CHAPTER 15.

# Oxidative stress and radical-induced signalling

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Throughout evolution, aerobic organisms have developed multiple defence systems to protect themselves against oxygen radicals (Benzie, 2000). One-, two-, and three-electron reductions of molecular oxygen give rise to, respectively, superoxide ($O_2^{\cdot -}$), hydrogen peroxide ($H_2O_2$, a radical precursor), and the highly reactive hydroxyl radical ($^{\cdot}OH$) or equivalent transition metal–oxygen complexes (Miller et al., 1990). Reactions of oxygen radicals with cellular components can deplete antioxidants, can cause direct oxidative damage to lipids, proteins, RNA, and DNA, and can result in the formation of a variety of other reactants with varying oxidative potentials, including carbon- or nitrogen-centred radicals (West and Marnett, 2006). A growing body of literature presents radicals as mediators of various cell signalling processes (Ma, 2010). An imbalance between the normal production of oxygen radicals and their capture and disposal by protective enzyme systems and antioxidants results in oxidative stress, and this condition has been proposed to be the basis of many deleterious chronic health conditions and diseases, including cancer.

## Sources of oxygen radicals

Mitochondrial oxidative phosphorylation is a major source of oxygen radicals of endogenous origin. Mitochondrial complex I (reduced nicotinamide adenine dinucleotide [NADH]:ubiquinone oxidoreductase) and complex III (ubiquinol:cytochrome c oxidoreductase) are sites of superoxide production, with as much as 1–2% of the electron flux shunted through one-electron reduction of molecular oxygen (St-Pierre et al., 2002). Peroxisomes are a source of $H_2O_2$, through reactions involving acyl-CoA oxidase (which is involved in oxidation of long-chain fatty acids), D-amino acid oxidase, and other oxidases (Schrader and Fahimi, 2006).

When stimulated, inflammatory cells such as neutrophils, eosinophils, and macrophages produce oxygen radicals during the associated respiratory burst (the rapid release of reactive oxygen species from cells) that involves nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Babior, 1999). This reaction produces superoxide, which is converted by superoxide dismutase to the more readily diffusible oxidant $H_2O_2$ and is involved in cell killing functions. Inflammatory cells such as macrophages are also capable of producing nitric oxide ($^{\cdot}NO$), through an inducible form of nitric

oxide synthase (Hibbs et al., 1988). ·NO is also involved in cell killing but can also react with superoxide at diffusion-limited rates to form peroxynitrite, a potent oxidant with a longer half-life and diffusion distance than the hydroxyl radical (Beckman, 1996).

Exogenous agents are also implicated in the generation of reactive oxygen. Metals such as cadmium and arsenic can participate in reactions that generate oxygen radicals (Liu et al., 2008; Kojima et al., 2009). Miller et al. (1990) presented a list of endogenous and exogenous agents that are capable of reducing oxygen to superoxide or that "autoxidize", probably through reactions catalysed by transition metals. Metabolism of many exogenous agents through cytochrome P450-mediated reactions can also result in the release of oxygen radicals (Hrycay and Bandiera, 2015), as can exposure to ionizing radiation. In addition, several lifestyle factors, such as obesity, tobacco smoking, and alcohol consumption, as well as chronic inflammatory conditions and viral infections are thought to involve radical-induced injury (Mena et al., 2009).

## Oxidative damage

The hydroxyl radical or equivalent transition metal–oxygen complexes (Bucher et al., 1983) are highly reactive entities, capable of abstracting electrons from lipids, proteins, or DNA (Miller et al., 1990), and the resulting target molecule radical can then combine with molecular oxygen to participate in subsequent radical reactions, such as propagation of lipid peroxidation. Radical reactions with DNA result in single- and double-strand breaks (Toyokuni and Sagripanti, 1996), cross-links, and modified bases. The oxidation product 8-oxo-2′-deoxyguanosine is often used as a marker of oxidative DNA damage, although other bases are also susceptible to oxidation. DNA bases can be modified by lipid peroxidation reaction products (*trans*-4-hydroxy-2-nonenal, 4-hydroperoxy-2-nonenal, and malondialdehyde) to form various pro-mutagenic exocyclic adducts (Bartsch and Nair, 2006).

## Defence mechanisms

Cytosolic and mitochondrial forms of superoxide dismutase catalyse the reduction of superoxide to $H_2O_2$, and when coupled with catalase within peroxisomes or with cytosolic glutathione peroxidase, can further convert these reactive species to water (Benzie, 2000). Sequestration of transition metals, principally iron and copper, in their oxidized forms through deposition in transport or storage proteins, or as chelates that do not support redox reactions, also limits radical reactions (Hatcher et al., 2009).

Dietary and endogenously produced antioxidants also contribute in the defence against radical damage by serving as radical scavengers. Theoretically any oxidizable substrate can act as a radical scavenger; ascorbic acid, tocopherols, uric acid, and sulfhydryl-containing amino acids provide considerable scavenging capacity (Benzie, 2000).

Interestingly, high concentrations of antioxidants in the presence of transition metals can actually drive formation of oxygen radicals (Tien et al., 1982). The importance of a balance between pro- and antioxidant capacities is also emphasized by an emerging understanding of the role of radical species in cellular signal transduction.

## Oxygen radicals in cancer

Hanahan and Weinberg (2011), in their landmark review "Hallmarks of cancer: the next generation", identified sustaining proliferative signalling, reprogramming of energy metabolism, evading growth suppressors and immune destruction, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis as signal transduction pathways key to unravelling the cancer phenotype. They also described how genomic instability and tumour-promoting inflammation are principal drivers of these events. Oxygen radicals clearly contribute to genomic instability, are produced by inflammation, and – along with other radical species – play key roles in many of the processes identified above as necessary for conversion of normal cells into cancer cells.

Oxidative damage is considered to be a major factor in the generation of mutations, which are estimated to occur at a frequency of 10 000 per cell per day in humans (Lu et al., 2001). More than 100 different oxidative DNA lesions (Klaunig et al., 2011) and at least 24 base modifications (Wilson et al., 2003) have been identified, along with DNA–protein cross-links (Cadet et al., 1997), all of which potentially lead to genomic instability. RNA has also been shown to be susceptible to radical attack (Li et al., 2006) but may be less chemically susceptible than DNA (Thorp, 2000). Oxidative damage to DNA can lead to point mutations, deletions, insertions, or chromosomal translocations, which may cause activation of oncogenes and inactivation of tumour suppressor genes and may lead to initiation of carcinogenesis (reviewed by Bartsch and Nair, 2006; Klaunig et al., 2011).

Clearly, high levels of oxygen radicals can be fatal to the cell through overt necrosis or the induction of apoptosis, but lower levels may also contribute to the process of carcinogenesis through stimulation of cellular proliferation and alterations in other cellular functions. There appear to be a myriad of potential mechanisms for these effects, involving induction of transcription factors for numerous signalling pathways, particularly nuclear factor erythroid 2-related factor 2 ($Nrf_2$), mitogen-activated protein kinase (MAPK)/AP1, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and hypoxia-inducible transcription factor 1 alpha (HIF-1α) (reviewed by Klaunig et al., 2011). Protein kinase C, which is also susceptible to activation by cellular oxidants, is a family of serine/threonine kinases that is central to the regulation of many cellular functions, including proliferation, cell-cycle control, differentiation, cytoskeletal organization, cell migration, and apoptosis (Wu, 2006). There is also evidence that the activated oncogene v-Ha-*Ras* may act as a sustained proliferative stimulus in transformed fibroblasts through superoxide-mediated signalling pathways (Irani et al., 1997).

## Vulnerable tissue sites and selected carcinogenic exposures

The synthesis of thyroid hormones requires iodination of thyroglobulin in a peroxidase-catalysed reaction that is dependent on $H_2O_2$. Krohn et al. (2007) reviewed evidence suggesting that the thyroid is particularly sensitive to formation of malignant nodules induced by oxidative stress. During active hormone synthesis, $H_2O_2$ levels are held in check with increased concentrations of glutathione peroxidases along with thyroid peroxidase, and high levels of glutathione peroxidases can interfere with the synthesis of thyroid hormones. Immunostaining for 8-oxo-2′-deoxyguanosine shows greater intensity in thyroid follicular cells near the lumen where $H_2O_2$ is generated than in the spleen, liver, or lung, suggesting a high level of oxidative DNA damage in the thyroid (Maier et al., 2006).

Environmental insults may augment oxidative DNA damage in the thyroid. Thyroid uptake of iodine-131 released during the accident with the Chernobyl Nuclear Power Plant in Ukraine is thought to be responsible for the high rate of papillary carcinoma observed in exposed children (Bennett et al., 2006). Rats and mice exposed to iodine-131 develop follicular cell tumours (IARC, 2012b). Thiocyanate from cigarette smoke may, by inhibiting uptake of iodine, cause oxidative damage through iodine deficiency. Production of thyroid-stimulating hormone is increased during periods of iodine deficiency, and this hormone stimulates $H_2O_2$ production in the thyroid. Levels of antioxidant enzymes have been shown to be elevated by iodine deficiency (Krohn et al., 2007).

The lung is also a vulnerable target for oxidative damage, by virtue of its exposure to air, which contains 21% oxygen, as opposed to the much lower oxygen concentrations in systemic tissues (Carreau et al., 2011). A key role of oxygen radicals in the pulmonary toxicity resulting from prolonged exposure to hyperoxia is demonstrated by the dramatic difference in the sensitivity of adult animals of various species, which succumb to oxygen toxicity after less than a week of exposure to 100% oxygen, compared with the ability of neonates of certain of these species to survive such exposures with little evidence of injury. The neonates of species resistant to pulmonary injury are capable of increasing their levels of antioxidant enzymes in response to hyperoxia, in contrast to the adults, which are incapable of mounting a similar response (Frank et al., 1978).

Several metals, metalloids, and fibres that contain metals or are frequently contaminated with metals have been demonstrated to cause cancer of the lung in humans and experimental animals (IARC, 2012a). These include certain forms of arsenic, asbestos, beryllium, cadmium, chromium, and nickel. Although carcinogenesis induced by metals appears to involve many mechanisms common to the process for other carcinogens, oxidative stress has been implicated as an important contributing factor for several metals (reviewed by Beyersmann and Hartwig, 2008).

Certain metals may undergo direct redox cycling, as demonstrated by the participation of nickel(II) in a Fenton-like reaction with $H_2O_2$, or in the metabolism of trivalent to pentavalent arsenic. Other metals, such as cadmium, may inhibit antioxidant enzymes or deplete antioxidants (cadmium and arsenic bind with sulfhydryl groups in glutathione) or potentially delocalize iron or copper from protected storage sites (e.g. trivalent arsenic can release iron from ferritin), making them available for participation in oxygen radical reactions. Still other metals, such as arsenic (reviewed by Shi et al., 2004), may activate signalling pathways through increased production of oxygen radicals, and potentially promote radical reactions through a variety of mechanisms. Finally, many studies have shown that asbestos or

asbestos-like materials (respirable elongated mineral particles or fibres) are capable of generating oxygen radicals, primarily through reactions catalysed by iron that is present in coordination bonding within the mineral structure, is associated with the surface, or is chelated and released from the fibre by various intracellular organic acids, such as citrate (Aust et al., 2011).

## Summary

Many substances recognized as carcinogens in both humans and experimental animals are capable of influencing redox processes and redox balance within target cells. Oxygen radicals are capable of interacting with and influencing many cellular processes believed to be involved in the dysregulation of normal cellular physiology, thus sending the cells down the pathway to cancer. Oxygen radical reactions are intimately involved in many well-recognized mechanisms of carcinogenesis, such as inflammation, genomic instability, and cell proliferation. Because of the fundamental involvement of the oxygen radical in many of these processes, substances that have been shown to promote cellular injury induced by oxygen radicals should be considered as putative human carcinogens until it has been adequately demonstrated otherwise (Bucher and Portier, 2004).

# References


Aust A, Cook PM, Dodson RF (2011). Morphological and chemical mechanisms of elongated mineral particle toxicities. J Toxicol Environ Health B Crit Rev. 14(1–4):40–75. http://dx.doi.org/10.1080/10937404.2011.556046 PMID:21534085

Babior BM (1999). NADPH oxidase: an update. Blood. 93(5):1464–76. PMID:10029572

Bartsch H, Nair J (2006). Chronic inflammation and oxidative stress in the genesis and perpetuation of cancer: role of lipid peroxidation, DNA damage, and repair. Langenbecks Arch Surg. 391(5):499–510. http://dx.doi.org/10.1007/s00423-006-0073-1 PMID:16909291

Beckman JS (1996). The physiological and pathological chemistry of nitric oxide. In: Lancaster J, editor. Nitric oxide: principles and actions. San Diego (CA), USA: Academic Press; pp. 1–82. http://dx.doi.org/10.1016/B978-012435555-2/50002-4

Bennett B, Repacholi M, Carr Z, editors (2006). Health effects of the Chernobyl accident and special health care programmes: report of the UN Chernobyl Forum Expert Group "Health". Geneva, Switzerland: World Health Organization. Available from: http://www.who.int/ionizing_radiation/chernobyl/WHO%20Report%20on%20Chernobyl%20Health%20Effects%20July%2006.pdf.

Benzie IFF (2000). Evolution of antioxidant defence mechanisms. Eur J Nutr. 39(2):53–61. http://dx.doi.org/10.1007/s003940070030 PMID:10918985

Beyersmann D, Hartwig A (2008). Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol. 82(8):493–512. http://dx.doi.org/10.1007/s00204-008-0313-y PMID:18496671

Bucher JR, Portier C (2004). Human carcinogenic risk evaluation, part V: the National Toxicology Program vision for assessing the human carcinogenic hazard of chemicals. Toxicol Sci. 82(2):363–6. http://dx.doi.org/10.1093/toxsci/kfh293 PMID:15456919

Bucher JR, Tien M, Aust SD (1983). The requirement for ferric in the initiation of lipid peroxidation by chelated ferrous iron. Biochem Biophys Res Commun. 111(3):777–84. http://dx.doi.org/10.1016/0006-291X(83)91366-9 PMID:6838585

Cadet J, Berger M, Douki T, Ravanat JL (1997). Oxidative damage to DNA: formation, measurement, and biological significance. Rev Physiol Biochem Pharmacol. 131:1–87. PMID:9204689

Carreau A, El Hafny-Rahbi B, Matejuk A, Grillon C, Kieda C (2011). Why is the partial oxygen pressure of human tissues a crucial parameter? Small molecules and hypoxia. J Cell Mol Med. 15(6):1239–53. http://dx.doi.org/10.1111/j.1582-4934.2011.01258.x PMID:730565

Frank L, Bucher JR, Roberts RJ (1978). Oxygen toxicity in neonatal and adult animals of various species. J Appl Physiol Respir Environ Exerc Physiol. 45(5):699–704. PMID:730565

Hanahan D, Weinberg RA (2011). Hallmarks of cancer: the next generation. Cell. 144(5):646–74. http://dx.doi.org/10.1016/j.cell.2011.02.013 PMID:21376230

Hatcher HC, Singh RN, Torti FM, Torti SV (2009). Synthetic and natural iron chelators: therapeutic potential and clinical use. Future Med Chem. 1(9):1643–70. http://dx.doi.org/10.4155/fmc.09.121 PMID:21425984

Hibbs JB Jr, Taintor RR, Vavrin Z, Rachlin EM (1988). Nitric oxide: a cytotoxic activated macrophage effector molecule. Biochem Biophys Res Commun. 157(1):87–94. http://dx.doi.org/10.1016/S0006-291X(88)80015-9 PMID:3196352

Hrycay EG, Bandiera SM (2015). Involvement of cytochrome P450 in reactive oxygen species formation and cancer. Adv Pharmacol. 74:35–84. http://dx.doi.org/10.1016/bs.apha.2015.03.003 PMID:26233903

IARC (2012a). Arsenic, metals, fibres, and dusts. IARC Monogr Eval Carcinog Risks Hum. 100C:1–499. Available from: http://publications.iarc.fr/120 PMID:23189751

IARC (2012b). Radiation. IARC Monogr Eval Carcinog Risks Hum. 100D:1–437. Available from: http://publications.iarc.fr/121 PMID:23189752

Irani K, Xia Y, Zweier JL, Sollott SJ, Der CJ, Fearon ER, et al. (1997). Mitogenic signaling mediated by oxidants in Ras-transformed fibroblasts. Science. 275(5306):1649–52. http://dx.doi.org/10.1126/science.275.5306.1649 PMID:9054359

Klaunig JE, Wang Z, Pu X, Zhou S (2011). Oxidative stress and oxidative damage in chemical carcinogenesis. Toxicol Appl Pharmacol. 254(2):86–99. http://dx.doi.org/10.1016/j.taap.2009.11.028 PMID:21296097

Kojima C, Ramirez DC, Tokar EJ, Himeno S, Drobná Z, Stýblo M, et al. (2009). Requirement of arsenic biomethylation for oxidative DNA damage. J Natl Cancer Inst. 101(24):1670–81. http://dx.doi.org/10.1093/jnci/djp414 PMID:19933942

Krohn K, Maier J, Paschke R (2007). Mechanisms of disease: hydrogen peroxide, DNA damage and mutagenesis in the development of thyroid tumors. Nat Clin Pract Endocrinol Metab. 3(10):713–20. http://dx.doi.org/10.1038/ncpendmet0621 PMID:17893690

Li Z, Wu J, Deleo CJ (2006). RNA damage and surveillance under oxidative stress. IUBMB Life. 58(10):581–8. http://dx.doi.org/10.1080/15216540600946456 PMID:17050375

Liu J, Qian SY, Guo Q, Jiang J, Waalkes MP, Mason RP, et al. (2008). Cadmium generates reactive oxygen- and carbon-centered radical species in rats: insights from in vivo spin-trapping studies. Free Radic Biol Med. 45(4):475–81. http://dx.doi.org/10.1016/j.freeradbiomed.2008.04.041 PMID:18501199

Lu AL, Li X, Gu Y, Wright PM, Chang DY (2001). Repair of oxidative DNA damage: mechanisms and functions. Cell Biochem Biophys. 35(2):141–70. http://dx.doi.org/10.1385/CBB:35:2:141 PMID:11892789

Ma Q (2010). Transcriptional responses to oxidative stress: pathological and toxicological implications. Pharmacol Ther. 125(3):376–93. http://dx.doi.org/10.1016/j.pharmthera.2009.11.004 PMID:19945483

Maier J, van Steeg H, van Oostrom C, Karger S, Paschke R, Krohn K (2006). Deoxyribonucleic acid damage and spontaneous mutagenesis in the thyroid gland of rats and mice. Endocrinology. 147(7):3391–7. http://dx.doi.org/10.1210/en.2005-1669 PMID:16627585

Mena S, Ortega A, Estrela JM (2009). Oxidative stress in environmental-induced carcinogenesis. Mutat Res. 674(1–2):36–44. http://dx.doi.org/10.1016/j.mrgentox.2008.09.017 PMID:18977455

Miller DM, Buettner GR, Aust SD (1990). Transition metals as catalysts of "autoxidation" reactions. Free Radic Biol Med. 8(1):95–108. http://dx.doi.org/10.1016/0891-5849(90)90148-C PMID:2182396

Schrader M, Fahimi HD (2006). Peroxisomes and oxidative stress. Biochim Biophys Acta. 1763(12):1755–66. http://dx.doi.org/10.1016/j.bbamcr.2006.09.006 PMID:17034877

ShiH, ShiX, LiuKJ (2004). Oxidative mechanism of arsenic toxicity and carcinogenesis. Mol Cell Biochem. 255(1–2):67–78. http://dx.doi.org/10.1023/B:MCBI.0000007262.26044.e8 PMID:14971647

St-Pierre J, Buckingham JA, Roebuck SJ, Brand MD (2002). Topology of superoxide production from different sites in the mitochondrial electron transport chain. J Biol Chem. 277(47):44784–90. http://dx.doi.org/10.1074/jbc.M207217200 PMID:12237311

Thorp HH (2000). The importance of being r: greater oxidative stability of RNA compared with DNA. Chem Biol. 7(2):R33–6. http://dx.doi.org/10.1016/S1074-5521(00)00080-6 PMID:10662699

Tien M, Bucher JR, Aust SD (1982). Thiol-dependent lipid peroxidation. Biochem Biophys Res Commun. 107(1):279–85. http://dx.doi.org/10.1016/0006-291X(82)91701-6 PMID:6289822

Toyokuni S, Sagripanti J-L (1996). Association between 8-hydroxy-2′-deoxyguanosine formation and DNA strand breaks mediated by copper and iron. Free Radic Biol Med. 20(6):859–64. http://dx.doi.org/10.1016/0891-5849(95)02184-1 PMID:8728035

West JD, Marnett LJ (2006). Endogenous reactive intermediates as modulators of cell signaling and cell death. Chem Res Toxicol. 19(2):173–94. http://dx.doi.org/10.1021/tx050321u PMID:16485894

Wilson DM 3rd, Sofinowski TM, McNeill DR (2003). Repair mechanisms for oxidative DNA damage. Front Biosci. 8(1–3):d963–81. http://dx.doi.org/10.2741/1109 PMID:12700077

Wu WS (2006). The signaling mechanism of ROS in tumor progression. Cancer Metastasis Rev. 25(4):695–705. http://dx.doi.org/10.1007/s10555-006-9037-8 PMID:17160708