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REVIEW ARTICLE

Oxidative stress and cancer: have we moved forward?

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'Reactive species' (RS) of various types are formed *in vivo* and many are powerful oxidizing agents, capable of damaging DNA and other biomolecules. Increased formation of RS can promote the development of malignancy, and the 'normal' rates of RS generation may account for the increased risk of cancer development in the aged. Indeed, knockout of various antioxidant defence enzymes raises oxidative damage levels and promotes age-related cancer development in animals. In explaining this, most attention has been paid to direct oxidative damage to DNA by certain RS, such as hydroxyl radical ($OH^{\bullet}$). However, increased levels of DNA base oxidation products such as 8OHdg (8-hydroxy-2′-deoxyguanosine) do not always lead to malignancy, although malignant tumours often show increased levels of DNA base oxidation. Hence additional actions of RS must be important, possibly their effects on p53, cell proliferation, invasiveness and metastasis. Chronic inflammation predisposes to malignancy, but the role of RS in this is likely to be complex because RS can sometimes act as anti-inflammatory agents.

Key words: antioxidant, cancer, DNA repair, nitric oxide, reactive oxygen species.

INTRODUCTION

In 1996, I co-authored in the *Biochemical Journal* an article speculating on the role of oxygen radicals and other 'reactive species' (RS; defined in the legend to Figure 1) in cancer [1]. Some 10 years later, have we established their role? Yes, the currently available data show them to be important players. But how do they act? We have greatly expanded our knowledge of the molecular mechanisms by which RS can modulate cellular processes relevant to cancer (reviewed in [2]), but have not really ascertained which are their most important actions. In this review, I will first present the evidence that RS are important in cancer, ask what RS are capable of doing, consider the evidence that some or all of these actions occur *in vivo*, and finally attempt to assess the importance of these events to the origin and progression of cancer.

EVIDENCE THAT REACTIVE SPECIES ARE IMPORTANT IN CANCER

It was shown as early as 1984 that exposure of mouse fibroblasts to ROS (reactive oxygen species) can lead to transformation [3,4]. However, just the right level of exposure is needed; too many RS can injure or kill cells (Figure 1). Indeed, ROS have a huge range of potential actions on cells (discussed further below; see also Figure 1), and one could easily envisage them as anti-cancer (e.g. by promoting cell-cycle stasis, senescence, apoptosis, necrosis or other types of cell death, and inhibiting angiogenesis) or as pro-cancer (promoting proliferation, invasiveness, angiogenesis, metastasis, and suppressing apoptosis). The same can be said about other types of RS, such as RNS (reactive nitrogen species) (Figure 1). For example, NO (nitric oxide) and other RNS can promote DNA damage and cause mutations, and inhibit caspases, delaying apoptosis. On the other hand, they can inhibit cytochrome oxidase and slow mitochondrial ATP formation, impairing cell proliferation and slowing tumour growth. Indeed, that might be the host's 'purpose' in $NO^{\bullet}$ production by iNOS (inducible nitric oxide synthase) in macrophages that sometimes gather around tumours [9,10].

So which predominates in cancer – good or bad effects of RS? One way to approach this question is to raise RS levels by decreasing antioxidant defences, and see what happens. The results are clear-cut. Knockout mice lacking CuZnSOD (copper- and zinc-containing superoxide dismutase), a major cellular scavenger of superoxide radicals ($O_2^{\bullet-}$), have increased rates of liver cancer development later in life [11]. Although CuZnSOD is present in most parts of the cell [2], the most important scavenger of $O_2^{\bullet-}$ in the mitochondrial matrix is MnSOD (manganese-containing superoxide dismutase) [12,13]. Absence of this enzyme in mice is lethal soon after birth, indicating the potential of excess mitochondrial $O_2^{\bullet-}$ to cause severe damage [12,13]. Heterozygous animals (50% of normal mitochondrial MnSOD) survive, but show increased risk of developing lymphomas, adenocarcinomas and pituitary adenomas as they age [14]. MnSOD and CuZnSOD catalyse the reaction:

$$2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$$

The H_2O_2 generated by SOD enzymes in mitochondria and cytosol is probably largely removed by peroxiredoxins, which are thioredoxin-dependent peroxidase enzymes, although GPxs (glutathione peroxidases) also contribute [2,5]. There are four GPx enzymes in animals; simultaneous knockout of two of them (GPx1 and GPx2) in mice led to intestinal cancers [15], whereas elimination of some of the peroxiredoxins promotes development of lymphomas, sarcomas and adenomas [16]. Embryonic fibroblasts from mice lacking peroxiredoxin-1 showed elevated ROS production, evidence of elevated c-*myc* expression, and increased susceptibility to transformation by *ras* overexpression [17]. Mice lacking catalase sometimes show increased incidence of 'spontaneous' mammary tumours [18].

The data seem clear: elevated RS can lead to more cancer. But how?

Abbreviations used: ATM, ataxia telangiectasia mutated; CuZnSOD, copper- and zinc-containing superoxide dismutase; DMEM, Dulbecco's modified Eagle's medium; GPx, glutathione peroxidase; 4-HNE, 4-hydroxynonenal; iNOS, inducible nitric oxide synthase; MnSOD, manganese-containing superoxide dismutase; MMP, matrix metalloproteinase; NF-κB, nuclear factor κB; 8-NG, 8-nitroguanine; NOX, NADPH oxidase; 8OHdG, 8-hydroxy-2′-deoxyguanosine; RS, reactive species; RNS, reactive nitrogen species; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor.

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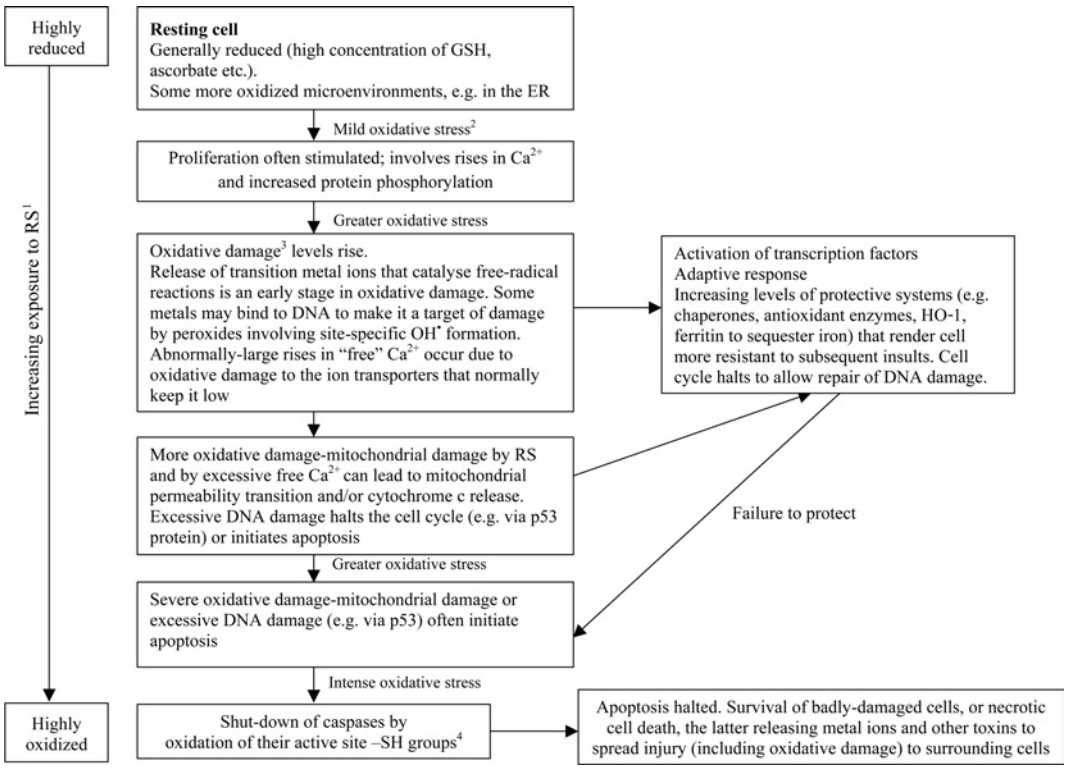

Figure 1 How cells respond to increasing exposure to reactive species

Adapted from Figure 4.1, p. 189, in How cells respond to oxidative stress from 'Free Radicals in Biology and Medicine' by Halliwell, B. and Gutteridge, J. M. C. (2006) by permission of Oxford University Press (www.oup.com). Stimulation of proliferation by low levels of RS is associated with increased net phosphorylation of multiple proteins, often because RS inactivate protein phosphatase enzymes [5] and sometimes because of increased protein kinase activity [6]. The cell is generally a reducing environment, especially the mitochondria (GSH/GSSG > 100) and cytosol (GSH/GSSG > 100), but less so in the endoplasmic reticulum lumen (GSH/GSSG ≈ 3), since a more oxidizing environment is required for optimal protein folding and disulfide bridge formation. ER, endoplasmic reticulum; HO-1, haem oxygenase 1. The following notes relate to the numbers in the Figure. [1]'Reactive species' is a collective term that includes reactive oxygen, nitrogen, halogen and sulfur species. For example, the term 'reactive oxygen species' (ROS) includes both oxygen-centred radicals (e.g. hydroxyl, superoxide, peroxyl) and certain non-radicals that are oxidizing agents and/or readily converted into radicals (e.g. peroxynitrite, hypochlorous acid). Similarly, reactive nitrogen species (RNS) includes the radicals nitric oxide, and nitrogen dioxide as well as non-radicals such as nitryl chloride and nitroxyl anion. Many (but not all) RS are powerful oxidizing agents, capable of damaging DNA and other biomolecules. For a full description of RS and discussion of terminology, please see [2]. [2]Oxidative stress is a serious imbalance between RS levels and antioxidants, leading to potential damage. [3]Oxidative damage is the biomolecular damage caused by direct attack of RS. Its cellular levels are controlled by the balance between rate of damage and rate of repair or replacement of damaged biomolecules. Please see [2] for further explanation. [4]Caspase activity can also be modulated by changes in intracellular pH caused by RS [7,8].

DIRECT DAMAGE TO DNA BY REACTIVE SPECIES

This is the most commonly offered explanation. Indeed, exposure to ionizing radiation has long been known to favour cancer development. Radiation-induced carcinogenesis appears to involve initiation, promotion, activation of proto-oncogenes and inactivation of stability- and tumour-suppressor genes. Some genetic damage by radiation occurs by direct absorption of energy by DNA, but much is mediated by the formation of the highly reactive hydroxyl radical ($OH^{\bullet}$) [19,20]. Thus $OH^{\bullet}$ could be involved in all stages of radiation-induced carcinogenesis. Hydroxyl radical attack upon DNA generates multiple mutagenic purine, pyrimidine and deoxyribose oxidation products [1,2,19–22]. One of the most studied is 8OHdG (8-hydroxy-2′-deoxyguanosine).

The incidence of most cancers rises with the fourth or fifth power of age in animals, e.g. approx. 35 % of humans have cancer by age 85. It has been hypothesized that this is largely due to a lifetime of attack by RS such as $OH^{\bullet}$ [23,24], generated endogenously and sometimes in addition by certain exogenous carcinogens, such as cigarette smoke [25]. Are endogenous RS production and oxidative DNA damage rates large enough to account for the age-related development of 'spontaneous' cancers? The steady-state levels of DNA base oxidation products in human cells vary according to the measurement method and between laboratories (reviewed in [20,22,24,26]). The average value for all lesions may be approx. 1 per 10^5 DNA bases or more. Is this enough to cause cancer? By comparison with the levels of adducts of known carcinogens that are detected in carcinogen-exposed animals, it could be (for a detailed analysis of the numbers, see [26]). Of course, one can always debate the relative mutagenicity of xenobiotic carcinogen adducts compared with that of RS-derived DNA lesions. However, many of the latter are significantly mutagenic, they are formed in our DNA since conception, and the levels present could have even more impact if damage is located in specific genes that are relevant to cancer, as has been suggested in some studies. For example, oxidative DNA damage in human gastric mucosa infected with *Helicobacter pylori* was unevenly distributed between genes, being especially elevated in the *p53* gene, for example [27]. Reactive chlorine, bromine, sulfur and nitrogen species can also attack DNA [2,9]. Some RNS (e.g. N_2O_3) deaminate DNA bases to mutagenic lesions, and peroxynitrite ($ONOO^-$, a product of reaction of $O_2^{\bullet -}$ with $NO^{\bullet}$), forms 8-NG (8-nitroguanine) (plus several other products) in DNA. 8-NG rapidly detaches from the DNA, leaving potentially mutagenic apurinic sites [9,28]. RNS have been suggested to be especially important in causing DNA damage (measured as 8-NG formation) in chronic inflammation

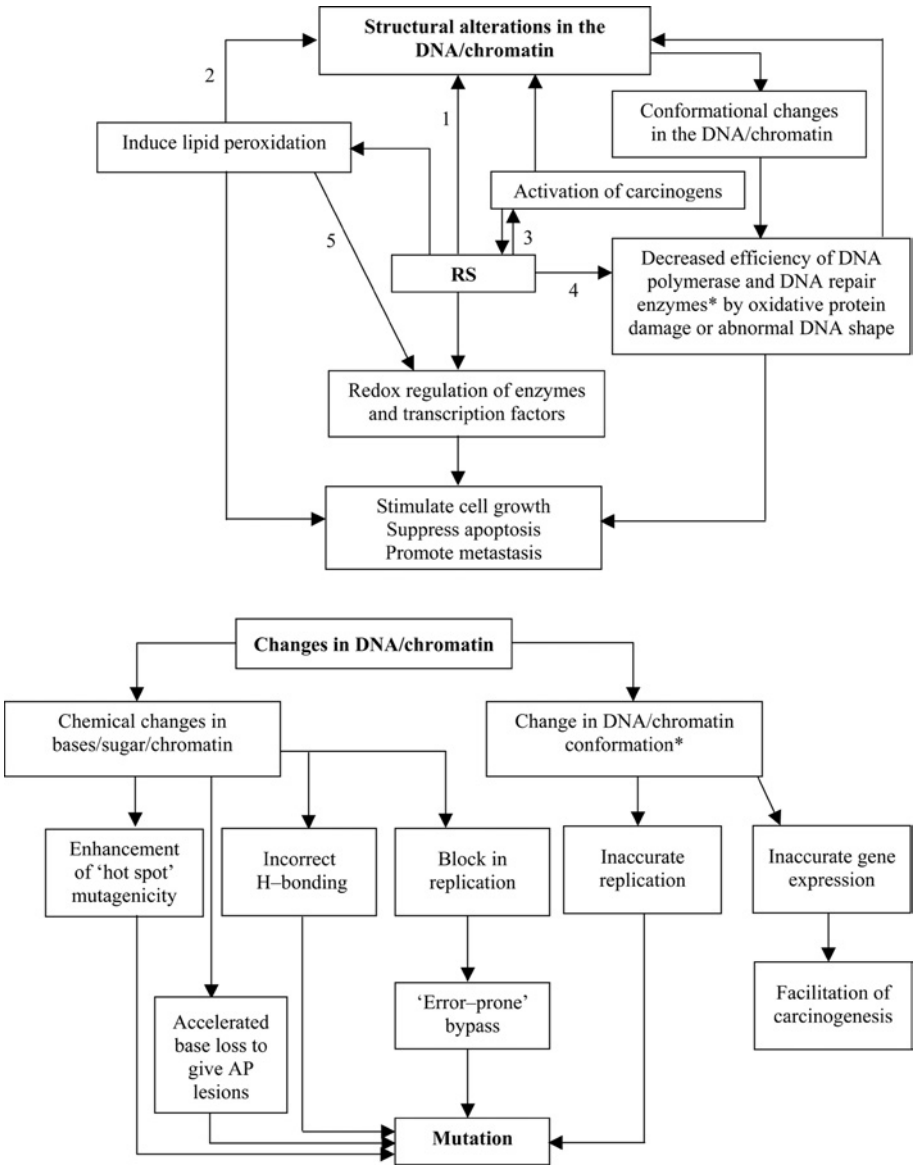

Figure 2 Some of the ways in which reactive species could facilitate cancer development

Upper panel: 1. Direct oxidative damage to DNA. 2. Aldehyde end-products of lipid peroxidation (e.g. malondialdehyde and 4-HNE) form mutagenic adducts with DNA bases. Radicals ($RO^{\bullet}$ and $RO_2^{\bullet}$) formed during lipid peroxidation might also attack DNA. 3. RS can help to convert pro-carcinogens into ultimate carcinogens, some of which then lead to more RS formation. 4. RS can damage proteins, e.g. chromatin proteins, DNA-repair enzymes and DNA polymerases (perhaps increasing the error rate of replication). 5. 4-HNE can be pro-proliferative and is a powerful redox modulator [29] and some other products of lipid peroxidation may act similarly [2]. Lower panel: how RS-induced changes in DNA can cause mutations. *Another possible mechanism (not shown) is for RS to affect gene methylation, e.g. oxidative DNA damage might modulate methylation, which could on the one hand cause inappropriate gene expression, and on the other silence genes encoding anti-tumour and antioxidant defences [30–33].

induced by the *Opisthorchis* (liver fluke) parasites and in the stomachs of humans infected with *H. pylori* [9,28]. Reactive chlorine species can chlorinate cytosine and adenine (reviewed in [2]). Attack of RS on proteins and lipids might also aggravate DNA damage (Figure 2).

DNA need not always be attacked directly to raise levels of mutagenic oxidation products. Defects in some of the enzymes that repair oxidative DNA damage lead to increased levels of 8OHdG and other mutagenic bases in cellular DNA and are associated with increased age-dependent cancer incidence in animals [34–36]. This evidence is less compelling, in general, than that provided by knockout of antioxidant defence enzymes, because many of the enzymes involved in base and nucleotide excision repair act on several lesions in DNA, not just oxidative damage, and so it is possible that non-oxidative mutagenic lesions accumulate as well. However, some data are clear-cut. Just as DNA can be attacked by RS, so can its precursors. Human and other animal cells contain an enzyme (MTH1) that hydrolyses 8OHdGTP, 8- and 2-OHdATPs and 8-chlorodGTP, preventing their incorporation into DNA. Mice lacking this enzyme show an increased rate of spontaneous tumorigenesis with age, especially in the lung, stomach and liver [34,35].

However, it is unlikely that the pro-cancer effects of RS are all due to elevated direct oxidative damage to DNA bases (Figures 1 and 2), simply because there are situations where 8OHdG levels can be elevated, but cancer rates do not increase (reviewed in

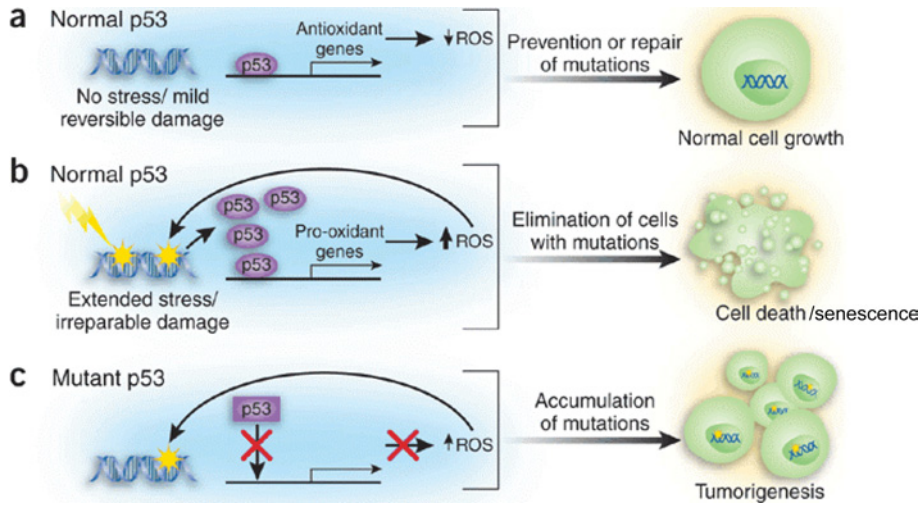

Figure 3 The p53 protein has both pro- and anti-oxidant functions

(**a**) In the absence of stress or after mild stress, low levels of p53 drive the expression of several genes which encode antioxidant proteins that decrease RS levels. p53 can also facilitate DNA repair. (**b**) Greater activation of p53 after severe or extended stress leads to RS formation by several mechanisms, resulting in elevated RS levels that might contribute to cell senescence or death. Both of these mechanisms might contribute to tumour suppression. (**c**) Loss of p53 function later in malignancy increases intracellular levels of RS, which damage DNA and might increase the mutation rate. The inability to activate p53-induced apoptosis in response to these oncogenic alterations ultimately results in the development of cancer. Reprinted by permission from Macmillan Publishers Ltd: *Nature Medicine* [40], copyright 2005 (http://www.nature.com/nm), by courtesy of Dr Karim Bensaad and Dr Karen Vousden.

[26,34], but also see [37]). Thus oxidative DNA damage may be necessary, but not sufficient, for cancer development, and RS must then act by additional mechanisms. Let us examine what these might be.

REACTIVE SPECIES AND CELL PROLIFERATION

As well as causing direct oxidative damage, RS affect cell behaviour in many ways. Depending on the type and level of RS present, time of exposure, the quantity of cellular antioxidant defences that remove RS and the activities of cellular repair systems that clear oxidative damage, cells exposed to RS can show increased proliferation, halted cell cycle, senescence, apoptosis or necrosis (Figure 1). Participation of RS in the initiation, promotion and progression of cancer could thus involve their effects on the cell cycle, gene expression, direct or indirect damage to DNA, and apoptosis and other forms of cell death (Figure 1). Angiogenesis, carcinogen metabolism and metastasis may also be affected by RS, as will be discussed below. The levels of RS at which cells 'switch' from proliferation to halted cell cycle, or undergo any of the other events, are cell-type-specific, e.g. fibroblasts proliferate rapidly at RS levels that impair chondrocyte proliferation [2]. For example, transfection of NIH-3T3 fibroblasts in culture with a *ras* oncogene led to production of excess superoxide radical ($O_2^{\bullet -}$) by the action of an NOX (NADPH oxidase) enzyme complex, and this $O_2^{\bullet -}$ promoted abnormal proliferation [38]. In some other cells, the same experiment (NOX expression in response to *ras* and formation of more $O_2^{\bullet -}$) can result in senescence [39]. Communication via gap junctions is generally decreased in tumour cells, which may facilitate their proliferation, and excess RS can aggravate this by decreasing gap-junctional communication [30].

p53 and reactive species

The actions of p53 are intimately linked with cancer and seem similarly intimately linked [40] with RS (Figure 3). First, the normal low levels of active p53 are involved in maintaining the cellular antioxidant defence network, promoting the transcription of genes encoding MnSOD, GPx1 and proteins that regenerate oxidized peroxiredoxins, among others [40].

Secondly, RS can increase p53 activity. Thus the expression of *ras* oncogenes can accelerate $O_2^{\bullet -}$ production, which under some circumstances can increase p53 activity and trigger senescence (although the $O_2^{\bullet -}$ can be pro-proliferative instead if p53 function is impaired) [40]. Thirdly, higher p53 activity can cause RS production by several mechanisms, and these RS might sometimes contribute to the cytostatic and pro-apoptotic effect of p53 (Figure 3). Thus the anti-proliferative effects of RS can involve activation of p53, not only indirectly (by producing DNA damage), but also by promoting p53 phosphorylation [10]. One protein involved in the actions of p53 in increasing RS production is $p66^{Shc}$, a protein that interacts with the mitochondrial electron-transport chain to promote ROS generation [41]. Indeed, mice lacking $p66^{Shc}$ live longer and show decreased levels of oxidative damage [41]. Other mechanisms include p53-dependent increases in the levels of proline oxidase, a mitochondrial enzyme that produces H_2O_2 as it converts proline into pyrroline 5-carboxylate [42]. Fourthly, p53 seems to promote mitochondrial respiration, by maintaining cytochrome oxidase activity (at least in cell lines) [43], and mitochondria are a major source of ROS [2]. Indeed, loss of p53 may account for the accelerated rates of glycolysis in some malignant cells [43].

However, if too many oxidizing RS are generated, they can inhibit p53 action. This protein has ten cysteine residues, some of which are involved in binding a zinc ion required for activity. Oxidation of cysteine inhibits the action of p53 as a transcription factor. For example, p53 can be oxidized and nitrated by $ONOO^-$, and nitrated p53 has been detected in human glioblastoma, a highly malignant brain cancer [44]. Oxidized cysteine residues on p53 can be re-reduced by Ref-1 and the thioredoxin system [5,40]. Oxidative inactivation of p53 will in turn raise cellular ROS levels (Figure 2). Thus the relationship of p53 with RS is complex and intimate, and it is hard to predict which way it will turn in a particular tumour.

RS are involved in the actions of at least some of the other proteins involved in cell-cycle regulation. One is the ATM (ataxia telangiectasia mutated) protein. Patients lacking functional ATM,

as well as *ATM*-knockout mice, show higher levels of oxidative damage, and some of the pathological defects in the mice can be diminished by antioxidant administration [45–48]. One of these effects, bone marrow failure, illustrates one consequence of too many RS; they induce excessive proliferation of haemopoietic stem cells, leading to eventual exhaustion of the stem cell pool [47].

REGULATING APOPTOSIS BY REACTIVE SPECIES

Moderate levels of RS (and what is 'moderate' depends on the cell type) tend to promote apoptosis (Figure 1), or cell death that has at least some of the features of apoptosis, in most 'normal' cells [49,50]. However, in some malignant cells, RS can have the opposite effect. For example, in the melanoma cell line M14, decreasing $O_2^{\bullet -}$ levels by overexpressing CuZnSOD promoted apoptosis, and decreasing CuZnSOD levels inhibited apoptosis [7]. Generation of ROS by an NOX enzyme system was found to be an anti-apoptotic, pro-proliferative stimulus in pancreatic cancer cell lines [51], and the ability of mitochondrial AIF (apoptosis-inducing factor) to oxidize NADH and make $O_2^{\bullet -}$ contributes to the viability of certain carcinoma cell lines [52].

How are these 'pro-survival' effects achieved? Oxidative inactivation of caspases might sometimes occur (Figure 1). In some cells, elevated $O_2^{\bullet -}$ leads to a higher cytosolic pH that deters caspase activation [8]. In contrast, H_2O_2 can promote apoptosis not only by attacking DNA (usually by its conversion into DNA-damaging $OH^{\bullet}$ by reacting with transition metal ions [2]) and by damaging mitochondria (Figure 1), but also by lowering the cytosolic pH. The actions of $O_2^{\bullet -}$ and H_2O_2 on cellular pH seem to involve increasing or decreasing respectively the activities of a plasma membrane Na^+/H^+ exchange system [8]. Another mechanism is for RS to inactivate PTEN (phosphatase and tensin homologue deleted on chromosome 10), leading to increased activation of the Akt pathway, which tends to promote cell survival [53]. However, the relationship of Akt signalling to the whole carcinogenic process is complex, e.g. some studies indicate that activation of this pathway could suppress invasion and metastasis [53].

A caution on cell-culture studies

Before going further, I must raise an important caveat. One must be very careful in extrapolating from experiments on malignant (or other) cells in culture to the *in vivo* state. The cell-culture process increases cellular ROS formation. Intracellular O_2 levels are low for most cells in the human body, usually <10 mmHg (1 mmHg $= 0.133$ kPa), which diminishes RS formation [2]. Yet most animal cells are cultured under 95 % air and 5 % CO_2. This is grossly hyperoxic (about 159 mmHg) and increases ROS production by mitochondria and cellular oxidase enzymes [54]. To make it worse, cell-culture media are often deficient in antioxidants (e.g. vitamins C and E) and antioxidant precursors (e.g. selenium) and contain 'free' iron ions, present as contaminants [2] or even added deliberately [e.g. iron(III) salts are added to DMEM (Dulbecco's modified Eagle's medium)]. These iron salts can catalyse RS formation in the medium. Indeed, the mild oxidative stress of cell culture may explain why many cell types proliferate so well in culture (Figure 1) [54–56]. Given the speed of 'natural selection' in rapidly dividing cultured cells, it is possible that, if such cells are grown in a pro-oxidant environment over several generations, they may evolve to use ROS for signalling pathways that promote proliferation and suppress cell death by mechanisms that may not normally operate *in vivo* [54,57]. The pro-oxidancy of cell culture has especially confused studies of cellular 'senescence' after repeated cell division in culture. Indeed, in mouse embryonic fibroblasts in culture, oxidative stress seemed to be more important than telomere shortening in causing senescence. Culture under hypoxic conditions preserves the replicative potency of many cells, i.e. the 'Hayflick limit' is in part an artefact of culture conditions [58–60]. Culture under ambient air may also confuse studies of sensitivity to anti-cancer agents. For example, when Ehrlich ascites cells taken from mouse peritoneum were transferred to cell culture under 21 % O_2, their sensitivities to such drugs altered radically within a few days, an effect prevented by culture under 4 % O_2 [61].

Many papers have appeared claiming that ascorbate and polyphenolic compounds such as flavonoids are toxic to malignant cells in culture and may have therapeutic potential. At least some of these claims are artefacts; the added compounds undergo *in vitro* oxidation in the culture medium (especially in DMEM) to produce H_2O_2 and other oxidation products that are the true toxic agents [54,62–64].

METASTASIS AND REACTIVE SPECIES

Are the cellular effects of RS, mostly demonstrated in laboratory-grown cells, really relevant to cancer? We do not really know, but some studies suggest that they are, in the content of invasiveness and metastasis [65–71]. One mechanism of RS action in this context is by modulating integrin expression; another is by suppressing anoikis, the apoptosis that normally results when cells separate from their normal environment. Facilitation of metastasis may also involve changes in cell-surface morphology, intercellular communication, cell mobility and increased vascular permeability. In addition, degradation of extracellular matrix involves MMPs (matrix metalloproteinases), some of which are secreted in latent forms (by both tumour cells and phagocytes, especially macrophages [72]) that can be activated by RS [2]. MMP enzymes can also liberate growth factors bound to matrix constituents. For example, levels of stromelysin-1 (MMP3) are increased in many breast tumours. The possibility of a 'vicious cycle' of events is suggested by the observation that exposing mouse mammary epithelial cells to this enzyme increased their ROS production, causing oxidative DNA damage and genomic instability [70]. Indeed, human metastatic prostate cancer cells seemed to be producing more RS than primary malignant cells [69], as is evident from higher levels of 8OHdG, 4-HNE (4-hydroxynonenal; an end-product of lipid peroxidation [29]), and 3-nitrotyrosine (a biomarker of attack on proteins by RNS [73]). H_2O_2 can stimulate not only the proliferation but also the migration of human prostate cancer cells, in part by modulating their expression of heparin affin regulatory peptide [74].

But are these effects important *in vivo*? Possibly; several studies indicate that experimentally increasing the levels of peroxide-metabolizing enzymes in or around malignant cells injected into animals decreases their metastatic potential [75–78]. The level of lysyl oxidase, an H_2O_2-producing enzyme, was increased in distant metastatic human breast cancer tissues as compared with primary cancer tissues, and levels were in both cases greater than in normal breast tissue [77]. In mice, weakly tumorigenic and non-metastatic cells became malignant and invasive after co-implantation with a gelatine sponge to induce inflammation, an effect abolished by instilling a SOD preparation [78]. The pro-metastatic effect was also diminished if the mice lacked NOX activity, also consistent with a role for RS *in vivo* [71].

Another possibility is that ROS could sometimes promote angiogenesis, e.g. by increasing cellular production of VEGF

(vascular endothelial growth factor) [66,79,80]. In addition, the actions of VEGF (and angiopoietin-1) on endothelial cells may themselves involve increased $O_2^{\bullet-}$ generation by a NOX [79,81,82]. In bladder cancer, elevated thymidine phosphorylase activity has been shown to correlate with increased angiogenesis and poor prognosis, an effect that may involve ROS-mediated effects on VEGF production [83]. There is the usual biphasic effect of RS, however: too much oxidative damage can impair angiogenesis [84]. Thus we do not know as yet the significance of RS in modulating angiogenesis *in vivo*.

ARE MALIGNANT CELLS IN A PRO-OXIDANT STATE?

It has been argued that some malignant cells use ROS to suppress apoptosis, accelerate proliferation, metastasis and angiogenesis, and possibly to promote genetic instability, e.g. by increased oxidative DNA damage [2,7,52,66–71,74,85]. Many malignant cells produce high levels of ROS in culture [86]. But is this a cell culture artefact or do malignant cells really make more ROS *in vivo*? Several experiments reveal that many of them do (reviewed in [20,28,87]). For example, chronic lymphocytic leukaemia cells freshly taken from patients showed increased ROS production compared with normal lymphocytes [88], as did B-cell lines from patients with Burkitt's lymphoma associated with Epstein–Barr virus infection [89] and malignant B-cells from patients with hairy cell leukaemia [90]. For solid tumours, however, demonstrating increased RS production *in vivo* is difficult to achieve owing to methodological inadequacies [2,91], so most investigators have examined oxidative damage levels rather than ROS production. Many such studies have shown increased levels of 8OHdG in human and other animal tumours [2,20,21,92–94]. This is seen both in 'spontaneous' cancers and in those induced by a range of carcinogens, despite the fact that the primary DNA-damaging mechanisms of many carcinogens do not appear to involve RS [2,20]. There are exceptions of course, such as the direct involvement of ROS in the carinogenicity of arsenic and of several metals [2,95,96]. In the few cases where it has been examined, elevation in the levels not only of 8OHdG but also of multiple other oxidative DNA base-damage products has been shown; such a pattern is diagnostic of DNA damage by $OH^{\bullet}$ [2,20].

Increased oxidative damage levels in malignant cells could result from: (i) more RS formation with unaltered antioxidant defences; (ii) unaltered RS formation with decreases in antioxidant defences; (iii) failure to repair oxidative damage, so that levels rise; or (iv) any combination of the above. Which is responsible?

Increased formation of reactive species?

Data suggest that at least some malignant tumours *in vivo* do generate more ROS than normal cells [65,71,77,85,87]. Sources of ROS include lysyl oxidase [77], proline oxidase [39], increased mitochondrial $O_2^{\bullet-}$ formation and $O_2^{\bullet-}$-generating NOX systems [90]. For example, some human prostate cancers contain mutations in mitochondrial DNA that may increase mitochondrial ROS production [97]. A caveat: do not assume that the increased RS production is an early event, e.g. in primary leukaemia cells, the mitochondrial DNA mutations seemed more associated with damage due to chemotherapy, an effect again perhaps due to increased ROS generation [98]. NOX systems are homologues of the phagocyte $O_2^{\bullet-}$-generating components, and are found in normal as well as malignant tissues. For example, NOX1 is normally present in colon, prostate, uterus and vascular smooth muscle, and appears to be constitutively active [65]. Excitement rose when overexpression of NOX1 in NIH-3T3 cells or prostate cancer cell lines was found to increase $O_2^{\bullet-}$ formation, followed by more cell proliferation and the development of some features of malignancy [99]. But is NOX1 more active in human colon cancer? It is not clear as yet; reports are variable (some even say that levels decrease) and its level may depend on the differentiation state of the tumour [39,65,100,101]. In contrast, NOX1 levels are elevated in human prostate cancers, hairy cell leukaemia and some skin cancers [69,90]. Indeed, several authors have suggested that RS may play an especially important role in the origin and progression of prostate cancer [102,103], although this remains a suggestion. Another caveat; NOX activities are usually carefully regulated by cytosolic proteins [39], so more protein does not necessarily equate to more $O_2^{\bullet-}$ production.

Altered DNA-repair rates?

Defects in DNA repair, and possibly polymorphisms in DNA-repair enzymes, contribute to the origins of some cancers [104]. However, for several cancers, some studies report increased activities of DNA-repair enzymes that can act on oxidative DNA damage, whereas others report the opposite [34,35,104,105]. DNA repair may sometimes be down-regulated in the later stages of malignancy, perhaps promoting genetic instability and tumour progression [104,106,107]. Indeed, variability in rates of DNA repair is an important determinant of the success of chemotherapy [104]. But be wary of drawing conclusions based on gene expression or protein levels for repair enzymes, since mRNA or protein do not always equate to activity, e.g. some repair enzymes can be inactivated by RS [2,21]. DNA-repair systems are complex and overlapping, so changes in a single enzyme may have little overall effect. Thus, in general terms, malignant cells do seem able to repair DNA, but it is hard to determine what contributions alterations in repair systems make to increased oxidative DNA-damage levels. Overall, the ability to repair oxidative DNA damage in many human cancers does not seem to be markedly impaired, and may sometimes be increased.

Changes in antioxidant defences?

Tumour cells in culture often show low MnSOD activity. Transfection of a MnSOD gene can suppress the malignant phenotype in some (but not all) of these cell lines [108,109]. Some authors suggest that elevated MnSOD generates more H_2O_2, which causes inhibition of proliferation, sometimes via p53 activation. Consistent with this, overexpression of catalase can often prevent the effects of excess MnSOD in cell lines [109]. Low activities of CuZnSOD, catalase and GPx1 are also often reported in transformed cell lines [109–111].

Again, one must be careful when extrapolating from cultured cells to those *in vivo*. Thus, if cells in culture 'select for' high levels of RS to help them proliferate, there might be a powerful selection pressure to decrease antioxidant levels (up to a point, that is; that point being when oxidative damage rises to a level that impairs cell function). What do direct measurements of antioxidants in malignant human tissues show? Results are variable [2,31,94,109,111–114], but, in general, there is no clear pattern of major decreases in MnSOD or other antioxidant enzymes in freshly obtained human cancerous tissue. Indeed, levels of MnSOD are sometimes (e.g. in mesothelioma, neuroblastoma, melanoma, stomach, ovarian and breast cancer) elevated [94,111–114]. Some investigators propose that high MnSOD activity is associated with increased tumour invasion, metastasis and a poor prognosis, possibly because a cellular redox imbalance induced by MnSOD overexpression leads to increased activities of MMPs [111]. The levels of peroxiredoxins may also be increased in some

(but not all [33]) malignant cells [111–114], but these enzymes are easily inactivated by ROS [5], so again remember that protein level or gene expression do not necessarily equate to activity. Probably what is important *in vivo* is not the level of any one antioxidant enzyme, but the balance of RS production, antioxidant defence (including the ratio of SOD to H_2O_2-consuming enzymes) and the repair systems in malignant cells.

Overall, the evidence supports the view that at least some malignant cells make more RS *in vivo*. Antioxidant defence and repair activities may sometimes rise, but not enough to cope with the extra RS. It is thus possible that malignant cells produce more RS for their own benefit, but must be careful not to exceed the level where oxidative damage becomes so severe that cell function is impaired (Figure 1).

AN ANTIOXIDANT PARADOX?

If RS are important in causing cancer, it appears paradoxical that consumption of antioxidant supplements does not decrease cancer incidence in humans, unless one starts with a nutrient-deficiency state [2,115–118]. The paradox is more apparent than real; these antioxidant supplements simply fail to significantly decrease levels of oxidative damage *in vivo*, and so their lack of effect is predictable [2,119–122]. On a related note, should cancer patients consume antioxidants? The clinical trials conducted to date to address this issue have given little evidence of benefit [2,123–125], again possibly because of failure of the administered antioxidants to decrease oxidative damage [2,119–122]. Indeed, in one study of head and neck cancer, patients were given α-tocopherol (400 units/day) and β-carotene (30 mg/day) during radiotherapy (which acts by increasing DNA damage, probably mostly oxidative, in the tumour cells to an intolerable level [19]) and for up to 3 years later. There was a tendency to have less severe acute adverse effects during therapy in the test group as compared with the placebo group. Unfortunately, the test group also showed a trend for cancer recurrence rates to be higher [126].

CACHEXIA AND REACTIVE SPECIES

Advanced malignant disease is accompanied by body wasting, including loss of muscle mass. Cytokines, especially TNFα (tumour necrosis factor α), play an important role, in part by inducing oxidative stress involving increased mitochondrial ROS production (reviewed in [2,127]). Indeed, levels of lipid peroxidation, protein oxidation and 3-nitrotyrosine are increased in the wasted muscles of mice with hepatoma [128].

CHRONIC INFLAMMATION, CANCER AND REACTIVE SPECIES

Overall, the data from knockout animals show that elevated RS levels can lead to cancer, probably by multiple mechanisms (Figures 1–3). Chronic inflammation is a well-known risk factor for cancer development. Examples include asbestosis, inflammatory bowel disease, pancreatitis, oesophagitis, bile duct inflammation, *H. pylori* infection, infection with the parasite *Schistosoma haematobium* (which produces chronic bladder inflammation with increased risk of bladder cancer) and hepatitis, often associated with chronic infection and inflammation caused by hepatitis B and C viruses, or liver flukes of the *Opisthorchis* genus (reviewed in [2,9,28]). Indeed, a transgenic mouse strain expressing a hepatitis B virus protein in the liver (provoking an immune response) showed chronic hepatitis and elevated liver 8OHdG levels, which preceded the development of hepatoma [129]. Where it has been measured, all of these inflammatory conditions in humans are accompanied by increased levels of oxidative (and sometimes nitrative) DNA damage [9,20,28]. In human melanoma patients, the presence of iNOS and nitrotyrosine was correlated with poor outcome [130]. Oxidative damage is usually measured as 8OHdG levels, although sometimes other oxidatively damaged bases are measured and found to be elevated as well. Methodological caution is needed in such studies, since a biopsy sample may include not only tumour cells but also inflammatory cells [72], which may be activated to damage their own DNA [131] and so raise bulk 8OHdG levels in the whole tissue sample. Nevertheless, the available evidence overall supports the occurrence of elevated DNA damage by RS in inflammation-related cancer [2,20].

Just as inflammation can promote cancer development, a tumour often induces an inflammatory response, e.g. it may secrete cytokines that recruit phagocytes and lymphocytes. Many tumours are infiltrated by macrophages [72]. Whether this response is good (helping to suppress tumour growth) or bad (facilitating more mutations in the tumour cells and promoting metastasis, e.g. by secreting proteolytic enzymes) is uncertain and may differ from tumour to tumour [72,130,132]. Animal studies have suggested that progression to malignancy often involves activation of NF-κB (nuclear factor κB), which (among its multitude of possible effects) can suppress apoptosis [133,134]. The effects of RS on NF-κB activation are complex; in some (but by no means all) cells, low to moderate RS levels activate NF-κB (often by increasing net protein phosphorylation), whereas higher RS levels tend to interfere with its action as a transcription factor by oxidizing the protein [2,135–137]. What is defined as 'moderate' or 'high' depends on the cell type [2]. For example, mice lacking uncoupling protein 2, which modulates mitochondrial $O_2^{\bullet -}$ generation, showed increased NF-κB activation, oxidative damage and more tumours in the colon after exposure to the carcinogen azoxymethane [138], consistent with a link between these events. However, it is not that simple: in a colitis model in mice, knockout of the IKKβ (inhibitory κB kinase β) gene, whose product is needed for NF-κB activation in intestinal epithelium, did not decrease the severity of inflammation (and presumably not therefore RS formation, although it would have been good to measure this), but tumour incidence was decreased by 80 %. Interestingly, knockout of the NF-κB system in macrophages in these animals also decreased tumour incidence (by 50 %), implying that (in this system at least) the host inflammatory response was pro-carcinogenic [134]. So, although some link of increased RS production to chronic inflammation-induced cancers seems likely, be wary of assuming that it is as simple as inflammation $\rightarrow$ more RS $\rightarrow$ cancer.

Mice lacking phagocyte NOX showed less metastasis after injection of fibrosarcoma cells [71], whereas, in contrast, some studies suggest that iNOS and $ONOO^-$ might be anti-metastatic in melanomas [132]. One complicating factor is that there is a feedback loop; whereas RS at the right level might promote carcinogenesis and contribute to inflammation, RS can sometimes suppress their own production. Thus too many RS can damage the phagocytes generating them and can also attack lymphocytes to damp down the immune response [131]. There are several cases in animal models where lowering RS levels leads to increased inflammation, as well as cases where such lowering is anti-inflammatory (reviewed in [131]). Indeed, it has been suggested that phagocyte RS production can impair T-cell functions in patients with advanced cancer [139,140]. These various contradictions may help to explain the generally very limited effect of antioxidants in the treatment of human chronic inflammations or in the prevention of the cancers that arise

from them; also contributing is their ineffectiveness in decreasing oxidative damage, as discussed above (reviewed in [2]).

So here we have a paradox. The commonly used antioxidant supplements do not scavenge RS well in *vivo*, and, even if they did, that might sometimes be pro-inflammatory (but it still could conceivably deter later cancer development, i.e. de-link cancer from the inflammation). So what interventions might work better? Anything that stops chronic inflammation, so we need better anti-inflammatory agents.

IMPLICATIONS FOR CHEMOTHERAPY

If malignant cells *in vivo* are under self-inflicted (and/or host-inflicted) oxidative stress, and that stress contributes to malignancy, then antioxidants that really do act to decrease oxidative damage *in vivo* might have anti-cancer effects. The opposite approach would be to stress these cells further by providing agents that generate RS. These should have limited effects on normal cells (provided that RS levels do not rise too high), but might, when added to their already elevated RS generation, push malignant cells 'over the edge', increasing oxidative damage to a point that the cell cannot cope [87,88,141]. Indeed, it was observed that human leukaemia cells with high ROS levels are more sensitive to 2-methoxyoestradiol than normal cells [142]. This agent appears to increase oxidative stress in cells [142], although exactly how it works is not clear [143].

Does increased RS formation thus contribute to the anti-tumour effects of chemotherapy and radiotherapy? Almost certainly so in radiotherapy [19]. Most agents used in chemotherapy act in specific ways, e.g. cytosine arabinoside and cisplatin by directly interfering with DNA replication, taxol by blocking mitotic spindle formation, methotrexate by interfering with the supply of DNA precursors, irinotecan and the anthracyclines by inhibiting topoisomerases (among other mechanisms), farnesyltransferase inhibitors by interfering with the action of small GTPases, adaphostin by inhibiting protein kinases, and proteasome inhibitors by interfering with cell division. Many (and possibly all) of these agents also cause oxidative stress, which has often been suggested to contribute to their side effects [142,144–156]. For example, it seems that mutations in the Bcr/Abl kinase domain involved in the resistance of leukaemic cells to imatinib also result in increased cellular ROS production, leading to DNA damage that contributes further to the development of resistance [156]. Does oxidative stress also aid the anti-cancer effects of the above compounds? It seems likely.

Of course, interfering with the RS–antioxidant balance in cancers has risks. If one gives too many RS generators, normal cells will be at risk. For example, arsenic trioxide (As_2O_3) may act against acute promyelocytic leukaemia by causing oxidative stress [152] (and indeed certain antioxidants can block its action in cell culture), but As_2O_3 can also be carcinogenic and RS are probably involved in the mechanism [95].

CONCLUSION

The data with knockout animals that lack antioxidant enzymes, supported by data from some animal knockouts of repair enzymes, strongly support the view that RS contribute to the age-related development of cancer. Direct damage to DNA is probably one key event, but is insufficient alone to produce cancer, suggesting that the ability of RS to suppress apoptosis, and promote proliferation, invasiveness and metastasis (and possibly angiogenesis) are also important. The relative contributions of these various mechanisms are unclear. Cancer associated with chronic inflammation may also involve RS, although they are by no means the only important element. Thus we understand more now than about the importance of RS than we did 10 years ago, but not exactly how they are acting. Our improved knowledge has not yet helped us to deliver better strategies for prevention and treatment (except to discourage healthy people and cancer patients from gobbling huge doses of 'antioxidant supplements'). Indeed, RS are both good and bad, so they may be difficult to modulate using an 'antioxidant' dosage that is expected to work on all subjects. The trend now in cancer treatment is to tailor-make therapies based on the gene expression, cell signalling and proteomic profiles of a tumour. Perhaps we need to do the same for its 'oxidative stress status'. If it is high, attack either with more ROS or with powerful antioxidants that actually work, perhaps? If it is low, seek another approach. Indeed, levels of gene expression for antioxidant defence enzymes and other proteins related to cellular redox balance have been said to constitute a 'redox signature score' that seemed to be predictive of outcome in patients with diffuse large B-cell lymphoma [157]. Techniques to measure oxidative stress and oxidative damage have improved enormously in the past few years (reviewed in [2,91]), although there is still a way to go. Thus development of such a score by combining parameters of oxidative damage with those of redox state, DNA-repair activity and antioxidant defence might be a useful therapeutic tool.

REFERENCES

1 Wiseman, H. and Halliwell, B. (1996) Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Biochem. J. **313**, 17–29

2 Halliwell, B. and Gutteridge, J. M. C. (2006) Free Radicals in Biology and Medicine (4th edn.), Clarendon Press, Oxford, in the press

3 Weitzman, S. A., Weitberg, A. B., Clark, E. P. and Stossel, T. P. (1985) Phagocytes as carcinogens: malignant transformation produced by human neutrophils. Science **227**, 1231–1233

4 Zimmerman, R. and Cerutti, P. (1984) Active oxygen acts as a promoter of transformation in mouse embryo C3H/10T1/2/C18 fibroblasts. Proc. Natl. Acad. Sci. U.S.A. **81**, 2085–2087

5 Kang, S. W., Rhee, S. G., Chang, T. S., Jeong, W. and Choi, M. H. (2005) 2-Cys peroxiredoxin function in intracellular signal transduction: therapeutic implications. Trends Mol. Med. **11**, 571–578

6 Storz, P., Doppler, H. and Toker, A. (2004) Protein kinase Cδ selectively regulates protein kinase D-dependent activation of NF-κB in oxidative stress signaling. Mol. Cell. Biol. **24**, 2614–2626

7 Pervaiz, S. and Clement, M. V. (2004) Tumor intracellular redox status and drug resistance: serendipity or a causal relationship?. Curr. Pharm. Des. **10**, 1969–1977

8 Akram, S., Teong, H. F., Fliegel, L., Pervaiz, S. and Clement, M. V. (2006) Reactive oxygen species-mediated regulation of the Na^+–H^+ exchanger 1 gene expression connects intracellular redox status with cells' sensitivity to death triggers. Cell Death Differ. **13**, 628–641

9 Sawa, T. and Ohshima, H. (2006) Nitrative DNA damage in inflammation and its possible role in carcinogenesis. Nitric Oxide **14**, 91–100

10 Hofseth, L. J., Saito, S., Hussain, S. P., Espey, M. G., Miranda, K. M., Araki, Y., Jhappan, C., Higashimoto, Y., He, P., Linke, S. P. et al. (2003) Nitric oxide-induced cellular stress and p53 activation in chronic inflammation. Proc. Natl. Acad. Sci. U.S.A. **100**, 143–148

11 Elchuri, S., Oberley, T. D., Qi, W., Eisenstein, R. S., Jackson Roberts, L., Van Remmen, H., Epstein, C. J. and Huang, T. T. (2005) CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. Oncogene **24**, 367–380

12 Li, Y., Huang, T. T., Carlson, E. J., Melov, S., Ursell, P. C., Olson, J. L., Noble, L. J., Yoshimura, M. P., Berger, C., Chan, P. H. et al. (1995) Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. Nat. Genet. **11**, 376–381

13 Melov, S., Doctrow, S. R., Schneider, J. A., Haberson, J., Patel, M., Coskun, P. E., Huffman, K., Wallace, D. C. and Malfroy, B. (2001) Lifespan extension and rescue of spongiform encephalopathy in superoxide dismutase 2 nullizygous mice treated with superoxide dismutase–catalase mimetics. J. Neurosci. **21**, 8348–8353

14 Van Remmen, H., Ikeno, Y., Hamilton, M., Pahlavani, M., Wolf, N., Thorpe, S. R., Alderson, N. L., Baynes, J. W., Epstein, C. J., Huang, T. T. et al. (2003) Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. Physiol. Genomics **16**, 29–37

15 Chu, F. F., Esworthy, R. S., Chu, P. G., Longmate, J. A., Huycke, M. M., Wilczynski, S. and Doroshow, J. H. (2004) Bacteria-induced intestinal cancer in mice with disrupted *Gpx1* and *Gpx2* genes. Cancer Res. **64**, 962–968

16 Neumann, C. A., Krause, D. S., Carman, C. V., Das, S., Dubey, D. P., Abraham, J. L., Bronson, R. T., Fujiwara, Y., Orkin, S. H. and Van Etten, R. A. (2003) Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. Nature **424**, 561–565

17 Egler, R. A., Fernandes, E., Rothermund, K., Sereika, S., de Souza-Pinto, N., Jaruga, P., Dizdaroglu, M. and Prochownik, E. V. (2005) Regulation of reactive oxygen species, DNA damage, and c-Myc function by peroxiredoxin 1. Oncogene **24**, 8038–8050

18 Ishii, K., Zhen, L. X., Wang, D. H., Funamori, Y., Ogawa, K. and Taketa, K. (1996) Prevention of mammary tumorigenesis in acatalasemic mice by vitamin E supplementation. Jpn. J. Cancer Res. **87**, 680–684

19 von Sonntag, C. (2006) Free Radical-Induced DNA Damage and its Repair: a Chemical Perspective, Springer, Basle

20 Evans, M. D., Dizdaroglu, M. and Cooke, M. S. (2004) Oxidative DNA damage and disease: induction, repair and significance. Mutat. Res. **567**, 1–61

21 Siomek, A., Rytarowska, A., Szaflarska-Poplawska, A., Gackowski, D., Rozalski, R., Dziaman, T., Czerwionka-Szaflarska, M. and Olinski, R. (2006) *Helicobacter pylori* infection is associated with oxidatively damaged DNA in human leukocytes and decreased level of urinary 8-oxo-7,8-dihydroguanine. Carcinogenesis **27**, 405–408

22 Senturker, S. and Dizdaroglu, M. (1999) The effect of experimental conditions on the levels of oxidatively modified bases in DNA as measured by gas chromatography–mass spectrometry: how many modified bases are involved?. Prepurification or not? Free Radical Biol. Med. **27**, 370–380

23 Totter, J. R. (1980) Spontaneous cancer and its possible relationship to oxygen metabolism. Proc. Natl. Acad. Sci. U.S.A. **77**, 1763–1767

24 Beckman, K. B. and Ames, B. N. (1997) Oxidative decay of DNA. J. Biol. Chem. **272**, 19633–19636

25 Halliwell, B. and Poulsen, H. E. (2006) Cigarette Smoke and Oxidative Stress, Springer, Basle

26 Halliwell, B. (2002) Effect of diet on cancer development: is oxidative DNA damage a biomarker? Free Radical Biol. Med. **32**, 968–974

27 Choi, J., Yoon, S. H., Kim, J. E., Rhee, K. H., Youn, H. S. and Chung, M. H. (2002) Gene-specific oxidative DNA damage in *Helicobacter pylori*-infected human gastric mucosa. Int. J. Cancer **99**, 485–490

28 Kawanishi, S., Hiraku, Y., Pinlaor, S. and Ma, N. (2006) Oxidative and nitrative DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis. Biol. Chem. **387**, 365–372

29 Leonarduzzi, G., Robbesyn, F. and Poli, G. (2004) Signaling kinases modulated by 4-hydroxynonenal. Free Radical Biol. Med. **37**, 1694–1702

30 Klaunig, J. E. and Kamendulis, L. M. (2004) The role of oxidative stress in carcinogenesis. Annu. Rev. Pharmacol. Toxicol. **44**, 239–267

31 Lee, O. J., Schneider-Stock, R., McChesney, P. A., Kuester, D., Roessner, A., Vieth, M., Moskaluk, C. A. and El-Rifai, W. (2005) Hypermethylation and loss of expression of glutathione peroxidase-3 in Barrett's tumorigenesis. Neoplasia **7**, 854–861

32 Valinluck, V., Tsai, H. H., Rogstad, D. K., Burdzy, A., Bird, A. and Sowers, L. C. (2004) Oxidative damage to methyl-CpG sequences inhibits the binding of the methyl-CpG binding domain (MBD) of methyl-CpG binding protein 2 (MeCP2). Nucleic Acids Res. **32**, 4100–4108

33 Furuta, J., Nobeyama, Y., Umebayashi, Y., Otsuka, F., Kikuchi, K. and Ushijima, T. (2006) Silencing of peroxiredoxin 2 and aberrant methylation of 33 CpG islands in putative promoter regions in human malignant melanomas. Cancer Res. **66**, 6080–6086

34 Nakabeppu, Y., Tsuchimoto, D., Furuichi, M. and Sakumi, K. (2004) The defense mechanisms in mammalian cells against oxidative damage in nucleic acids and their involvement in the suppression of mutagenesis and cell death. Free Radical Res. **38**, 423–429

35 Nakabeppu, Y., Sakumi, K., Sakamoto, K., Tsuchimoto, D., Tsuzuki, T. and Nakatsu, Y. (2006) Mutagenesis and carcinogenesis caused by the oxidation of nucleic acids. Biol. Chem. **387**, 373–379

36 Russo, M. T., De Luca, G., Degan, P. and Bignami, M. (2006) Different DNA repair strategies to combat the threat from 8-oxoguanine, Mutat. Res., doi:10.1016/j.mrfmmm.2006.03.007

37 Arai, T., Kelly, V. P., Minowa, O., Noda, T. and Nishimura, S. (2006) The study using wild-type and *Ogg1* knockout mice exposed to potassium bromate shows no tumor induction despite an extensive accumulation of 8-hydroxyguanine in kidney DNA. Toxicology **221**, 179–186

38 Irani, K., Xia, Y., Zweier, J. L., Sollott, S. J., Der, C. J., Fearon, E. R., Sundaresan, M., Finkel, T. and Goldschmidt-Clermont, P. J. (1997) Mitogenic signaling mediated by oxidants in Ras-transformed fibroblasts. Science **275**, 1649–1652

39 Geiszt, M. and Leto, T. L. (2004) The Nox family of NAD(P)H oxidases: host defense and beyond. J. Biol. Chem. **279**, 51715–51718

40 Bensaad, K. and Vousden, K. H. (2005) Savior and slayer: the two faces of p53. Nat. Med. **11**, 1278–9

41 Giorgio, M., Migliaccio, E., Orsini, F., Paolucci, D., Moroni, M., Contursi, C., Pelliccia, G., Luzi, L., Minucci, S., Marcaccio, M. et al. (2005) Electron transfer between cytochrome *c* and $p66^{Shc}$ generates reactive oxygen species that trigger mitochondrial apoptosis. Cell **122**, 221–233

42 Rivera, A. and Maxwell, S. A. (2005) The p53-induced gene-6 (proline oxidase) mediates apoptosis through a calcineurin-dependent pathway. J. Biol. Chem. **280**, 29346–29354

43 Matoba, S., Kang, J. G., Patino, W. D., Wragg, A., Boehm, M., Gavrilova, O., Hurley, P. J., Bunz, F. and Hwang, P. M. (2006) p53 regulates mitochondrial respiration. Science **312**, 1650–1653

44 Cobbs, C. S., Samanta, M., Harkins, L. E., Gillespie, G. Y., Merrick, B. A. and MacMillan-Crow, L. A. (2001) Evidence for peroxynitrite-mediated modifications to p53 in human gliomas: possible functional consequences. Arch. Biochem. Biophys. **394**, 167–172

45 Reichenbach, J., Schubert, R., Schindler, D., Muller, K., Bohles, H. and Zielen, S. (2002) Elevated oxidative stress in patients with ataxia telangiectasia. Antioxid. Redox Signaling **4**, 465–469

46 Browne, S. E., Roberts 2nd, L. J., Dennery, P. A., Doctrow, S. R., Beal, M. F., Barlow, C. and Levine, R. L. (2004) Treatment with a catalytic antioxidant corrects the neurobehavioral defect in ataxia-telangiectasia mice. Free Radical Biol. Med. **36**, 938–942

47 Ito, K., Hirao, A., Arai, F., Takubo, K., Matsuoka, S., Miyamoto, K., Ohmura, M., Naka, K., Hosokawa, K., Ikeda, Y. et al. (2006) Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. Nat. Med. **12**, 446–451

48 Erker, L., Schubert, R., Elchuri, S., Huang, T. T., Tarin, D., Mueller, K., Zielen, S., Epstein, C. J. and Wynshaw-Boris, A. (2006) Effect of the reduction of superoxide dismutase 1 and 2 or treatment with α-tocopherol on tumorigenesis in *Atm*-deficient mice. Free Radical Biol. Med. **41**, 590–600

49 Chandra, J., Samali, A. and Orrenius, S. (2000) Triggering and modulation of apoptosis by oxidative stress. Free Radical Biol. Med. **29**, 323–333

50 Tang, S. Y., Whiteman, M., Jenner, A., Peng, Z. F. and Halliwell, B. (2004) Mechanism of cell death induced by an antioxidant extract of *Cratoxylum cochinchinense* (YCT) in Jurkat T cells: the role of reactive oxygen species and calcium. Free Radical Biol. Med. **36**, 1588–1611

51 Vaquero, E. C., Edderkaoui, M., Pandol, S. J., Gukovsky, I. and Gukovskaya, A. S. (2004) Reactive oxygen species produced by NAD(P)H oxidase inhibit apoptosis in pancreatic cancer cells. J. Biol. Chem. **279**, 34643–34654

52 Urbano, A., Lakshmanan, U., Choo, P. H., Kwan, J. C., Ng, P. Y., Guo, K., Dhakshinamoorthy, S. and Porter, A. (2005) AIF suppresses chemical stress-induced apoptosis and maintains the transformed state of tumor cells. EMBO J. **24**, 2815–2826

53 Toker, A. and Yoeli-Lerner, M. (2006) Akt signaling and cancer: surviving but not moving on. Cancer Res. **66**, 3963–3966

54 Halliwell, B. (2003) Oxidative stress in cell culture: an under-appreciated problem?. FEBS Lett. **540**, 3–6

55 Burdon, R. H., Gill, V. and Rice-Evans, C. (1990) Oxidative stress and tumour cell proliferation. Free Radical Res. Commun. **11**, 65–76

56 Burdon, R. H., Alliangana, D. and Gill, V. (1995) Hydrogen peroxide and the proliferation of BHK-21 cells. Free Radical Res. **23**, 471–486

57 Halliwell, B. (1996) Free radicals, proteins and DNA: oxidative damage versus redox regulation. Biochem. Soc. Trans. **24**, 1023–1027

58 Itahana, K., Campisi, J. and Dimri, G. P. (2004) Mechanisms of cellular senescence in human and mouse cells. Biogerontology **5**, 1–10

59 Wright, W. E. and Shay, J. W. (2002) Historical claims and current interpretations of replicative aging. Nat. Biotechnol. **20**, 682–688

60 Ben-Porath, I. and Weinberg, R. A. (2004) When cells get stressed: an integrative view of cellular senescence. J. Clin. Invest. **113**, 8–13

61 Lin, H. and D'Rosario, T. (2003) Pretreatment growth environments alter the sensitivity of tumor cells to cytotoxic agents. Free Radical Biol. Med. **35**, 277–283

62 Long, L. H., Clement, M. V. and Halliwell, B. (2000) Artifacts in cell culture: rapid generation of hydrogen peroxide on addition of (−)-epigallocatechin, (−)-epigallocatechin gallate, (+)-catechin, and quercetin to commonly used cell culture media. Biochem. Biophys. Res. Commun. **273**, 50–53

63 Lapidot, T., Walker, M. D. and Kanner, J. (2002) Can apple antioxidants inhibit tumor cell proliferation? Generation of H_2O_2 during interaction of phenolic compounds with cell culture media. J. Agric. Food Chem. **50**, 3156–3160

64 Clement, M. V., Ramalingam, J., Long, L. H. and Halliwell, B. (2001) The *in vitro* cytotoxicity of ascorbate depends on the culture medium used to perform the assay and involves hydrogen peroxide. Antioxid. Redox Signaling **3**, 157–163

65 Lambeth, J. D. (2004) NOX enzymes and the biology of reactive oxygen. Nat. Rev. Immunol. **4**, 181–189

66 Storz, P. (2005) Reactive oxygen species in tumor progression. Front. Biosci. **10**, 1881–1896

67 Savaraj, N., Wei, Y., Unate, H., Liu, P. M., Wu, C. J., Wangpaichitr, M., Xia, D., Xu, H. J., Hu, S. X. and Tien Kuo, M. (2005) Redox regulation of matrix metalloproteinase gene family in small cell lung cancer cells. Free Radical Res. **39**, 373–381

68 Mori, K., Shibanuma, M. and Nose, K. (2004) Invasive potential induced under long-term oxidative stress in mammary epithelial cells. Cancer Res. **64**, 7464–7472

69 Oberley, T. D. (2002) Oxidative damage and cancer. Am. J. Pathol. **160**, 403–408

70 Radisky, D. C., Levy, D. D., Littlepage, L. E., Liu, H., Nelson, C. M., Fata, J. E., Leake, D., Godden, E. L., Albertson, D. G., Nieto, M. A. et al. (2005) Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. Nature **436**, 123–127

71 Okada, F., Kobayashi, M., Tanaka, H., Kobayashi, T., Tazawa, H., Iuchi, Y., Onuma, K., Hosokawa, M., Dinauer, M. C. and Hunt, N. H. (2006) The role of nicotinamide adenine dinucleotide phosphate oxidase-derived reactive oxygen species in the acquisition of metastatic ability of tumor cells. Am. J. Pathol. **169**, 294–302

72 van Kempen, L. C., de Visser, K. E. and Coussens, L. M. (2006) Inflammation, proteases and cancer. Eur. J. Cancer **42**, 728–734

73 Halliwell, B. (1997) What nitrates tyrosine? Is nitrotyrosine specific as a biomarker of peroxynitrite formation *in vivo*? FEBS Lett. **411**, 157–160

74 Polytarchou, C., Hatziapostolou, M. and Papadimitriou, E. (2005) Hydrogen peroxide stimulates proliferation and migration of human prostate cancer cells through activation of activator protein-1 and up-regulation of the heparin affin regulatory peptide gene. J. Biol. Chem. **280**, 40428–40435

75 Nishikawa, M., Hyoudou, K., Kobayashi, Y., Umeyama, Y., Takakura, Y. and Hashida, M. (2005) Inhibition of metastatic tumor growth by targeted delivery of antioxidant enzymes. J. Controlled Release **109**, 101–107

76 Heirman, I., Ginneberge, D., Brigelius-Flohe, R., Hendrickx, N., Agostinis, P., Brouckaert, P., Rottiers, P. and Grooten, J. (2006) Blocking tumor cell eicosanoid synthesis by GPx4 impedes tumor growth and malignancy. Free Radical Biol. Med. **40**, 285–294

77 Payne, S. L., Fogelgren, B., Hess, A. R., Seftor, E. A., Wiley, E. L., Fong, S. F., Csiszar, K., Hendrix, M. J. and Kirschmann, D. A. (2005) Lysyl oxidase regulates breast cancer cell migration and adhesion through a hydrogen peroxide-mediated mechanism. Cancer Res. **65**, 11429–11436

78 Okada, F., Shionoya, H., Kobayashi, M., Kobayashi, T., Tazawa, H., Onuma, K., Iuchi, Y., Matsubara, N., Ijichi, T., Dugas, B. et al. (2006) Prevention of inflammation-mediated acquisition of metastatic properties of benign mouse fibrosarcoma cells by administration of an orally available superoxide dismutase. Br. J. Cancer **94**, 854–862

79 Huang, S. S. and Zheng, R. L. (2006) Biphasic regulation of angiogenesis by reactive oxygen species. Pharmazie **61**, 223–229

80 Krop, I., Marz, A., Carlsson, H., Li, X., Bloushtain-Qimron, N., Hu, M., Gelman, R., Sabel, M. S., Schnitt, S., Ramaswamy, S. et al. (2005) A putative role for psoriasin in breast tumor progression. Cancer Res. **65**, 11326–11334

81 Kim, Y. M., Kim, K. E., Koh, G. Y., Ho, Y. S. and Lee, K. J. (2006) Hydrogen peroxide produced by angiopoietin-1 mediates angiogenesis. Cancer Res. **66**, 6167–6174

82 Ushio-Fukai, M. (2006) Redox signaling in angiogenesis: role of NADPH oxidase. Cardiovasc. Res. **71**, 226–235

83 Brown, N. S., Streeter, E. H., Jones, A., Harris, A. L. and Bicknell, R. (2005) Cooperative stimulation of vascular endothelial growth factor expression by hypoxia and reactive oxygen species: the effect of targeting vascular endothelial growth factor and oxidative stress in an orthotopic xenograft model of bladder carcinoma. Br. J. Cancer **92**, 1696–1701

84 Haendeler, J. and Dimmeler, S. (2006) Inseparably tied: functional and antioxidative capacity of endothelial progenitor cells. Circ. Res. **98**, 157–158

85 Toyokuni, S., Okamoto, K., Yodoi, J. and Hiai, H. (1995) Persistent oxidative stress in cancer. FEBS Lett. **358**, 1–3

86 Szatrowski, T. P. and Nathan, C. F. (1991) Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Res. **51**, 794–798

87 Pelicano, H., Carney, D. and Huang, P. (2004) ROS stress in cancer cells and therapeutic implications. Drug Resist. Updates **7**, 97–110

88 Zhou, Y., Hileman, E. O., Plunkett, W., Keating, M. J. and Huang, P. (2003) Free radical stress in chronic lymphocytic leukemia cells and its role in cellular sensitivity to ROS-generating anticancer agents. Blood **101**, 4098–4104

89 Cerimele, F., Battle, T., Lynch, R., Frank, D. A., Murad, E., Cohen, C., Macaron, N., Sixbey, J., Smith, K., Watnick, R. S. et al. (2005) Reactive oxygen signaling and MAPK activation distinguish Epstein–Barr Virus (EBV)-positive versus EBV-negative Burkitt's lymphoma. Proc. Natl. Acad. Sci. U.S.A. **102**, 175–179

90 Kamiguti, A. S., Serrander, L., Lin, K., Harris, R. J., Cawley, J. C., Allsup, D. J., Slupsky, J. R., Krause, K. H. and Zuzel, M. (2005) Expression and activity of NOX5 in the circulating malignant B cells of hairy cell leukemia. J. Immunol. **175**, 8424–8430

91 Halliwell, B. and Whiteman, M. (2004) Measuring reactive species and oxidative damage *in vivo* and in cell culture: how should you do it and what do the results mean?. Br. J. Pharmacol. **142**, 231–255

92 Neeley, W. L. and Essigmann, J. M. (2006) Mechanisms of formation, genotoxicity, and mutation of guanine oxidation products. Chem. Res. Toxicol. **19**, 491–505

93 Kasai, H. (2002) Chemistry-based studies on oxidative DNA damage: formation, repair, and mutagenesis. Free Radical Biol. Med. **33**, 450–456

94 Sanchez, M., Torres, J. V., Tormos, C., Iradi, A., Muniz, P., Espinosa, O., Salvador, A., Rodriguez-Delgado, J., Fandos, M. and Saez, G. T. (2006) Impairment of antioxidant enzymes, lipid peroxidation and 8-oxo-2′-deoxyguanosine in advanced epithelial ovarian carcinoma of a Spanish community. Cancer Lett. **233**, 28–35

95 Hei, T. K. and Filipic, M. (2004) Role of oxidative damage in the genotoxicity of arsenic. Free Radical Biol. Med. **37**, 574–581

96 Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M. and Mazur, M. (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact. **160**, 1–40

97 Petros, J. A., Baumann, A. K., Ruiz-Pesini, E., Amin, M. B., Sun, C. Q., Hall, J., Lim, S., Issa, M. M., Flanders, W. D., Hosseini, S. H. et al. (2005) mtDNA mutations increase tumorigenicity in prostate cancer. Proc. Natl. Acad. Sci. U.S.A. **102**, 719–724

98 Carew, J. S., Zhou, Y., Albitar, M., Carew, J. D., Keating, M. J. and Huang, P. (2003) Mitochondrial DNA mutations in primary leukemia cells after chemotherapy: clinical significance and therapeutic implications. Leukemia **17**, 1437–1447

99 Arbiser, J. L., Petros, J., Klafter, R., Govindajaran, B., McLaughlin, E. R., Brown, L. F., Cohen, C., Moses, M., Kilroy, S., Arnold, R. S. et al. (2002) Reactive oxygen generated by Nox1 triggers the angiogenic switch. Proc. Natl. Acad. Sci. U.S.A. **99**, 715–720

100 Fukuyama, M., Rokutan, K., Sano, T., Miyake, H., Shimada, M. and Tashiro, S. (2005) Overexpression of a novel superoxide-producing enzyme, NADPH oxidase 1, in adenoma and well differentiated adenocarcinoma of the human colon. Cancer Lett. **221**, 97–104

101 Szanto, I., Rubbia-Brandt, L., Kiss, P., Steger, K., Banfi, B., Kovari, E., Herrmann, F., Hadengue, A. and Krause, K. H. (2005) Expression of NOX1, a superoxide-generating NADPH oxidase, in colon cancer and inflammatory bowel disease. J. Pathol. **207**, 164–176

102 Lim, S. D., Sun, C., Lambeth, J. D., Marshall, F., Amin, M., Chung, L., Petros, J. A. and Arnold, R. S. (2005) Increased Nox1 and hydrogen peroxide in prostate cancer. Prostate **62**, 200–207

103 Shand, R. L. and Gelmann, E. P. (2006) Molecular biology of prostate-cancer pathogenesis. Curr. Opin. Urol. **16**, 123–131

104 Madhusudan, S. and Middleton, M. R. (2005) The emerging role of DNA repair proteins as predictive, prognostic and therapeutic targets in cancer. Cancer Treat. Rev. **31**, 603–617

105 Cooke, M. S., Rozalski, R., Dove, R., Gackowski, D., Siomek, A., Evans, M. D. and Olinski, R. (2006) Evidence for attenuated cellular 8-oxo-7,8-dihydro-2′-deoxyguanosine removal in cancer patients. Biol. Chem. **387**, 393–400

106 Kitajima, Y., Miyazaki, K., Matsukura, S., Tanaka, M. and Sekiguchi, M. (2003) Loss of expression of DNA repair enzymes MGMT, hMLH1, and hMSH2 during tumor progression in gastric cancer. Gastric Cancer **6**, 86–95

107 Matsukura, S., Miyazaki, K., Yakushiji, H., Ogawa, A., Chen, Y. and Sekiguchi, M. (2003) Combined loss of expression of O^6-methylguanine–DNA methyltransferase and hMLH1 accelerates progression of hepatocellular carcinoma. J. Surg. Oncol. **82**, 194–200

108 Weydert, C. J., Waugh, T. A., Ritchie, J. M., Iyer, K. S., Smith, J. L., Li, L., Spitz, D. R. and Oberley, L. W. (2006) Overexpression of manganese or copper-zinc superoxide dismutase inhibits breast cancer growth. Free Radical Biol. Med. **41**, 226–237

109 Oberley, L. W. (2005) Mechanism of the tumor suppressive effect of MnSOD overexpression. Biomed. Pharmacother. **59**, 143–148

110 Wang, M., Kirk, J. S., Venkataraman, S., Domann, F. E., Zhang, H. J., Schafer, F. Q., Flanagan, S. W., Weydert, C. J., Spitz, D. R., Buettner, G. R. et al. (2005) Manganese superoxide dismutase suppresses hypoxic induction of hypoxia-inducible factor-1α and vascular endothelial growth factor. Oncogene **24**, 8154–8166

111 Kinnula, V. L. and Crapo, J. D. (2004) Superoxide dismutases in malignant cells and human tumors. Free Radical Biol. Med. **36**, 718–744

112 Soini, Y., Kallio, J. P., Hirvikoski, P., Helin, H., Kellokumpu-Lehtinen, P., Kang, S. W., Tammela, T. L., Peltoniemi, M., Martikainen, P. M. and Kinnula, V. L. (2006) Oxidative/nitrosative stress and peroxiredoxin 2 are associated with grade and prognosis of human renal carcinoma. APMIS **114**, 329–337

113 Sander, C. S., Hamm, F., Elsner, P. and Thiele, J. J. (2003) Oxidative stress in malignant melanoma and non-melanoma skin cancer. Br. J. Dermatol. **148**, 913–922

114 Kondo, S., Toyokuni, S., Iwasa, Y., Tanaka, T., Onodera, H., Hiai, H. and Imamura, M. (1999) Persistent oxidative stress in human colorectal carcinoma, but not in adenoma. Free Radical Biol. Med. **27**, 401–410

115 Bjelakovic, G., Nikolova, D., Simonetti, R. G. and Gluud, C. (2004) Antioxidant supplements for prevention of gastrointestinal cancers: a systematic review and meta-analysis. Lancet **364**, 1219–1228

116 Lawlor, D. A., Davey Smith, G., Kundu, D., Bruckdorfer, K. R. and Ebrahim, S. (2004) Those confounded vitamins: what can we learn from the differences between observational versus randomised trial evidence? Lancet **363**, 1724–1727

117 Miller, 3rd, E. R., Pastor-Barriuso, R., Dalal, D., Riemersma, R. A., Appel, L. J. and Guallar, E. (2005) Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. Ann. Intern. Med. **142**, 37–46

118 Bjelakovic, G., Nagorni, A., Nikolova, D., Simonetti, R. G., Bjelakovic, M. and Gluud, C. (2006) Meta-analysis: antioxidant supplements for primary and secondary prevention of colorectal adenoma. Aliment. Pharmacol. Ther. **24**, 281–291

119 Halliwell, B. (1999) Establishing the significance and optimal intake of dietary antioxidants: the biomarker concept. Nutr. Rev. **57**, 104–113

120 Moller, P. and Loft, S. (2006) Dietary antioxidants and beneficial effect on oxidatively damaged DNA. Free Radical Biol. Med. **41**, 388–415

121 Prieme, H., Loft, S., Nyyssonen, K., Salonen, J. T. and Poulsen, H. E. (1997) No effect of supplementation with vitamin E, ascorbic acid, or coenzyme Q_{10} on oxidative DNA damage estimated by 8-oxo-7,8-dihydro-2′-deoxyguanosine excretion in smokers. Am. J. Clin. Nutr. **65**, 503–507

122 Halliwell, B. (2006) Dietary polyphenols: good, bad or indifferent for health? Cardiovasc, Res., in the press

123 Pathak, A. K., Bhutani, M., Guleria, R., Bal, S., Mohan, A., Mohanti, B. K., Sharma, A., Pathak, R., Bhardwaj, N. K., Prasad, K. N. et al. (2005) Chemotherapy alone vs. chemotherapy plus high dose multiple antioxidants in patients with advanced non small cell lung cancer. J. Am. Coll. Nutr. **24**, 16–21

124 Lesperance, M. L., Olivotto, I. A., Forde, N., Zhao, Y., Speers, C., Foster, H., Tsao, M., MacPherson, N. and Hoffer, A. (2002) Mega-dose vitamins and minerals in the treatment of non-metastatic breast cancer: an historical cohort study. Breast Cancer Res. Treat. **76**, 137–143

125 Ladas, E. J., Jacobson, J. S., Kennedy, D. D., Teel, K., Fleischauer, A. and Kelly, K. M. (2004) Antioxidants and cancer therapy: a systematic review. J. Clin. Oncol. **22**, 517–528

126 Bairati, I., Meyer, F., Gelinas, M., Fortin, A., Nabid, A., Brochet, F., Mercier, J. P., Tetu, B., Harel, F., Abdous, B. et al. (2005) Randomized trial of antioxidant vitamins to prevent acute adverse effects of radiation therapy in head and neck cancer patients. J. Clin. Oncol. **23**, 5805–5813

127 Mantovani, G. and Madeddu, C. (2006) Oxidative stress and cancer cachexia. In Oxidative Stress, Disease and Cancer (Singh, K. K., ed.), pp. 733–759, World Scientific Publishing Co., London

128 Barreiro, E., de la Puente, B., Busquets, S., Lopez-Soriano, F. J., Gea, J. and Argiles, J. M. (2005) Both oxidative and nitrosative stress are associated with muscle wasting in tumour-bearing rats. FEBS Lett. **579**, 1646–1652

129 Hagen, T. M., Huang, S., Curnutte, J., Fowler, P., Martinez, V., Wehr, C. M., Ames, B. N. and Chisari, F. V. (1994) Extensive oxidative DNA damage in hepatocytes of transgenic mice with chronic active hepatitis destined to develop hepatocellular carcinoma. Proc. Natl. Acad. Sci. U.S.A. **91**, 12808–12812

130 Ekmekcioglu, S., Tang, C. H. and Grimm, E. A. (2005) NO news is not necessarily good news in cancer. Curr. Cancer Drug Targets **5**, 103–115

131 Halliwell, B. (2006) Phagocyte-derived reactive species: salvation or suicide? Trends Biochem. Sci. **141**, 312

132 Ahmed, B. and Van Den Oord, J. J. (2000) Expression of the inducible isoform of nitric oxide synthase in pigment cell lesions of the skin. Br. J. Dermatol. **142**, 432–440

133 Karin, M. (2006) Nuclear factor-κB in cancer development and progression. Nature **441**, 431–436

134 Li, Q., Withoff, S. and Verma, I. M. (2005) Inflammation-associated cancer: NF-κB is the lynchpin. Trends Immunol. **26**, 318–325

135 Pahl, H. L. and Baeuerle, P. A. (1994) Oxygen and the control of gene expression. BioEssays **16**, 497–502

136 Hayakawa, M., Miyashita, H., Sakamoto, I., Kitagawa, M., Tanaka, H., Yasuda, H., Karin, M. and Kikugawa, K. (2003) Evidence that reactive oxygen species do not mediate NF-κB activation. EMBO J. **22**, 3356–3366

137 Robbesyn, F., Salvayre, R. and Negre-Salvayre, A. (2004) Dual role of oxidized LDL on the NF-κB signaling pathway. Free Radical Res. **38**, 541–551

138 Derdak, Z., Fulop, P., Sabo, E., Tavares, R., Berthiaume, E. P., Resnick, M. B., Paragh, G., Wands, J. R. and Baffy, G. (2006) Enhanced colon tumor induction in uncoupling protein-2 deficient mice is associated with NF-κB activation and oxidative stress. Carcinogenesis **27**, 956–961

139 Schmielau, J. and Finn, O. J. (2001) Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of T-cell function in advanced cancer patients. Cancer Res. **61**, 4756–4760

140 Hellstrand, K. (2003) Melanoma immunotherapy: a battle against radicals? Trends Immunol. **24**, 232–233

141 Kong, Q., Beel, J. A. and Lillehei, K. O. (2000) A threshold concept for cancer therapy. Med. Hypotheses **55**, 29–35

142 Huang, P., Feng, L., Oldham, E. A., Keating, M. J. and Plunkett, W. (2000) Superoxide dismutase as a target for the selective killing of cancer cells. Nature **407**, 390–395

143 Kachadourian, R., Liochev, S. I., Cabelli, D. E., Patel, M. N., Fridovich, I. and Day, B. J. (2001) 2-Methoxyestradiol does not inhibit superoxide dismutase. Arch. Biochem. Biophys. **392**, 349–353

144 Pan, J. and Yeung, S. C. J. (2005) Recent advances in understanding the antineoplastic mechanisms of farnesyltransferase inhibitors. Cancer Res. **65**, 9109–9112

145 Kishida, O., Miyazaki, Y., Murayama, Y., Ogasa, M., Miyazaki, T., Yamamoto, T., Watabe, K., Tsutsui, S., Kiyohara, T., Shimomura, I. et al. (2005) Gefitinib ("Iressa", ZD1839) inhibits SN38-triggered EGF signals and IL-8 production in gastric cancer cells. Cancer Chemother. Pharmacol. **55**, 393–403

146 Appenroth, D., Frob, S., Kersten, L., Splinter, F. K. and Winnefeld, K. (1997) Protective effects of vitamin E and C on cisplatin nephrotoxicity in developing rats. Arch. Toxicol. **71**, 677–683

147 Geller, H. M., Cheng, K. Y., Goldsmith, N. K., Romero, A. A., Zhang, A. L., Morris, E. J. and Grandison, L. (2001) Oxidative stress mediates neuronal DNA damage and apoptosis in response to cytosine arabinoside. J. Neurochem. **78**, 265–275

148 Ramanathan, B., Jan, K. Y., Chen, C. H., Hour, T. C., Yu, H. J. and Pu, Y. S. (2005) Resistance to paclitaxel is proportional to cellular total antioxidant capacity. Cancer Res. **65**, 8455–8460

149 Minotti, G., Menna, P., Salvatorelli, E., Cairo, G. and Gianni, L. (2004) Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol. Rev. **56**, 185–229

150 Fattman, C. L., Chang, L. Y., Termin, T. A., Petersen, L., Enghild, J. J. and Oury, T. D. (2003) Enhanced bleomycin-induced pulmonary damage in mice lacking extracellular superoxide dismutase. Free Radical Biol. Med. **35**, 763–771

151 Halliwell, B. (2006) Proteasomal dysfunction: a common feature of neurodegenerative diseases? Implications for the environmental origins of neurodegeneration. Antioxid. Redox Signaling **8**, 2007–2019

152 Chou, W. C., Jie, C., Kenedy, A. A., Jones, R. J., Trush, M. A. and Dang, C. V. (2004) Role of NADPH oxidase in arsenic-induced reactive oxygen species formation and cytotoxicity in myeloid leukemia cells. Proc. Natl. Acad. Sci. U.S.A. **101**, 4578–4583

153 Doroshow, J. H. (2006) Redox modulation of chemotherapy-induced tumor cell killing and normal tissue toxicity. J. Natl. Cancer Inst. **98**, 223–225

154 Alexandre, J., Batteux, F., Nicco, C., Chereau, C., Laurent, A., Guillevin, L., Weill, B. and Goldwasser, F. (2006) Accumulation of hydrogen peroxide is an early and crucial step for paclitaxel-induced cancer cell death both *in vitro* and *in vivo*. Int. J. Cancer **119**, 41–48

155 Kay, N. E. (2006) ROS: double-edged sword for leukemic cells. Blood **107**, 2212–2213

156 Koptyra, M., Falinski, R., Nowicki, M. O., Stoklosa, T., Majsterek, I., Nieborowska-Skorska, M., Blasiak, J. and Skorski, T. (2006) BCR/ABL kinase induces self-mutagenesis via reactive oxygen species to encode imatinib resistance. Blood **108**, 319–327

157 Tome, M. E., Johnson, D. B., Rimsza, L. M., Roberts, R. A., Grogan, T. M., Miller, T. P., Oberley, L. W. and Briehl, M. M. (2005) A redox signature score identifies diffuse large B-cell lymphoma patients with a poor prognosis. Blood **106**, 3594–3601

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