Review

Role of oxidative stress and DNA damage in human carcinogenesis

Thomas B. Kryston[a], Anastassiya B. Georgiev[a], Polycarpos Pissis[b],
Alexandros G. Georgakilas[a,b,*]

[a] Department of Biology, Thomas Harriot College of Arts and Sciences, East Carolina University, Greenville, NC 27858, USA
[b] Department of Physics, National Technical University of Athens, Zografou Campus, GR 15773 Athens, Greece

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ABSTRACT

Cells in tissues and organs are continuously subjected to oxidative stress and free radicals on a daily basis. This free radical attack has exogenous or endogenous (intracellular) origin. The cells withstand and counteract this occurrence by the use of several and different defense mechanisms ranging from free radical scavengers like glutathione (GSH), vitamins C and E and antioxidant enzymes like catalase, superoxide dismutase and various peroxidases to sophisticated and elaborate DNA repair mechanisms. The outcome of this dynamic equilibrium is usually the induction of oxidatively induced DNA damage and a variety of lesions of small to high importance and dangerous for the cell i.e. isolated base lesions or single strand breaks (SSBs) to complex lesions like double strand breaks (DSBs) and other non-DSB oxidatively generated clustered DNA lesions (OCDLs). The accumulation of DNA damage through mis-repair or incomplete repair may lead to mutagenesis and consequently transformation particularly if combined with a deficient apoptotic pathway. In this review, we present the current status of knowledge and evidence on the mechanisms and involvement of intracellular oxidative stress and DNA damage in human malignancy evolution and possible use of these parameters as cancer biomarkers. At the same time, we discuss controversies related to potential artifacts inherent to specific methodologies used for the measurement of oxidatively induced DNA lesions in human cells or tissues.

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Contents

1. Sources of intracellular oxidative stress

DNA and cells of the human body are constantly exposed to attacks of oxidative nature. These attacks can be divided into two broad categories: exogenous and endogenous (Fig. 1). Exogenous and environmental sources of oxidation relate to specific exposures of the organism to ionizing radiations like X-, γ- or cosmic rays and α-particles from radon decay, oxidizing chemicals and UVA solar light. Endogenous (intracellular) attacks correspond to natural origin such as through cellular signaling and metabolic processes or during inflammation [1–4]. These endogenously induced DNA lesions can often reach a level much higher than the ones induced by environmental factors like even low doses of ionizing radiation (<0.3 Gy) contributing significantly in the accumulation of mutations in cells and tissues [5–7]. During these attacks, although not in all cases, the primary damage is being induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS). Examples range from the hydroxyl radical (*OH), $O_2^{\bullet-}$, singlet oxygen ($^1O_2$) and peroxynitrite to others [1,8]. Intracellularly, ROS are created as a natural byproduct of oxidative metabolism (Fig. 1). ROS are constantly generated in mitochondria as respiration byproducts (1–5% of consumed O_2) and in general are accepted as the major source of oxidative injury in all aerobic organisms. Another source of constant generation of free radicals which is usually underestimated is the chronic exposure to viral infections [9]. For example, in the case of hepatitis viruses there is an established connection between chronic infection and induction of oxidative stress. Different groups have associated a variety of viruses with increased oxidative stress, DNA damage and mutagenic rate [9]. This high intracellular oxidation status in viral infections consists of decreased antioxidant enzymes like catalase, glutathione peroxidase, glutathione reductase as well as high level of hydroxyl radicals. Although there are numerous differences between exogenous and endogenous attacks, the main differences are the levels and complexity of DNA damage which both are expected to be lower in the case of intracellular stress [4,10].

Oxygen is an important element that plays significant roles in many processes of the human body including cellular metabolism, intercellular and intracellular signaling and acts as a key component for an effective immune system and response [11]. Although beneficial, it is accepted that oxygen, through ROS generation, can react with DNA, proteins and other cellular components and can become problematic. The body is constantly trying to maintain homeostasis with the utilization of the immune system. The immune system is divided into two categories: adaptive and innate. In adaptive immunity, highly complex cells are deployed and recognize antigens on foreign cells. Innate immunity is much broader and is designed to recognize common features on foreign cells and ultimately release more expansive white blood cells such as macrophages and neutrophils. These cells are capable of releasing cytokines which are chemicals that signal other cells to a specific site of damage or injury and aid with the induction of inflammation [12]. The phagocytic capability of macrophages utilizes oxidants and enzymes to degrade the foreign intruder. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is a complex inside the macrophage that sanctions this to occur i.e. to the production of free radicals [13]. Inside this complex, the covalent bond between atoms is severed, creating an imbalance of electrons and when oxygen is one of these entities, creation of free radicals occurs. When this free radical formation transpires with oxygen as one of the elements, it is known as reactive oxygen species (ROS). ROS interact with the biological molecules and disrupt the normal synthesis and repair of DNA. This disruption is primarily associated with inhibition/inactivation of antioxidant key proteins as well as DNA repair enzymes induced by ROS-damage to these biomolecules [14,15] Macrophages are one source of ROS, but it is important to note that there are many other sources of the creation of ROS. ROS can impact key mechanisms securing cell survival and the avoidance of genomic instability [16]. Tumor growth increases inflammation and recent studies have indicated that in the presence of a tumor or a malignancy in general in the organism, a high oxidative stress status can be detected [12]. Through the bystander effect, the microenvironment surrounding the tumor is not the only location where this elevation is seen but also in distant organs. Recent evidence by Redon et al. suggests the induction of complex DNA damage i.e. double strand DNA breaks (DSBs) and non-DSB oxidatively generated clustered DNA lesions (OCDLs) by tumors growing in mice is not limited to close proximity, but also to distant proliferative organs. This is seen primarily via the inflammatory response pathway and specific cytokines such as chemokine (C–C) ligand 2 (CCL2) [17]. Exposure of an organism to chronic inflammation stress can result to imbalances of tissue homeostasis and possible tumor formation [18]. Typically most ROS have a short half-life and cause damage locally but for example H_2O_2 has a relatively long half-life and can travel long distances, causing DNA damage at distant sites [19]. In addition, it may be pointed out that mostly hydroxyl radical (*OH) and to a lesser extent the lower-energy singlet molecular oxygen ($^1O_2$) through specific targets (guanine, histidine, tryptophan, tyrosine) may react with DNA and proteins. In contrast O_2^* is completely unreactive towards biomolecules while H_2O_2 requires the presence of reduced transition metals such as Fe^{2+} to promote the Fenton type reaction [20].

2. Types of oxidatively induced DNA lesions

The oxidatively induced DNA damage associated with ROS typically are apurinic/apyrimidinic (abasic) DNA sites, oxidized purines and pyrimidines, single strand (SSBs) and double strand (DSB) DNA breaks. Two of the most common endogenous DNA base modifications are 8-oxo-7,8-dihydroguanine (8-oxoGua) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine. Both originate from the addition of the hydroxyl radical to the C8 position of the guanine ring producing a 8-hydroxy-7,8-dihydroguanyl radical which can be either oxidized to 8-oxoGua or reduced to give the ring-opened FapyGua [2,21]. The frequency of the damage is dependent on the quality and level of oxidative stress as well as other factors. It is accepted that the alterations in DNA from 8-oxodG is part of the recognition site utilized by DNA glycosylases to detect damaged guanine bases. For example, FapydG is currently considered as the most prevalent guanine-derived lesions formed under low oxygen (O_2) conditions i.e. hypoxia [22–24]. Interaction of hydroxyl radical with pyrimidines (thymine and cytosine) at positions 5 or 6 of the ring, can produce several base lesions and two of the most abundant and well known products, 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol, Tg) and 5,6-dihydroxy-5,6-dihydrocytosine (cytosine glycol). As discussed in the next section, 8-oxodG and Tg are often chosen as reliable markers of oxidative stress in a variety of biological systems ranging from bacteria up to human cancer patients. Especially in the case of 8-oxodG, this lesion has been reported to exist at high steady levels in genomic, mitochondrial and telomeric DNA and RNA [25]. In addition, 8-oxodG and Tg have been both utilized as markers for indication of high levels of oxidative stress and damage in the human body in association with human cancer [2]. These lesions ultimately are not lethal to the cell, but are considered to be highly mutagenic. Although the creation of an altered base or base loss is not expected to result into a significant destabilization of the DNA molecule, a localized perturbation of the stacking forces, hydrogen bonds and interaction with water molecules and/or positive ions like Na^+ surrounding the DNA double helix is expected [22,26]. It is generally accepted that this localized destabilization and conformational changes of
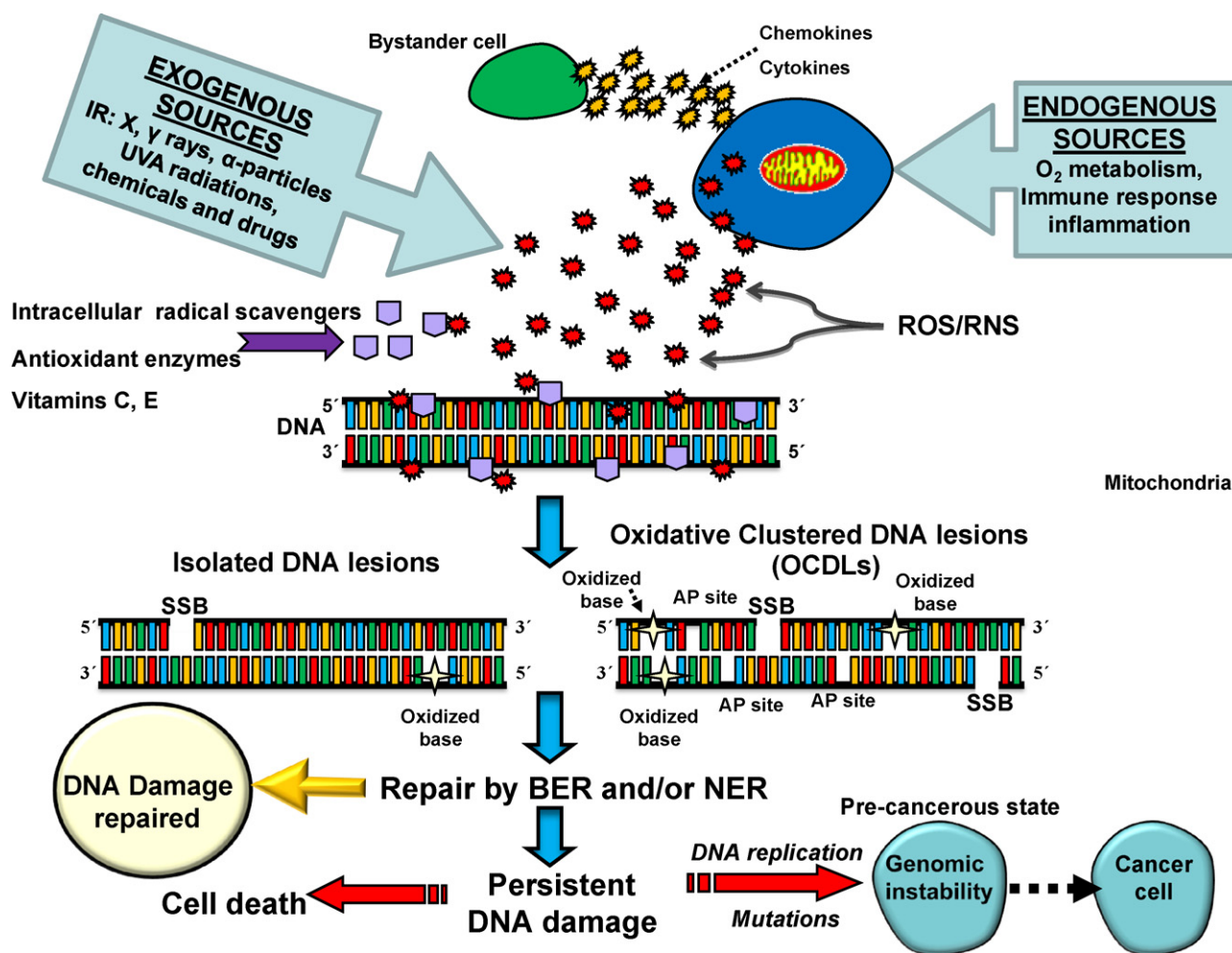
Fig. 1. Association of persistent oxidatively generated DNA lesions with human cancer. Cells in every organism are getting exposed on an everyday-basis to various oxidizing and damaging agents ranging from exogenous sources like environmental, medical, diagnostic ionizing (IR) and non-ionizing radiations (X- or γ-rays, α-particles from radon decay, UVA radiation) or chemicals to intracellular (endogenous) sources of oxidative stress primarily produced by O_2 metabolism, immune responses and inflammation. The final outcome is the production of reactive oxygen/nitrogen species (ROS, RNS) reacting with the DNA and producing various lesions and adducts (indirect effect). IR can damage DNA also by direct energy deposition and ionizations. DNA damage can be induced also in neighboring or distant cells via an inflammation based mechanism (bystander/distal effects). The first frontier of cellular defense against DNA damage consists of endogenous non-enzymatic radical scavengers like glutathione (GSH) and vitamins like C, E, antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) as well as sophisticated and highly specified DNA repair pathways. According to current status of knowledge, the major types of DNA damage and their repair are expected to be several DNA lesions like single strand breaks (SSBs) and oxidized bases like 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol, Tg) in a clustered (OCDLs) or single (isolated) formation. All these lesions are expected to be processed primarily by base excision repair (BER) while the involvement of nucleotide excision repair (NER) cannot be excluded. Single DNA lesions are expected to be repaired more efficiently than OCDLs which can be very challenging for the cell to repair. In many cases, the cell will bypass DNA damage using specific DNA polymerases and enter DNA replication creating mutations and chromosomal lesions. Alternatively the presence of unrepaired DNA lesions can induce cell death through the apoptotic pathway. Chronic exposure to DNA lesions can lead to mutations and genomic instability (pre-cancerous state) and eventually to malignant transformations (cancerous state).

the DNA at the site of the DNA lesion are part of the recognition mechanisms used by the DNA glycosylases to detect the altered guanine bases like 8-oxodG or FapydG [21,22]. Two other pyrimidine lesions often detected in human patients as the result of the interaction of the hydroxyl radical *OH with the methyl group of the thymine, are the 5-(hydroxymethyl)uracil and 5-formyluracil [2]. Again, although all these purine and pyrimidine oxidation products are not considered lethal for the cell, they are usually noncoding and highly mutagenic [27]. Through the hydrolysis of the *N*-glycosidic bond of nucleotides in the DNA, the DNA base is released and the phosphodiester backbone remains intact, ultimately creating an AP site [28]. It must be stated that the most prevalent and characteristic abasic sites formed under oxidative stress are expected to be 2-deoxyribonolactone and the so-called C4′ oxidized abasic site that arise from *OH-mediated hydrogen abstraction at C1 and C4 of the 2-deoxyribose moiety of DNA respectively [29]. Normal (transient) abasic sites arise mostly from

the spontaneous (non-enzymatic) hydrolysis of the *N*-glycosidic bond of purine 2′-deoxyribonucleosides that is much more labile than that of pyrimidine 2′-deoxyribonucleosides. These transient apurinic sites though cannot be considered *per se* as oxidatively generated damage to DNA but they have been shown to accumulate in cells and tissue at significant numbers that can reach or exceed ~10,000/cell/day [30–33]. It is likely that a few *OH-mediated damage to DNA including FapyGua and FapyAde may be considered as potential precursors of apurinic sites since the opening of the imidazole ring of the purine bases is known to lead to a pronounced increase in the hydrolytic lability of their *N*-glycosidic bonds. This occurrence is very common and can occur spontaneously or also enzymatically as 'repair intermediates' after the removal (excision) of the damage base by a DNA glycosylase in base excision repair (BER) [27,34,35]. AP sites are not considered lethal unless in high levels and, if present are expected to block DNA polymerases therefore having a high mutagenic potential [36–38]. The

two main repair pathways for the processing of oxidatively generated DNA lesions are considered to be the BER and to a smaller extent nucleotide excision repair (NER) (Fig. 1).

With the interaction of the hydroxyl radicals with DNA, SSBs may occur. The mechanism consists of hydrogen abstraction from the 2-deoxyribose leading to the formation of carbon based radicals which under the presence of oxygen can be converted to peroxyl radicals (ROO•). The peroxyl radicals through different reactions, can also abstract hydrogen atoms from sugar moieties thus leading to DNA strand breaks. The most possible pathway though for induction of strand breaks involves the *OH-mediated hydrogen abstraction at C3′, C4′ and C5′ [29,39]. Peroxyl radicals are also implicated in lipid peroxidation mediated DNA damage and carcinogenesis especially under the presence again of oxygen [40]. Through a process known as Fenton type reaction, hydrogen peroxide may be reduced by Fe^{2+} creating the reactive hydroxyl radicals which attack the DNA inducing base lesions and SSBs [28]. The simultaneous attack of hydroxyl radicals to DNA and can cause two neighboring SSBs i.e. a DSB [41]. Pioneering experiments by Ward et al. have shown [41] that exposure of mammalian cells to low-moderate concentration of H_2O_2 (50 μM) although producing SSBs, is not efficient to kill cells and therefore suggesting for the first time the idea of 'locally multiply damage sites' (LMDSs) [41,42]. Only with much higher H_2O_2 concentrations (50 mM) cell killing was observed due to the induction of DNA lesions (DSBs and other non-DSB lesions) presumably of higher complexity. The damage to DNA is said to be clustered if the sites of impediment or damage are within two or more bases within few helical turns (Fig. 1). Since then, quite a few laboratories have reported the presence of non-DSB DNA clustered lesions in human cells or tissues and their accumulation under persistent or chronic oxidative stress or DNA repair deficiencies as reviewed in [10]. The direct association between these DNA lesions and the occurrence of endogenous or exogenous oxidative stress lead to the most properly known idea of oxidatively generated non-DSB bistranded clustered DNA lesions (OCDLs) [43]. Although the induction of OCDLs with radiation is expected to be significant based on theoretical and experimental evidence [44], the situation is still unclear pertaining the mechanism(s) leading to accumulation of clusters endogenously as a result of high oxidative stress in the cell or tissue [10]. Different studies suggest a great variation at the levels of intracellular OCDLs ranging from a few clusters up to several hundred per Gbp (10–1000 clusters/Gbp) depending on the measurement method followed and the type of cells or tissues used for detection [17,45–49]. We believe that the microenvironment (redox status and repair efficiency) and origin of the cell or tissue is also significant since mouse tissues for example are expected to undergo always a higher state of oxidative stress compared to human tissues based simply on the higher metabolic rate and oxygen consumption [50]. Although a possible overestimation of endogenous clusters maybe present at least in some cases, the highly varying numbers published on the steady-state levels of single (non-clustered) oxidatively generated DNA damage (like 8-oxodG: 0.2–8 lesions/Mbp) [3,51,52] would support the accumulation of clusters in tissues. Overall, many review studies [1,51–53] reporting expected levels of endogenous oxidatively generated DNA lesions in tissues or cells tend to agree that an expected lowest number for one of the most frequent oxidative DNA lesions, 8-oxodG, is $\sim0.1/10^6$ normal bases or 0.2 (8-oxodG)/Mbp. The specific frequency is presumably indicative of insignificant artifactual oxidation due to DNA isolation or measurement method. Considering the fact that 8-oxodG constitutes only ~5% of the total number of oxidized base lesions [52], we can derive the minimum number of ~4 total oxidative lesions/Mbp. This number of total oxidative lesions gives an expected frequency of 0.4–0.8 oxybase clusters/Mbp i.e. 400–800 clusters/Gbp (2400–4800 per cell assuming an average genome size of ~6 Gbp) based on the suggested ratio of 5:1 to 10:1 of total oxidative lesions to oxybase clusters [54]. Of course in these calculations, one should add the abasic DNA lesions (AP sites) which are expected to be prevalent and repair resistant [55,56] and also contribute to the total OCDL load present in the cell or tissue. Recent studies by Chastain et al. [56] suggest a preferential AP site formation also in a clustered formation in genome areas undergoing replication. The recent studies by Redon et al. [17], also conclude that highly proliferative tissues are more susceptible to DNA damage induction (DSBs and OCDLs). Mechanistically, we believe that the formation of OCDLs can be explained by the abundance of intracellular ROS and oxidation events which can be present also in a cluster formation [5]. In addition, several independent studies suggest the possible formation of tandem lesions (closely spaced in one strand) in cells through Fenton type reactions and only one radical hit [57–59]. All these studies propose the induction of these tandem lesions through electron transfer reactions and oxidation of peroxyl radicals through intrastrand hole migration. We cannot exclude such a mechanism also involved in the induction of bistranded DNA clusters. But the existence of tandem lesions by itself in cellular DNA would be detected as OCDLs by all methodologies used for the measurement of bistranded lesions. Interestingly and even in the case of OCDLs by ionizing radiation, clusters are expected to be induced not really by a 'two radical hit' but one event [60,61]. Finally different studies have also reported occurrence of endogenous DSBs (a form of bistranded clustered DNA damage) in various cells or tissues [4,62–65].

It must be mentioned that *in vivo*, the final outcome of the interaction of DNA with ROS/RNS and distribution of DNA lesions is practically determined by several factors like DNA repair, the levels of antioxidant enzymes, DNA sequence and accessibility of free radicals to react with DNA which is surrounded and usually protected by histone proteins and tightly bound molecules (polyamines, thiols, etc.) [66–69]. In addition, ROS/RNS can also attack except of the DNA, various key cellular proteins like those participating in DNA repair, cell cycle control etc. and severely affect their binding to their DNA substrates i.e. damaged DNA or other proteins [15]. This latter phenomenon of possible cellular protein destruction and/or activation by free radicals is usually underestimated in cases where the overall outcome and results of chronic exposure to oxidative stress is studied.

3. Measurement of DNA damage in human cancer

Accumulating evidence supports the identification of oxidatively damaged DNA as a potential reliable indicator of oxidative stress in an organism under the presence of a malignancy. Many studies as presented in Table 1, show that in a variety of cancers and tumors there is an ongoing battle between the persistence of oxidative stress and generation of free radical species attacking the DNA in one hand and the cellular defense mechanisms (radical scavengers, antioxidant enzymes and DNA repair) on the other. This can be manifested by the significant overexpression of several antioxidant/repair enzymes in some malignant tissues compared to controls in response to this high-oxidant status [70]. In many cases, the cellular defense network is overwhelmed by the oxidative attack and the result is the induction of DNA lesions at much higher levels compared to controls. The accepted importance and high necessity of accurate measurement of endogenous DNA damage in different types of cells or tissues led to the employment of a variety of techniques and methods for the detection of single oxidatively generated DNA lesions like 8-oxodG, Tg and AP sites such as high performance liquid chromatography (HPLC), liquid chromatography/tandem mass spectrometry (LC-MS/MS), alkaline filter elution, single cell gel electrophoresis (SCGE or Comet assay), adaptations of agarose gel electrophoresis and others

Table 1
Major documented cases with elevated levels of oxidative stress and/or DNA damage in human malignancies.

Type of cancer	Study model	Findings
Benign prostatic hyperplasia (BPH)	Human prostate glands	Majority of patients had higher endogenous levels of typical *OH-induced products of DNA bases and lower activities of antioxidant enzymes in BPH tissues than in surrounding disease-free tissues of the prostate gland. When both catalase (CAT) and superoxide dismutase (SOD) had decreased activities in BPH tissues, the increases in the endogenous levels of DNA base products were most prominent [98].
Breast	Human mammary tissues: normal, benign hyperplasia (BH), ductal carcinoma *in situ* (DCIS) and invasive breast cancer (IBC)	A number of oxidative stress proteins, DNA repair proteins, and damage markers overexpressed in human breast cancer tissue [99].
Breast	Human breast cancer patients	Significantly higher ($P < 0.0001$) levels of 8-hydroxydeoxyguanosine (8-oxodG) in DNA from tumor compared to non-malignant adjacent tissue [100].
Breast	Human breast cancer patients and cell lines	Significantly elevated levels of 8-oxo-dG ($P < 0.001$) in malignant breast tissue (invasive ductal carcinoma); also levels significantly greater ($P = 0.007$) in oestrogen receptor positive (ORP) vs. ORP negative malignant tissue and cancer cell lines [101].
Breast	Human breast cancer cell lines	Defective DNA repair of 8-oxo-7,8-dihydroguanine in mitochondria of MCF-7 and MDA-MB-468 human breast cancer cell lines [102]. Reduced repair of 8-oxo-7,8-dihydroguanine in the human breast cancer cell line, HCC1937 [103]. Accumulation of oxidatively induced DNA damage in human breast cancer cell lines following treatment with hydrogen peroxide [104].
Breast	Human breast cancer cell lines	Higher levels of endogenous oxidatively induced clustered DNA lesions (OCDLs) in human breast cancer cell line MCF-7 compared to non-malignant MCF-10A [43].
Breast	Breast cancer patients	Mean levels of 5-(hydroxymethyl)-2′-deoxyuridine were significantly higher in blood of women who had high risk or invasive breast lesions vs. women with benign lesions [105].
Breast carcinoma	Breast carcinoma patients	In a study monitoring patients' serum level of oxidative DNA damage prior and following chemotherapy, thiobarbituric acid reacting substances (TBARS), total nitrite/nitrate (NOx), nitrotyrosine (NT), and 8-oxodG concentrations significantly increased prior to and following chemotherapy. Antioxidant enzyme activities and total antioxidant capacity (TAS) were significantly decreased prior and following chemotherapy [106].
Cervical cancer	Cervical tissues in human patients	Levels of 8-oxodG significantly increased ($P < 0.001$) in DNA from low-grade and high-grade levels of dysplasia, compared to normal, although this did not correlate with human papillomavirus status [107].
Colorectal cancer	Colorectal cancer (CRC), benign adenoma (AD) patients	Vitamin A, C, E levels decreased gradually in AD and CRC patients. 8-OxodG was found increased in leukocytes and urine of CRC and AD patients. 8-OxoGua was higher only in the urine of CRC patients while mRNA levels of OGG1 and APE1 increased in CRC and AD patients [108].
Colorectal cancer	Sporadic colorectal tumors patients	Malondialdehyde and 8-hydroxy-2-deoxyguanosine (8-oxodG) levels were two-fold higher in colorectal tumors compared to normal mucosa ($P < 0.005$). Seven of 10 DNA tumor samples (70%) showing higher values of 8-oxodG also had genetic alterations at different chromosomal loci [109].
Colorectal cancer	Colorectal tumor patients	Significantly higher levels of 8-oxodG in nuclear DNA of primary adenocarcinoma, compared to surrounding non-tumorous tissue ($P < 0.005$) [110]. 8-oxodG-specific lyase activity and expression were significantly up-regulated in carcinoma; A proportional association between 8-oxodG levels and either 8-oxodG lyase activity ($P < 0.05$) or expression ($P < 0.05$) present [111].
Colorectal cancer	Colon cancer patients	Significantly elevated levels of 8-oxodG lymphocyte DNA in colorectal cancer patients, compared to controls accompanied by reduced levels of antioxidant vitamins [112].
Colorectal cancer	Colorectal cancer patients	Immunostaining for pATM, gammaH2AX and pChk2 revealed that all were significantly expressed during tumor progression in advanced carcinoma (vs. normal tissue for pATM ($P < 0.05$); vs. normal and adenoma for gammaH2AX ($P < 0.05$); and vs. normal tissue for pChk2 ($P < 0.05$). Western blot analysis of gammaH2AX and pChk2 revealed that their level increased gradually during tumor progression and was maximal in advanced carcinoma (vs. normal tissue; $P < 0.05$) [113].
Colorectal adenoma and cancer	Colorectal adenoma and cancer patients	Enzyme-linked immunosorbent assay revealed significant increased levels of 8-oxodG ($P = 0.045$) associated with development of colorectal adenoma and cancer [114].
Gastric	Gastric cancer patients	Significantly higher levels of 8-oxodG in DNA from tumor-adjacent and tumor adenocarcinoma tissues than in normal tissue ($P < 0.001$) of gastric cancer patients [115].
Gastric	Human patients with chronic gastritis and gastric cancer.	Levels of 8-oxodG significantly elevated in DNA from chronic atrophic gastritis ($P = 0.0009$), intestinal metaplasia ($P = 0.035$) and *Helicobacter pylori* infected ($P = 0.001$) tissues, compared to unaffected controls [116].
Gynecologic cancer	Female cancer patients	Significantly higher ($P \leq 0.05$) levels of urinary 8-oxodG in patients with gynecological cancer compared to control subjects [117].
Hepatocellular carcinoma (HCC)	HCC Patients	Significantly ($P < 0.005$) elevated levels of 8-oxo-dG in DNA from peritumoral tissue compared to tumor tissue in HCC. In contrast, patients with hepatic metastases (non-HCC) or end-stage alcoholic liver disease showed no differences between the corresponding two regions [118].
Acute lymphoblastic leukemia (ALL)	Human lympocytes from ALL patients and controls	Lymphocyte DNA levels of FapyGua, 8-oxoGua, FapyAde, 8-oxoAde, 5-OH-Cyt, 5-OH-5-MeHyd and 5-OH-Hyd significantly ($P < 0.05$) elevated in ALL compared to control subjects [119].
Adult T cell leukemia lymphoma; lymphoma, acute leukemia and myelodysplastic syndrome	Human leukemia and lymphoma patients	Significant difference in levels of urinary 8-oxodG between adult T cell leukemia/lymphoma and controls ($P < 0.05$); no significant difference in levels of urinary 8-oxo-dG between lymphoma, acute leukemia and myelodysplastic syndrome [120].

Table 1 (*Continued*)

Type of cancer	Study model	Findings
Lung	Human patients (18 with lung cancer and 3 with normal cell lines)	H1650 and H226 cell lines presented the lowest expression of hOGG1 mRNA expression therefore having severe reduction in 8-oxoG incision in nuclear and mitochondrial extracts. Under expression of hOGG1 mRNA and hOgg1 protein associates with decrease in mitochondrial DNA repair to oxidative damaging agents [121].
Lung	Lung cancer patients	Lymphocyte DNA levels of 8-oxo-dG significantly elevated ($P < 0.05$) compared to controls [122].
Lung	Lung cancer patients	An increase in urinary 8-oxodG/creatinine was found in non-small-cell carcinoma (non-SCC) patients during the course of radiotherapy. SCC patients showed higher levels of urinary 8-oxodG/creatinine than the controls ($P < 0.05$) [123].
Lung	Patients with lung squamous cell carcinoma (SCC)	In a pilot study of five subjects: levels of 8-OH-Ade elevated in tumor tissue of all SCC patients vs. controls; levels of 8-oxoGua elevated in 4/5b patients; levels of FapyGua elevated in 3 patients; 5-OHMeUra, 5-OH-Ura, 5-OH-Cyt, 2-OH-Ade levels elevated in 3/5 patients; 5-OH-Hyd, 5,6-diOH-Ura, FapyAde (DNA)-levels elevated in only 1/5 or 2/5 patients. Antioxidant enzyme (GPx, SOD and CAT) levels were lower in cancerous tissues [124].
Liver, ovary, kidney, breast, and colon	Tumor and adjacent normal tissues from human cancer patients.	Higher non-DSB clustered oxidative DNA lesions (OCDLs) in many tumor vs. normal tissues, importance of endogenous non DSB clusters in human cancer and their potential use as cancer biomarkers [45].
Melanoma (cutaneous)	Melanoma patients	Nuclear and cytoplasmic 8-oxodG staining were evaluated in combination, total 8-oxodG resulted significantly associated with p53 ($P = 0.026$) and with nuclear or total (nuclear and cytoplasmic staining evaluated in combination) surviving immunoreactivity, with borderline significance ($P = 0.095$). In survival analysis, Kaplan-Meier univariate analysis demonstrated that patients with tumors negative for nuclear 8-oxodG had significantly longer survival time compared with those with nuclear 8-oxodG-positive tumors ($P = 0.032$) [125].
Nasopharyngeal	Human NPC cells	All cases of NPC were positive for 8-NitroG, 8-oxodG and 94.7% were positive for INOS. NPC samples exhibited significantly more intense staining for 8-NitroG, 8-oxodG and INOS than those of chronic nasopharyngitis. Pathological stimulation of nasopharyngeal tissue, caused by bacterial, viral, or parasitic inflammation, may lead to nitrative and oxidative DNA lesions, caused by nitric oxide [126].
Prostate	Male prostate cancer patients	Significant increased risk was observed for individuals who carried 1 or 2 copies of the variant allele of the XRCC-1 Arg399Gln polymorphism, compared with those who only harbored the wild-type allele. Variability in the capacity of repairing oxidative DNA damage influences susceptibility to prostate cancer [127].
Ovarian carcinoma	Invasive ovarian carcinomas	8-oxodG, to be a powerful prognostic factor in ovarian carcinoma (Kaplan-Meier survival log-rank-analysis $P = 0.003$). 8-oxodG also associated with poor differentiation ($P = 0.053$), higher stage ($P < 0.001$) and non-optimal surgical outcome ($P = 0.002$) [128].
Renal cell carcinoma (RCC)	RCC patients	A 54% higher content of 8-oxodG was found in RCC than in the corresponding non-tumorous kidney, suggesting that the DNA of RCC is more exposed to ROS than is the DNA of non-tumorous kidneys [129].

[71–75]. Experimental evidence suggests the presence of 'background' oxidatively generated DNA lesions (e.g., abasic sites or oxidized bases) in human or animal cells and tissues at values ranging from 100–10,000 lesions/Gbp [3,31,76–79]. In all cases, DNA has to be isolated from the cells or tissues and therefore some artefactual DNA damage may be measured due to the unavoidable oxidation of DNA during extraction. There is a lot of controversy and debate about the steady-state levels of 8-oxoGua and in general about the oxidized bases steady-state level as reviewed in [4,10]. The measurement of 8-oxo-7,8-dihydroguanine (8-oxoGua) or its related 2′-deoxyribonucleoside (8-oxodGuo) is often used as an indicator of oxidation reactions to cellular DNA. Again, evidence that has been accumulated during the last decade suggests that several of the methods used for detecting 8-oxoGua in cellular DNA gave rise to erroneous conclusions due to artefactual oxidation reactions for chromatographic methods (erroneous GC–MS measurements) and lack of specificity for the immunoassays [52,80]. In addition another identified drawback although of much lower amplitude than the previous, deals with artefactual oxidation reactions during DNA extraction and subsequent work-up. There is a consensus for a higher steady-state values that are close to ~1 8-oxodGuo lesion per 10^6 normal nucleosides following several ESCODD reports [52,80] a few years ago and reviewed in [1]. However there is a discrepancy between the latter HPLC measurements and the values obtained using enzymatic assays which in fact are about 15-fold lower. This may be partially explained by a relatively small contribution of occurrence of artefactual oxidation in the HPLC assays and an incomplete digestion of oxidatively generated tandem bases modifications as recently proposed by Bergeron et al. [57]. Similar artifacts maybe present up to an extent with all the electrophoretic methodologies used for the measurement of DSBs and OCDLs in cells or tissues like non-denaturing Comet assay or pulsed field gel electrophoresis [10,44]. In addition, the prolonged periods of cell-lysis at temperatures equal and especially higher to 37 °C during DNA preparation can introduce the problem of 'heat-labile' sites. These sites can be induced by ionizing radiation or other chemical agents within a clustered DNA damage site and then are thermally converted to SSBs or DSBs and therefore can be mistakenly measured as DNA damage [81]. Even in the case of the γ-H2AX foci methodology which can be used very successfully for the *in situ* detection of DSBs, different studies suggest the possible induction of these foci at non-DSB sites like degraded telomeres or in the absence of DNA damage [63,82]. Current electrophoretic techniques although rendering significant information on the spectrum of clustered DNA damage have several intrinsic problems especially related to the detection of small DNA fragments leading to the underestimation of damage levels [44]. All these experimental difficulties point to the need for the development of an independent method for the accurate detection of clustered DNA lesions. Dynamic dielectric relaxation and conductivity methods have been used in the past for the sensitive detection of DNA damage and degradation, structure changes and apoptosis-induced DNA fragmentation but have not been applied in the case of complex DNA damage detection [26,83,84]. Specifically, the technique of broadband dielectric relation spectroscopy (DRS) is well established and one of the most widely applied non-invasive

techniques used for probing the structure and function of a wide variety of biopolymers like DNA, proteins etc. as reviewed in [85]. Over the last thirty years, the DNA dielectric behavior in solution has been investigated by many research groups. Measurements of dc conductivity have also been used in the past for the study of radiation induced breaks in single stranded polynucleotides and DNA molecules. The observed changes in conductivity have been attributed to the liberation of counterions from these polymers as a result of the induced single strand breaks [86–88]. Dielectric measurements have been also applied for the successful differentiation of breast carcinoma to surrounding normal breast tissues [89]. But as with all methods, a major difficulty for the use of DRS is the usual high conductivity of cell or DNA solutions giving rise to electrode polarization phenomena [90].

4. Conclusions and future directions

Many characteristic studies (Table 1) support the implication of persistent or chronic oxidative stress and damage in human carcinogenesis. Although they may suffer in some cases from a lack of accuracy and a relative overestimation of the DNA lesions detected, they pinpoint to the existence of a phenomenon i.e. the parallel existence of a malignancy and oxidative DNA injury. The idea of using several oxidatively generated DNA lesions (like 8-oxodG, Tg, AP sites and potentially OCDLs) as novel biomarkers of oxidative stress, chronic inflammation and susceptibility to cancer gains more ground. The many well documented cases of higher levels of DNA damage in malignant cells or tissues compared to non-malignant controls definitely reveals a great potential in the usage of oxidatively generated DNA damage biomarkers towards prognostic and curative applications in cancer and inflammation as shown in Table 1 and also in [72]. The alternative presentation of these data also as a percentage (%) of damage increase in cancer patients (relative to controls) in many cases makes the above mentioned trend even more pronounced [91] and further emphasizes the potential application of these novel biomarkers associated with oxidative stress in a malignancy. Although no knowledge of a definite mechanism exists on the occurrence of elevated DNA damage in the presence of cancerous cells or a tumor in the organism usually referred as 'reverse-causation' effect initiated by tumor growth [92], all the above discussed mechanisms of deficient DNA repair and/or antioxidant systems as well as the induction of inflammatory responses may be involved. Oncogenic changes induce a chronic inflammatory microenvironment within and surrounding tumors including presence of inflammatory cells like macrophages and inflammatory mediators such as chemokines, cytokines and prostaglandins [12] as well as elevated levels of endogenous oxidative stress and ROS production [3,93]. These ROS, produced either directly by tumors, or indirectly via inflammatory responses, can cause DNA damage in healthy neighboring cells as well as distant sites (Fig. 1). Although no direct and specific mechanistic insights can be derived from all the above studies towards the exact role of oxidatively induced DNA damage, the conversion of unrepaired or misrepaired DNA lesions to mutations seems to be the driving force directing a cell(s) to transformation and carcinogenesis [94]. The abrogation and/or deregulation of key DNA repair, cell growth and apoptosis related proteins play definitely a governing role in the promotion of chromosomal instability and malignancy [16,95]. In addition, new molecular approaches like epigenetics and proteomics are needed that will allow us gain further mechanistic insights [96,97]. At the same time, the exploitation of new 'cancer biomarkers' in the future will not only contribute significantly in early prognosis but also in the structural design of new and more efficient therapeutic regimes especially in oxidative stress-related malignancies.

Conflict of interest

There are no conflicts of interest.

Acknowledgments

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