residue and fills the one-nucleotide gap (short-patch BER). Finally, the nick is sealed by the DNA ligase III/XRCC1 complex [40]. This repair process results in the removal and replacement of a single damaged nucleotide with a proper one [41]. In addition to the above pathway an alternative pathway may occur. DNA glycosylases that process oxidative DNA lesions have an intrinsic AP lyase activity that incises abasic sites 3′ to the baseless sugar leaving a 3′(2,3-didehydro-2,3-dideoxyribose) termini that is then removed by AP endonuclease. As in the main pathway, the gap is filled by DNA polymerase and the nick is sealed by DNA ligase [17,42–44]. Short-patch BER accounts for 80–90% of all BER. Long-patch BER, which replaces 2–10 nucleotides of DNA, is utilized when the oxidized lesion is refractory to the AP lyase activity of DNA polymerase β. Long-patch BER is dependant on PCNA and FEN1 and DNA synthesis is thought to be mediated by several DNA polymerases including polymerases β, δ and ε. SSBs can also be repaired by the long-patch pathway after processing by the XRCC1/PARP1 complex [45] (Fig. 2A).

Typically, the rate of formation of ROS-induced lesions is balanced by their rate of repair. However, chronic exposure to oxidative stress due to deficiencies in cellular repair processes or changes in mitochondrial redox potential can result in persistently high levels of DNA lesions [25]. Numerous studies suggest that ROS play a role in normal and pathological aging, cancer and a wide variety of aging-related diseases such as some neurological diseases, type 2 diabetes, autoimmune, reproductive disorders and cardiovascular diseases,

and others [12,46–51] (Fig. 3). In addition, ROS have been implicated in intracellular communication of stress. In the next sections we will discuss the contribution of oxidatively induced DNA damage in aging and cancer, and its role in stress signaling.

2. Oxidative DNA damage and aging

There is considerable evidence suggesting that oxidative stress plays a critical role in both *in vitro* senescence and *in vivo* aging [52,53]. Cells of laboratory mice were reported to reach senescence after 4–5 population doublings under standard cell culture conditions, however, the onset of senescence was substantially delayed when the O_2 level was reduced from 21% to 3% [54]. The discovery that lower O_2 increased plating efficiencies [55] was an important milestone in development of the experimental conditions for culturing bone marrow stem cells [56]. These findings may not be totally unexpected, given the natural hypoxic environment of stem cells [57], which could be interpreted as a strategy to avoid oxidative damage and senescence. In fact, the average life span of mice which are treated with antioxidant drugs increases up to 25% [58], and mice lacking the antioxidant enzyme super oxide dismutase 1 exhibit a 30% decrease in life expectancy [59]. Likewise, although mice lacking either Ogg1 or Myh, both members of the BER pathway, exhibit normal life spans, mice lacking both enzymes exhibit a 50% reduction in life expectancy [60]. Other evidence has also suggested that DNA lesions induced by the oxidative stress play an important role in mammalian aging [53,61–65]. These observations implicate oxidative stress in cellular senescence and aging, and further suggest that antioxidants and efficient repair of oxidative damage may extend life span.

Oxidative DNA lesions can be difficult to quantitate in situ. However, as mentioned above, they may lead to the formation of DSBs which can more easily be quantified by immunocytochemical detection of phosphorylated histone H2AX (γ-H2AX). When a DSB forms, many H2AX molecules become phosphorylated within a few minutes of break formation to form a γ-H2AX focus, a highly amplified response which enables the individual DSB site to be visualized in situ [32]. The chemical nature of the DSBs marked by γ-H2AX varies; reflecting different mechanisms of generation (reviewed in [32])(Fig. 2A). For example, prompt strand breaks induced by ionizing radiation generally arise from oxidative cleavage of the deoxyribosyl moiety, generating termini of various sorts (which are later excised by repair enzymes); two such breaks, in close proximity on opposite strands, comprising the DSB. Other DSBs arise solely by the action of endonucleases, and others by a combination; for example when topoisomerases cleave next to a prompt SSB, and when ROS-induced DNA damage interferes with both DNA replication and transcription. A DSB can be generated during DNA repair when excision of a modified base takes place near an unrepaired SSB. Oxidative DNA lesions can also interfere with reversible topoisomerase cleavage complexes during DNA replication and RNA transcription. In such cases, DNA/RNA polymerase forks run off the DNA to generate DSBs. Finally, DSBs can also appear when transcription and replication forks collide directly with SSBs or other ROS-induced lesions. Rarely, interference during DNA repair by BER also leads to DSB formation. γ-H2AX is a key component of the DNA damage response. Upon DSB formation, optimal kinase activity is required for the phosphorylation of H2AX as well as for activation of many other DNA repair and checkpoint proteins. Following DSB induction by irradiation, cells respond by activating the ATM signal transduction pathway, while replication-induced DSBs trigger an ATR response [66]. The same amplified response occurs when telomeres become critically shortened and uncapped, exposing a DNA double-stranded end. As with frank DSBs, the γ-H2AX foci formed on these eroded telomeres include the accumulation of DNA repair proteins [52,66,67].

These two types of γ-H2AX foci can be differentiated by their position on metaphase chromosomes. Foci on chromosome arms can be classified as marking DSBs, while those on chromosome ends as marking eroded telomeres. A combination of immunocytochemical γ-H2AX detection to monitor DSB formation and fluorescent in situ hybridization (FISH) for telomere-specific probes on metaphase chromosomes permitted direct visualization of oxidative stress-induced DNA DSBs during both *in vitro* and *in vivo* aging as foci on chromosome arms [52,67] (Fig. 2B). During mouse aging and senescence in culture, γ-H2AX foci were found to accumulate predominantly on chromosome arms, suggesting that oxidative stress-induced DNA DSBs may occur during both *in vitro* and *in vivo* aging [52]. Senescence-associated γ-H2AX foci on chromosome arms decrease when cells are transferred to low oxygen (3%) or treated with antioxidants, supporting the notions that oxidative stress is involved in aging-associated DNA DSB induction and that these DSBs may be repairable [52].

Another important target of oxidative stress is telomeric DNA which in vertebrates contains multiple TTAGGG repeats [68]. Since guanine is the most easily oxidized of the four DNA nucleobases [69], especially runs of consecutive guanines [70], telomeres are particularly susceptible to ROS [71]. Telomeric DNA becomes shorter with each cell division [68,71] and below a threshold length, the telomeric proteins dissociate, resulting in uncapped telomeres, which in turn trigger cellular growth arrest. Oxidized bases destabilize telomeric DNA resulting in accelerated telomere shortening during cell proliferation and even in the absence of DNA replication [72,73]. In contrast to the oxidative stress-induced DNA DSBs localized on chromosome arms which are potentially repairable under low oxidative stress conditions, critically short telomeric DNA requires telomerase to add TTAGGG repeats [52,74]. Since somatic cells generally lack telomerase activity, telomere dysfunction is irreversible. Because the rate of telomeric DNA shortening can be slowed under conditions of low oxidative stress, reduction of ROS levels may be an effective anti-aging strategy [75].

While many external agents are capable of generating ROS in cells, there are also important internal agents of ROS generation. Mitochondria are considered to be the primary source of endogenous ROS, which are released during ATP production. Mice deficient in the synthesis of Cytochrome C Oxidase 2, which functions in the regulation of the mitochondrial Cytochrome C Oxidase complex, exhibited increased mitochondrial oxidative stress and premature aging [76]. Mitochondria may also present a target of ROS because they contain a circular DNA (mtDNA) molecule with 16,569 bp which lacks protective histones, perhaps making it more vulnerable to oxidative stress [77]. While the magnitude of the role of oxidative stress-induced mtDNA damage in aging is not clear, evidence does suggest that the accumulation of mitochondrial genomic damage by oxidative stress may be related to aging [77]. In humans, point mutations in the control/D-loop region of mtDNA accumulate during aging, and age-related large rearrangements of mtDNA have been reported [78]. In addition, numerous human diseases including premature aging, cancer, diabetes and neurodegenerative disorders, have been linked to mutations in mtDNA [79]. Thus, there is ample circumstantial evidence that oxidative stress leads to the accumulation of DNA damage during aging. Future research will clarify the roles this type of damage plays in the causes of aging.

3. Oxidative DNA damage and cancer

That oxidative stress-induced DNA lesions may contribute to carcinogenesis is suggested by the increased cancer susceptibility of persons with a variety of chronic inflammatory diseases, such as ulcerative colitis, viral hepatitis, prostatitis, *Helicobacter pylori* infection, parasitic diseases, and others [3]. In these diseases, cancer induction may be a pathological consequence of elevated ROS levels which lead to increased steady-state levels of oxidative

DNA damage which in turn leads to a higher risk of mutations that may activate oncogenes or inactivate tumor-suppressor genes [7,80].

In addition to cancer induction, cancer progression may also be a consequence of high levels of endogenous ROS. Elevated ROS levels, DNA damage, and defective DNA repair have been reported in different malignancies [10,14,15,26,27,41,81,82]. Nearly 20 purine- and pyrimidine-derived oxidative lesions have been implicated in cancer development. One, the 8-oxo-dG lesion is an accepted biomarker of oxidative stress and DNA damage [10,83]. Elevated levels of 8-oxo-dG and other characteristic DNA base lesions like thymine glycol, 4,6-diamino-5-formamidopyrimidine, and 2,6-diamino-4-hydroxy-5-formamidopyrimidine have been found in several pre-malignant and malignant cells or tissues including acute lymphoblastic leukemia, breast (invasive ductal carcinoma), cervical, colorectal, gastric adenocarcinoma, hepatocellular carcinoma, renal cell carcinoma, and small cell lung carcinoma [10,82]. Comparison of the levels of OCDLs in surgically removed malignant tumors of different origins and normal adjacent tissues demonstrated increased oxidative DNA damage in the tumor tissues [25]. In addition, the levels of DSBs in these tissues exhibited significant increases in tumor samples compared to corresponding normal tissues [84]. Tumor cells may contain increased levels of ROS due to higher cellular metabolism rates and deficiency in redox systems [85–87], characteristics which may aid cancer progression. Since cancer cells generally already have mutations in their DNA repair machinery and cell cycle checkpoints, elevated ROS levels continue to generate elevated levels of oxidative DNA lesions which disrupt normal cellular replication and lead to DSBs and further chromosome abnormalities [88] (Fig. 3).

Elevated DNA DSB levels have been widely reported in cancer cells and pre-malignant lesions, often by measuring the levels of γ-H2AX [84,89–92]. Two characteristics of cancer cells in culture are the different average numbers of γ-H2AX foci in different tumor lines, and the heterogeneity of focal numbers in individual cells of a tumor line [91,92]. However, as described above, γ-H2AX is induced by two types of DNA double-strand damage, the DSBs and the eroded telomere, which can be distinguished by γ-H2AX/telomere-FISH staining of metaphase chromosomes. This technique reveals that the differences in the average number of γ-H2AX foci among different tumor lines and the large heterogeneity of focal numbers in cells of the same culture are both primarily due to differences in foci associated with chromosome ends. Differences in average numbers of γ-H2AX foci among tumor lines correlate inversely with their levels of telomerase. Chromosome arm-associated DNA damage was present at more constant levels in these tumor lines. These results suggest that critically short telomeres are giving rise to the differences observed in these tumor lines [93]. The origins of telomere heterogenity may be numerous. In addition to differences of telomerase levels in tumor cells and the environmental impact such as oxidative stress level, telomere length is tissue-specific and age-dependent even in normal cells [94,95], and there is considerable heterogeneity between individuals [96].

The presence of persistent DNA lesions in tumor cells has led many to propose that DNA damage markers are potential biomarkers for screening ongoing oxidative stress and for cancer diagnostics and prediction [84]. Additionally, because ROS levels are higher in cancer cells, inhibiting redox machinery may be an effective and specific way to induce cell death in tumors [97].

4. Oxidative DNA damage is induced in bystander cell populations

Intercellular communication has been well studied in relation to bystander effects *in vitro* and *in vivo*. Bystander effects are seen in cell populations neighboring or sharing media with damaged or stressed cells [98] including those under biological stresses such as aging and

cancer [99]. Some examples of bystander effects include increased mutations, DNA DSB formation, and apoptosis [98,100,101].

The signaling *in vitro* has been shown to be reminiscent of the inflammatory response mediated by COX-2 related pathways, involving cytokines, growth factors, and membrane-permeable ROS, including nitric oxide (NO) [98,102]. There are several lines of evidence that support a role for ROS in the production of bystander DNA damage. ROS can be produced in directly damaged cells (Fig. 1) or indirectly via inflammatory process, and can pass to neighboring cells through passive diffusion, gap junctions, or active transport [103,104]. Although most ROS have a short half-life and cause damage locally, H_2O_2 has a relatively long half-life and can travel long distances, causing DNA damage at distant sites [105]. Once in neighboring cells, ROS can cause oxidative lesions by directly acting on the DNA or indirectly through lipid peroxidation or protein damage [3,106]. Alternatively, the damaged or stressed cells may release cytokines that bind to bystander cells, inducing the local production of ROS possibly by activating transcription of NO synthase and cyclooxygenase II [3,107]. That NO can function as an agent of the bystander effect is evidenced by the findings that NO synthase inhibitors mitigate DSB formation in bystander cells though not in directly hit cells [99,108,109] and that diethylamine NONOate (DEANO), a compound that releases NO induces a comparable increase in DNA DSBs when added to the media of otherwise undamaged cells [99,108–110]. Other reactive species including superoxide anion, hydrogen peroxide, and hydroxyl radicals have also been proposed as agents of the bystander effect, since antioxidants and DMSO have been shown to reduce bystander cellular damage [99,107,111,112]. Additionally, mitochondrial damage or dysfunction has also been implicated in bystander effects [103,113]. Though the precise mechanism of bystander-induced DSB formation has yet to be elucidated, several groups have proposed that the bystander DNA DSBs are initially oxidative lesions that are converted to DSBs upon collision with moving replication machinery [98,99,103,104,114–116] (Fig. 2).

The potentially deleterious effects of bystander signaling are obvious. Any increase in DNA DSB frequency may increase the risk of mutation and other forms of genome instability [114]. This, in turn, increases the risk for cell transformation and tumor development [3,106], especially when the DNA damage repair machinery is altered by mutation [117,118]. However, the possibility remains that ROS production and bystander signaling in response to stress might be beneficial to the organism. For instance, it has been reported that bystander signaling from nontransformed damaged cells selectively kills precancerous transformed cells in culture [119]. In a fully developed organism, most cells are not cycling and while capable of generating bystander signals, they are less susceptible to bystander effects [99,115,116]. Therefore, the susceptibility of cycling cells to bystander signaling [113,114] might result in mitigating tumor development by eliminating cells that have begun to replicate aberrantly [119]. Another possibility is that bystander signaling acts to induce an adaptive response in cycling cell populations, in which a small amount of damage permits cells to better survive larger amounts of damage that may occur in the future.

Finally, there is evidence that tumors growing *in vivo*, even at early stages of development, produce oxidative damage in neighboring tissues through similar bystander mechanisms [120]. Additionally, mice deficient in oxidative DNA damage repair mechanisms accumulate DSBs in bystander tissue after local direct irradiation, suggesting that ROS bystander signaling in response to other forms of stress exists *in vivo* [121]. This information potentially has important implications for the study of genomic instability resulting from ROS signaling from tumors and other damage.

5. Conclusions

There is an abundance of evidence implicating ROS as one source of DNA damage associated with aging, cancer, stress signaling, and other conditions. However, ROS are essential to numerous cellular processes including apoptosis [122], cell growth [123] and the activation of redox system proteins [97]. In addition, ROS play a role in acquired immunity, killing bacteria and other pathogens, when produced by macrophages and neutrophils [124]. Moreover, extreme hypoxia (less than 1% O_2) also activates DNA damage responses including γ-H2AX focus formation [125,126]. Counterintuitively, a recent study demonstrated that certain types of antioxidant supplementation may increase mortality [127]. These results highlight how little is known about the roles played by ROS in cellular metabolism. Nevertheless, maintenance of the balance of ROS levels appears to be an important consideration for the prevention of human disease.

Understanding ROS roles in cellular homeostasis could help improve human health. Clinical data suggests that in some cases of human pathology, monitoring oxidatively induced DNA lesions can be used for optimizing disease treatment including targeted radio-or chemotherapy [32,128,129]. These lesions could also be used to detect exposures to various toxic chemicals and metals [4]. The presence of persistent DNA lesions in tumor cells has led many to propose that DNA damage markers are potential biomarkers for screening ongoing oxidative stress and for cancer diagnostics and prediction [84]. Additionally, because ROS levels are higher in cancer cells, it has been suggested that inhibiting redox machinery may be an effective and specific way to induce cell death in tumors [97]. There are many opportunities for elucidating the role of oxidatively induced DNA lesions in human health and disease. Better understanding of the mechanism of ROS-induced human pathology will help to develop effective strategies to prevent and/or treat human disease.

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Abbreviations

8-oxo-dG	8-oxo-2′-deoxyguanosine
BER	base excision repair
DEANO	diethylamine NONOate
DSB	double-strand break
FISH	fluorescent in situ hybridization
Mbp	mega base pair
mtDNA	mitochondrial DNA
NO	nitric oxide
OCDL	oxidative clustered DNA lesions
ROS	reactive oxygen species
SSB	single-strand break
Topo	topoisomerase

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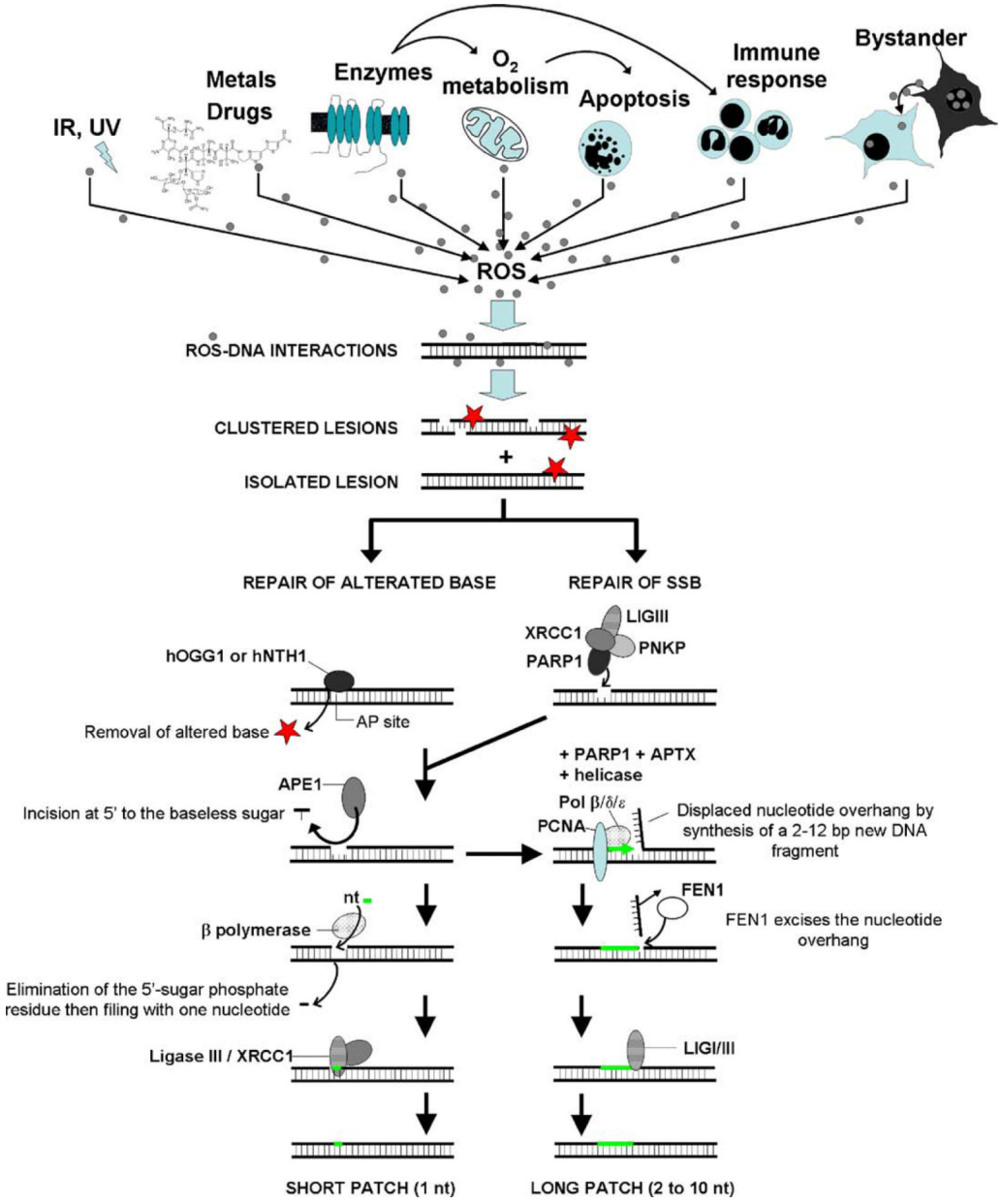

Fig. 1.

ROS have different origins. ROS can arise following exposure to ionizing radiation or light (IR, UV), drugs and other chemicals such as metals. Enzymes, oxygen metabolism and apoptosis also account for ROS production. Finally, the inflammatory responses involving the immune system and bystander signaling also utilize ROS. When ROS enter the nuclear cell compartment, they interact with DNA creating lesions ranging from base or sugar modifications to abasic sites (represented by red stars) and SSBs. ROS-induced DNA lesions can appear in an isolated or clustered form and they are primarily repaired by two BER subpathways: the short-patch and the long-patch pathways. The short-patch or single-nucleotide pathway is initiated by a DNA glycosylase (hOGG1 or hNTH1) that cleaves and removes the altered base, giving an abasic site. This abasic site is then processed by an endonuclease (APE1) allowing DNA polymerase β to process the next step, catalyzing the elimination of the 5′-sugar phosphate residue and filling the gap with a nucleotide. Finally,

the nick is sealed by the ligase III/XRCC1 complex. To simplify, only the branch of the short-pathway utilizing a monofunctional glycosylase is represented. SSBs can be repaired by the long-patch pathway (replacing approximately 2–12 nucleotides). This subpathway is dependant on PCNA and FEN1. It contains many of the same factors as the short-patch pathway but in contrast to the short-patch subpathway, DNA synthesis is thought to be mediated by several DNA polymerases including polymerases β, δ and ε. nt: nucleotide.

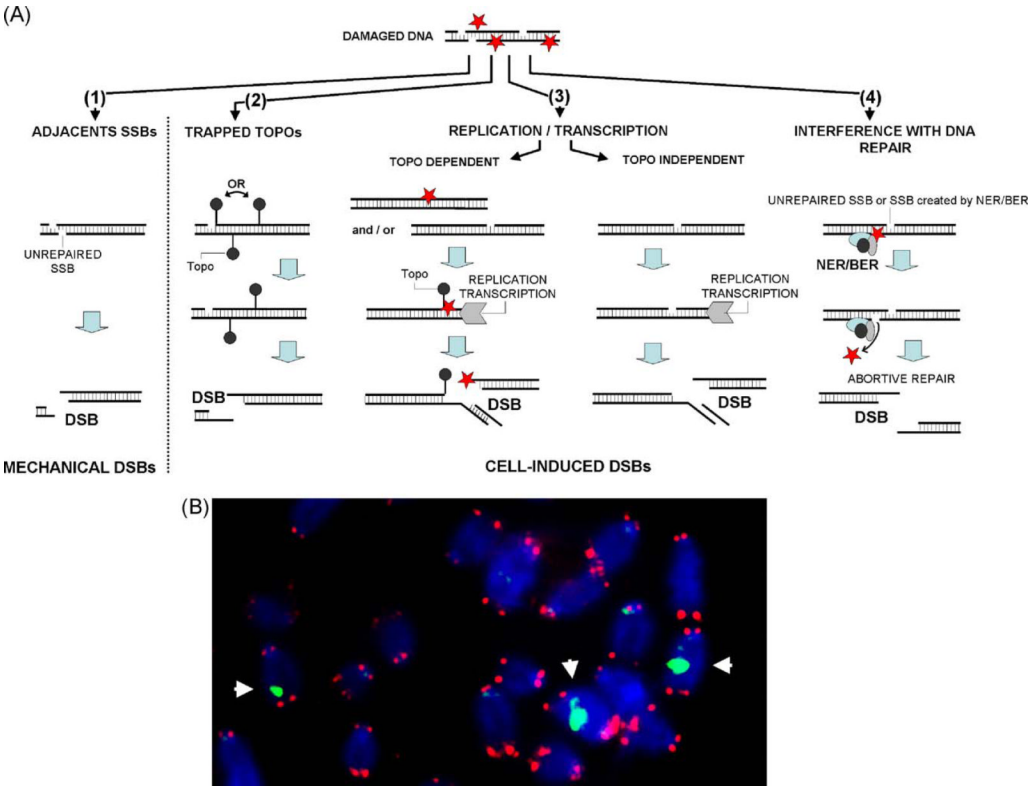

Fig. 2.

Oxidative stress can lead to DNA DSB formation. (A) A DSB can arise when two SSBs form close to each other (1), when topos cleave next to a SSB (2) and when ROS-induced DNA damage interferes with both DNA replication and transcription (3). A DSB is generated during DNA repair when excision of a modified base takes place near an unrepaired SSB. Oxidative DNA lesions can also interfere with reversible topo cleavage complexes during DNA replication and RNA transcription. In such cases, DNA/RNA polymerase forks run off the DNA to generate DSBs. Finally, DSBs can also appear when transcription and replication forks collide directly with SSBs or other ROS-induced lesions. Rarely, interference during DNA repair by BER also leads to DSB formation (4). (B) Representative image showing DNA DSBs induced by oxidative stress on mouse chromosomes. Blue: DNA; red: FISH signal indicating telomeres; green: DSBs visualized by γ-H2AX foci. White arrows indicate chromosomes containing a DSB.

Fig. 3.

The role of oxidative stress in human pathogenesis. Many studies suggest that ROS have a role in normal aging, cancer, infertility, and a wide variety of age-related diseases such as some neurological diseases, type 2 diabetes, autoimmune and cardiovascular diseases. In healthy cells, the steady-state level of the ROS-induced lesions depends on the relative rates for their formation and repair. Chronic exposure to ROS and/or deficiencies in DNA repair processes or redox machinery can result in persistent DNA lesions. Accumulation of DNA lesions can lead to point mutations and/or chromosomal aberrations via the formation of DNA DSBs leading to the development of disease. Diseased cells, in turn, develop increased ROS production and/or decreased efficiency of DNA repair processes or redox systems. Red arrows represent the possible negative feedback of pathology on ROS production. ➨: increase of ROS production. T: decrease of DNA repair or redox capacity.