Role of reactive oxygen species in apoptosis: implications for cancer therapy

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Abstract

Reactive oxygen species are widely generated in biological systems. Consequently humans have evolved antioxidant defence systems that limit their production. Intracellular production of active oxygen species such as −OH, O2Î’ and H2O2 is associated with the arrest of cell proliferation. Similarly, generation of oxidative stress in response to various external stimuli has been implicated in the activation of transcription factors and to the triggering of apoptosis. Here we review how free radicals induce DNA sequence changes in the form of mutations, deletions, gene amplification and rearrangements. These alterations may result in the initiation of apoptosis signalling leading to cell death, or to the activation of several proto-oncogenes and/or the inactivation of some tumour suppressor genes. The regulation of gene expression by means of oxidants, antioxidants and the redox state remains as a promising therapeutic approach. Several anticarcinogenic agents have been shown to inhibit reactive oxygen species production and oxidative DNA damage, inhibiting tumour promotion. In addition, recombinant vectors expressing radical-scavenging enzymes reduce apoptosis. In conclusion, oxidative stress has been implicated in both apoptosis and the pathogenesis of cancer providing contrived support for two notions: free radical reactions may be increased in malignant cells and oxidant scavenging systems may be useful in cancer therapy. © 2000 Published by Elsevier Science Ltd. All rights reserved.

Keywords: Antioxidant enzymes; Apoptosis; Cancer; Oxidative damage; Reactive oxygen species

Abbreviations: ALL, acute lymphoblastic leukaemia; AP-1, activated protein-1; ASK1, apoptosis signal-regulating kinase 1; c-AMP, adenosine cyclic monophosphate; CAT, catalase; CLL, chronic lymphatic leukaemia; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; GPX, glutathione peroxidase; GSH, glutathione; LDLs, low-density lipoproteins; LPS, lipopolysaccharide; MAPKs, mitogen-activated protein kinases; MPO, myeloperoxidase; NFkB, nuclear transcription factor kappa B; oxLDL, oxidized low-density lipoproteins; ROS, reactive oxygen species; SOD, superoxide dismutase; TGFβ, transforming growth factor beta; TNF, tumour necrosis factor.

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1. Introduction

As a consequence of aerobic metabolism small amounts of reactive oxygen species (ROS), including hydroxyl radicals (‘OH), superoxide anions (O$_2^-$), singlet oxygen ($^1$O$_2$) and hydrogen peroxide (H$_2$O$_2$), are constantly generated in organisms [1]. Cellular antioxidants act in concert to detoxify these species but, when the balance is disrupted, a condition referred to as oxidative stress exists. If oxidative stress persists, oxidative damage to critical biomolecules (including oxidant-induced damage to the genome) accumulates and eventually results in several biological effects ranging from alterations in signal transduction and gene expression to mitogenesis, transformation, mutagenesis and cell death [2,3].

Apoptosis and cancer are opposed phenomena, but ROS have been widely reported to play a key role in both. Evidences that apoptosis can be induced by ROS is provided by studies in which mediators of apoptosis, induce intracellular production of ROS or are inhibited by the addition of antioxidants. Although the mechanism involved is still controversial redox status and/or hydrogen peroxide have both been proposed as critical factors [4,5]. In addition, induction of carcinogenesis has been clearly linked to oxidative DNA damage [3] and the DNA oxidative product, 8-oxo-2'-deoxyguanosine, has been reported to be highly mutagenic [6]. ROS are thought to contribute to carcinogenesis through interference with signal cascade systems, including among others, the nuclear transcription factor kappa B (NFkB), activated protein-1 (AP-1), phospholipase A$_2$, mitogen-activated protein kinases (MAPKs) and c-Jun kinase [7–10].

Cells react rapidly to redox imbalance with a plethora of biological responses, including cell cycle-specific growth arrest, gene transcription, initiation of signal transduction pathways and repair of damaged DNA. These early events are likely to determine whether a cell will necrose, senesce, apoptose or survive and proliferate [11].

Many tumours have been associated with inhibition of apoptosis, follicular lymphomas, carcinomas with p53 mutations: medullary breast carcinoma, lung cancer, colorectal cancer; and hormone-dependent tumours: such as breast, prostate and ovarian cancer [12–14].

2. Source and control of ROS production

In aerobic cells, the most important sources of O$_2^-$ are the electron transport chains of mitochondria and endoplasmic reticulum. In mitochondria, ROS formation is significantly increased by uncouplers of oxidative phosphorylation, hyperbaric O$_2$ treatment, pathologic conditions such as ischemia/reperfusion syndrome, ageing, etc and alterations of mitochondrial lipids occurring during deficiency of polyunsaturated fatty acids and lipoperoxidation processes. In the endoplasmic reticulum (RE) NADPH-cytochrome P450 reductase can leak electrons onto O$_2$ generating O$_2^-$ (Fig. 1). It can also be formed during activity of the desaturase system, which introduce C–C double bonds into unsaturated fatty acids. The system contains FADH$_2$ and cytochrome b$_5$ that can leak electrons onto O$_2$ [15].

Other biological sources of O$_2^-$ are the nuclear membrane (containing an electron transport chain that in the presence of NAD(P)H is able to leak electrons onto O$_2$ with formation of O$_2^-$), the decomposition of oxyhaemoglobin, photo-irradiation of tryptophan, eumelanin and pheomelanin by UV-light, endothelium, autoxidation of catecholamines, thiols, a reduced form of riboflavin and its derivatives, enzymes such as xanthine oxidase, dyoxygenases and oxidases and phagocyte cells as neutrophils and macrophages [16].

Generation of hydrogen peroxide takes place through the dismutation of superoxide. Therefore any biological system generating O$_2^-$ will produce H$_2$O$_2$. However, there are enzymes localized in peroxisomes that produce H$_2$O$_2$ without intermedation of O$_2^-$. Contrary to O$_2^-$, H$_2$O$_2$ is able to cross cell membranes and inside the cells it can react with Fe$^{2+}$ or Cu$^+$ to form hydroxyl radicals via Fenton reaction [17,18].
3. Mechanisms for ROS detoxification

To avoid redox imbalance and oxidative DNA damage, a wide array of enzymatic and nonenzymatic antioxidant defences exist. Primary defence mechanisms prevent oxidative damage by scavenging reactive species directly. The primary defence system includes superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and thioredoxin reductase. Secondary defence's combat processes elicited by free radicals. Main compounds belonging to the secondary defence system are ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), β-carotene, vitamin A, NADPH and urate [19–23].

Superoxide dismutase (EC 1.15.1.1) destroys the highly reactive radical superoxide by conversion into the less reactive peroxide (H₂O₂), that can be destroyed by catalase or glutathione peroxidase reactions [24,25].
Catalase (EC 1.11.1.6) is a highly reactive enzyme, reacting with \( \text{H}_2\text{O}_2 \) to form water and molecular oxygen; and with H donors methanol, ethanol, formic acid or phenols [25].

\[
\text{\text{O}_2}^- + \text{O}_2^- + 2\text{H}^+ \xrightarrow{\text{SOD}} \text{H}_2\text{O}_2 + \text{O}_2
\]

Glutathione peroxidase (EC 1.11.1.19) catalyses the reduction of a variety of hydroperoxides (\( \text{ROOH} \) and \( \text{H}_2\text{O}_2 \)) using GSH, thereby protecting mammalian cells against oxidative damage and, reducing, among others, cellular lipid hydroperoxides [26].

\[
\text{\text{ROOH}} + 2\text{GSH} \xrightarrow{\text{GPX}} \text{ROH} + \text{GSSG} + \text{H}_2\text{O}
\]

The flavin containing thioredoxin reductase (EC 1.6.4.5) is an ubiquitous enzyme able to reduce \( \text{O}_2^- \) and NO by using thioredoxin as a substrate. Transferrine and ferritin sequester iron ions, while ceruloplasmin sequesters copper ions so that the ions are not available to catalyse Haber–Weiss reaction generating \( \cdot \text{OH} \) or to perform the decomposition of hydroperoxides. Ceruloplasmin has also ferroxidase activity: it oxidizes \( \text{Fe}^{2+} \) to \( \text{Fe}^{3+} \) and so inhibits \( \cdot \text{OH} \) formation from \( \text{H}_2\text{O}_2 \) and iron dependent lipoperoxidation [17].

\( \alpha \)-Tocopherol is concentrated inside the membranes, in blood lipoproteins and adrenal glands. It quenches and reacts with \( \cdot \text{O}_2 \) and is a scavenger of \( \cdot \text{OH} \), able to protect membranes from these extremely reactive species. However, its major antioxidant action in biological membranes is to act as a chain breaking antioxidant, donating labile hydrogen to peroxy and alkoxy radicals, thereby breaking the radical chain. It has been proposed that \( \alpha \)-TO' may be reduced by ascorbic acid or reduced glutathione. These are scavengers of ROS and other reactive free radicals [27]. \( \beta \)-Carotene is a powerful scavenger of \( \cdot \text{O}_2 \). Urate binds iron and copper and scavenges \( \cdot \text{OH} \), \( \cdot \text{O}_2 \) and peroxyl radicals [16].

4. ROS and activation of apoptosis

In the apoptotic process initial stress-induced damage does not kill cells directly, rather it triggers an apoptotic signalling programme that leads to cell death [28].

Apoptotic cell death is characterized by controlled autodigestion of the cell. This differs from necrosis by distinct morphological and biochemical features, such as chromatin condensation, membrane surface blebbing, oligonucleosomal DNA fragmentation and finally, the breakdown of the cell into a series of smaller units (membrane-bound fragments). These are called apoptotic bodies and in most tissues are phagocytosed by adjacent cells [29]. Such events are associated with activation of specific proteases termed caspases and loss of membrane phospholipid asymmetry resulting in phosphatidylserine externalization [30]. Apoptosis can be initiated by a variety of stimuli, including hyperthermia, growth-factor or hormone withdrawal, glucocorticoids, oxidants, ionizing radiation and multiple classes of chemotherapeutic agents [31,32]. Cell viability depends on the type of stress exerted on them. Following an apoptotic signal, cells sustain progressive lipid peroxidation. Thus, ROS and oxidative damage have been implicated in the induction of apoptosis [33–36]. The Bcl-2 proto-oncogene is unique among cellular genes for its ability in many contexts to block apoptotic deaths. Moreover, a mechanism has been proposed in which Bcl-2 regulates antioxidant pathways at sites of free radical generation [31]. The protein Bcl-2 protects against apoptosis by blocking cytochrome c release (preventing superoxide production when it is overexpressed) hence this protein may have an antioxidant function [37].

Previously reports suggest that oxygen inhibits the proliferation of human lymphocytes and fibroblasts [38]. Several lines of evidence implicate oxidative stress as a putative mediator of apoptosis. This acts by decreasing intracellular glutathione, the major buffer of the cellular redox status and/or by increasing cellular reactive species [32,39]. \( \text{H}_2\text{O}_2 \) at low doses induces apoptosis via production of \( \cdot \text{OH} \) radicals and alteration of the oxidant/antioxidant pathway [40].
Curiously, similar low doses also cause cell proliferation even in the absence of serum. However, these stimulatory effects do not appear to involve radicals as they are enhanced by inclusion of mannitol or DMSO in the medium [41]. In fact, hydrogen peroxide and superoxide appear to be important regulatory signals. This is suggested by the growth inhibitory effects of CAT and SOD. ROS may contribute a novel redox system of regulatory control superimposed upon established growth signal pathways. Levels of GSH may also be involved in these processes as CAT or SOD treatment of fibroblasts increase cellular levels of GSH [42]. In addition, α-tocopherol stimulates growth. Thus, whilst hydrogen peroxide may have a role in promoting the growth of transformed and immortalized cells oxidant protection is important [43]. On the other hand, Murrell (1992) [44] found how free radicals stimulated fibroblast proliferation and Burdon et al. (1996) [45] show that higher oxidant concentrations not only depress proliferation rates but actually lead to an increase in the appearance of apoptotic-like cells. Inhibitors of GPX, SOD and CAT have a similar effect. Therefore intracellular conditions that are considered more prooxidant than normal, appear to favour apoptosis over proliferation in fibroblasts.

5. Apoptosis, oxidative injury and pathogenesis

O$_2$ therapy, a widely used component in life-saving intensive care, can cause lung injury, although hyperoxia kills cells via necrosis, not apoptosis [46]. Nevertheless, cellular oxidant injury can occur without apoptosis and certain apoptotic mechanisms (i.e. fas-mediated) do not have requirements for ROS [47,48], apoptosis, oxidant injury and ROS are strongly related.

Formation of ROS following irradiation is thought to be a major determinant of cellular damage. Recombinant adenoviral vectors expressing the radical-scavenging enzymes Mn-SOD, Cu and Zn-SOD reduce the level of apoptosis [49]. Ferric/ferrous iron via the generation of ROS may mediate the UVB response, finally leading to tissue degradation, a hallmark in carcinogenesis, ageing and diseases [50,51]. Inorganic iron, in concert with chemical and physical inducers of the heat shock response, may trigger apoptosis. Accumulation of iron in injured tissue may thereby predispose to accelerated apoptosis and account in part for poor wound healing and organ failure [52].

Alkalosis is a clinical complication resulting from various pathological and physiological conditions. Although it is well established that reducing the cellular proton concentration is lethal, the mechanism leading to cell death is unknown. Mitochondrial respiration generates a proton gradient and superoxide radicals, suggesting a possible link between oxidative stress, mitochondrial integrity and alkaline-induced cell death [53]. Manganese superoxide dismutase removes superoxide radicals in mitochondria and thus protects mitochondria from oxidative injury. Therefore, overexpression of manganese superoxide dismutase reduced the levels of intracellular reactive oxygen species and prevents cell death [54].

Myeloid cells are a major source of superoxide and other oxygen metabolites. As a protective mechanism, cells express antioxidant enzymes such as Mn-SOD, Cu, Zn-SOD and GPX. Myeloid leukaemic lines, normal peripheral blood lymphocytes and monocytes are sensitive to cytotoxic effects of tumour necrosis factor (TNF) dramatically increased their levels of Mn-SOD RNA in the presence of TNF. In contrast Cu, Zn-SOD and GPX RNA levels do not increase in these cells. Kizaki et al., (1993) [55] reported that Mn-SOD may provide protection against cytotoxicity of TNF in hematopoietic cells. TNF-induced antiproliferative effects and caspase-3 activation, indicators of apoptosis are also completely suppressed by transfection of cells with Mn-SOD. Suppression of apoptosis induced by okadaic acid, hydrogen peroxide and taxol is inhibited by Mn-SOD but not that induced by vincristine, vinblastine or daunomycin. Activation of the p53-mediated DNA damage response induces either G1 cell cycle arrest or apoptosis. Data suggest that p21 may serve as a critical checkpoint regulator during the p53-mediated DNA damage response [56]. Overall, these results demonstrate that in ad-
dition to several recently identified signalling molecules, reactive oxygen intermediates play a critical role in activation of NFkB, activated protein-1, c-Jun kinase and apoptosis induced by TNF and other agents (Manna et al., 1998).

Gotoh and Cooper (1998) [57] suggest that TNF-induced activation of the apoptosis signal-regulating kinase 1 (ASK1) is mediated by ROS. They examine how ASK1 activity is regulated by ROS and find that ASK1 forms dimers or higher order oligomers in 293 cells. TNF or hydrogen peroxide treatment increases the dimeric form of ASK1, whilst pretreatment with N-acetylcysteine reduces it. However, synthetic dimerization of an ASK1-gyrase B fusion protein by coumermycin results in substantial activation of ASK1. This suggests that dimerization of ASK1 is sufficient for its activation. We may deduce from these results that TNF causes ASK1 activation via ROS-mediated dimerization.

The antioxidant superoxide dismutase but not catalase inhibited apoptosis induced by either oxidised low-density lipoproteins (oxLDL) or 25-hydroxycholesterol. This suggests not only that superoxide plays an important role but that a critical interaction between oxLDL and the cell takes place on the outer surface of the membrane, because superoxide dismutase is not membrane-permeable [58].

TGFβ may play an important part in the inhibition of cell proliferation and in the regulation of apoptosis. This may be induced by TGFβ preceded by reduction in p26-Bcl-2 protein levels. Therefore, TGFβ regulates Bcl-2 expression in adenoma cells undergoing apoptosis in response to TGFβ [59].

Apoptosis of neutrophils may be mediated by endogenous oxidative products. This suggestion is confirmed by observation that apoptosis of normal neutrophils is markedly inhibited by reduction of intracellular hydrogen peroxide levels. Inhibition of apoptosis in normal neutrophils by addition of catalase also occurs [60]. Activation of cell death is blocked by a variety of antioxidants. Although reactive oxygen intermediates do not act as mediators in the execution phase of CD95-mediated apoptosis, they are involved in the transcriptional regulation of CD95L expression [61]. A potential role of CD in oxidative stress-mediated cell death, ischemia/reperfusion and other diseases characterised by a disturbed redox balance has been recently reported [62]. Caspar-Bauguil et al. (1998) [63] show that activated T-lymphocytes are present in early atherosclerotic lesions where they may interact with oxLDLs. They concluded that mildly oxidized LDLs inhibit the proliferation and CD25 expression of activated T-lymphocytes. This suggests that oxLDLs may slow down the T-cell response in atherosclerotic lesions.

Programmed cell death produced by the spermidine/spermine N1-acetyltransferase-inducing polyamine analogues can be delayed by the inhibition of polyamine catabolism or the expression of the Bel-2. Natural polyamines may stimulate malignant transformation of immortalised cells [64]. These organic polycations have two opposing functions. First, the production of ROS during the catabolism of polyamines leading to programmed cell death. This shows how a decrease of intracellular spermine levels is involved in sensitization towards apoptosis induced by TNF [65]. Secondly, spermine has the ability to act directly as a free radical scavenger protecting DNA from ROS species attack [66,67].

6. Antioxidants against tumours

Antioxidant enzymes can antagonize initiation and promotion phases of carcinogenesis and they are reduced in many malignancies. The most commonly decreased enzyme is the mitochondrial Mn-SOD. This has led to suggestions that Mn-SOD might be a new type of tumour-suppressor gene. However, observations tend to ascribe the deficiency of the Mn-SOD activity to a defect in the expression of the gene rather than to its deletion. Transition metals (Mn, Fe) have been found to be highly deficient in some tumours. It is proposed that in the early stage of carcinogenesis an impairment of the signal transduction machinery might cause the defect in the Mn-SOD gene expression. Owing to a second messenger function of ROS activating transcription factors.
Combined with the ability of Mn to facilitate the dismutation of $O_2^{-}$ to $H_2O_2$ and Fe’s participation in the Fenton reaction. This may result in the limitation to binding of transcription factors like AP-1 and NFκB to the DNA as a consequence of the metal deficiency [68].

The generation of large amounts of reactive oxygen intermediates (as shown in Fig. 2) may contribute to the ability of some tumours to mutate, inhibit antiproteases and injure local tissues. Therefore promoting tumour heterogeneity, invasion and metastasis [1,69].

Several recent results suggest temporal relationships between oxidative stress, genomic instability and the development of cancer [70–73]. Free radicals may induce several DNA sequence changes: point mutations, deletions, gene amplification and rearrangements that result in the activation of several proto-oncogenes and/or the activation of some tumour suppressor genes [74].

In support of this, the steady state levels of one or more base damage products have been observed in DNA isolated from cancerous tumour biopsies of human lung, colon, kidney, breast, liver and bladder. DNA repair is also re-
sponsible for one of the most common cancers, hereditary nonpolyposis colon cancer [1].

DNA damage by ROS can cause multiple lesions, including single and double strand breaks and modified pyrimidines and purines. Repair of these lesions occurs primarily by means of base excision although nucleotide excision repair may also be involved. There are several different pathways leading from initial DNA base damage by ROS to subsequent mutation [1].

The simplest one is the chemical modification of base DNA. Additionally, ring-opened purines and a number of pyrimidine fragmentation products can block replication and may thus be mutagenic (Fig. 2). Singlet oxygen induced DNA damage is targeted selectively at guanine residues. DNA polymerase is known to be sensitive to damage-induced errors at guanines. The contribution of oxidative damage to polymerase-specific ‘hot-spots’, which is probably the major contributor to DNA polymerase mediated mutagenesis, is possibly a second mechanism. A third mechanism is linked to conformational change in the DNA template (Fig. 2). Although direct studies of the effect of base modifications on DNA conformation are just commencing, it is known that many oxidized bases are nonplanar and could change local DNA structure [1].

Observations of various types of cancer, present a possible link between decreased activities of antioxidant enzymes and increased levels of hydroxylated DNA base, due to oxidative damage. Supporting the idea that active oxygen may be increased in tumoural cells (Table 1). In fact, the levels of the antioxidant enzymes glutathione peroxidase, catalase and superoxide dismutase in lymphocytes of acute lymphoblastic leukaemia (ALL) patients are lower than in lymphocytes of controls [77]. In addition, the individual kinetic of DNA repair varies significantly between specimens derived from healthy individuals and chronic lymphatic leukaemia (CLL) patients; large differences are also found in the DNA repair half-time ($t_{1/2}$). Methoxyamine is a DNA repair modifier, which blocks the base excision repair pathway. Pretreatment of cells with this agent reveals a similar base excision repair/ independent DNA incision in almost all normal lymphocyte samples. In contrast, this portion varies to a great extent both relatively and absolutely among individual samples of CLL lymphocytes, suggesting a loss of stringent control of DNA repair processes in these cells.

The fact that a reduced activity of the selenium-dependent enzyme glutathione peroxidase in blood is associated with an increased risk and poor prognosis of cancer in humans is still controversial [85]. An association between low selenium level and advanced tumour disease exists, but it yet to be decided whether this phenomenon is more likely to be a consequence or a causative factor for development and course of the disease [86]. In neoplastic human cell lines, two bipolar factors appear to influence the activities of CAT, Mn-SOD, GPX, Cu and Zn-SOD. Potentially low superoxide production and intrinsically low peroxidizability of tumour cell membranes underlie the peculiar variation of antioxidant enzyme activities in tumour cells [87].

Macrophages have two mechanisms for destroying cancer cells: one mediated by proteolytic activity and a second that depends on the generation of oxygen-derived free radicals [88]. Digitonin stimulates activated macrophages to produce superoxide, hydrogen peroxide and possibly other free radicals that can increase macrophage-induced tumour cell cytotoxicity [89].

Results hint that H$_2$O$_2$ may act as a physiological mediator of intracellular response or as a
second messenger in mammalian cells. In fact, H2O2 has been implicated as an indirect activator of NFκB [8,90]. Over expression of manganese superoxide dismutase in human breast cancer MCF-7 cells completely abolishes TNF-mediated NFκB activation, IkB degradation, p65 nuclear translocation and NFκB-dependent reporter gene expression. Besides TNF, phorbol ester-, okadaic acid-, ceramide- and LPS-induced activation of NFκB is blocked by Mn-SOD, indicating a common pathway of activation. Inhibition of both NFκB binding activity and oxidative DNA damage hint that its antioxidant potential outweighs its oxidative capacity in a cellular environment. This may contribute to anticarcinogenic effects [10]. Additionally, Mn-SOD blocks the TNF mediated activation of activated protein-1, stress-activated c-Jun protein kinase and mitogen-activated protein kinase [91].

Expression of the genes encoding antioxidant enzymes Mn-SOD and GPX are increased in the lungs of influenza virus infected animals, whereas Cu, Zn-SOD and CAT mRNA are not induced by viral infection. Activation of the transcriptional regulatory proteins AP-1, C/EBP and NFκB (which are known to be affected by oxidant stress) is demonstrated by electrophoretic mobility shift assay after viral infection [92].

Described above, oxidants can trigger the activation of multiple signalling pathways that influence the cytotoxicity observed in affected cells. This includes phosphorylation cascades, leading to the activation of MAPKs, NFκB [93] and a multiprotein complex that regulates a variety of genes important for immunity, inflammation and cancer. Activation of these genes, induced by silica, lipopolysaccharide (LPS) and PMA, is blocked by catalase [94].

Oxidative processes in tumour cells may have a strong influence on the host response against tumours. Thus, in H-2kb-transformed tumour cells reduction of superoxide is associated with a significant increase in the level of Cu, Zn-SOD and GPX and a reduction in the DNA-binding form of NFκB [95].

Mn-SOD is reduced in a variety of tumour cells and has been proposed to be a new type of tumour suppressor gene. The mechanism(s) by which Mn-SOD suppresses cancer development is currently unknown. However, expression of this antioxidant might play a significant role in maintaining cellular redox status. The relationship between Mn-SOD expression, modulation of DNA-binding activity, transcriptional activation of redox-sensitive oncoproteins and tumour suppressor proteins has been recently studied [96]. Electrophoretic mobility shift assay and transcriptional activation studies revealed an inverse correlation between Mn-SOD expression and activity of c-Jun-associated transcription factors, activator protein 1 and c-AMP-responsive element binding protein. Expression of Bcl-xL (an activator protein 1 target gene and antiapoptotic member of the Bcl-2 family) is decreased in Mn-SOD-transfected cell lines. Thus, over expression of Mn-SOD may exert its tumour suppressor activity in part by modulation of specific oncogenes [97].

7. Directions for future research

Critical steps in the signal transduction cascade are sensitive to oxidants and antioxidants. At least two well-defined transcription factors (NFκB and AP-1) have been identified to be regulated by the intracellular redox state. Binding sites of these redox-regulated transcription factors are located in the promoter region of a large variety of genes that are directly involved in the pathogenesis of cancer and other diseases. Biochemical and clinical studies indicate that antioxidant therapy may be useful in the treatment of several diseases [98]. Moreover, a number of structurally different anticarcinogenic agents inhibit ROS production and oxidative DNA damage as they inhibit inflammation and tumour promotion [99]. The above statements underline the importance of ROS and oxidative genetic damage to the carcinogenic process. Also pointing to the possibility that some types of cancer may be preventable if the cycles of tumour promotion can be interrupted [100]. The mechanism through which ROS plays an important role in the initiation and progression of cancer and its ability to induce apoptosis is not yet fully under-
stood. Therefore, further efforts are also necessary to fully elucidate the importance of free radical scavengers in the therapy of several diseases. Therefore we consider it important to continue investigation on the biochemical roles of these antioxidant enzymes which clearly related to among other cellular processes both apoptosis and pathogenesis of cancer.

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