Oxy-radicals and cancer

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Many human cancers are caused by radiation and by exogenous carcinogens in the air and in the diet. Whilst xenobiotics and nutritional carcinogens of natural origin are potentially removable from the environment, oxy-radicals, which are produced from molecular oxygen and solar radiation, are ubiquitous unavoidable carcinogens to which we are constantly exposed. Under physiological conditions oxy-radicals are part of normal regulatory circuits and the cellular redox state is tightly controlled by antioxidants. However, increased flux of oxy-radicals and loss of cellular redox homoeostasis can be tumorigenic. Most carcinogens operate by common cellular mechanisms. However, the relative importance of the individual steps that lead to malignant transformation depends on the type of carcinogen, the target tissue, and individual genetic susceptibility. Crucial properties of carcinogens are their capacity to cause permanent structural changes in DNA as base-pair mutations, deletions, insertions, rearrangements, and sequence amplification, to activate cytoplasmic and nuclear signal transduction pathways, and to modulate the activity of stress proteins and stress genes that regulate effector genes related to growth, differentiation, and cell death. Oxy-radicals possess all three properties.

Mutation of cancer-related genes

The most important cancer-related genes are *ras*-family proto-oncogenes and the *P53* tumour-suppressor gene. The mutations observed suggest causative mutagens, and in some instances oxy-radicals might have a role. For example, G→T transversions are frequently observed in the middle position of hotspot codon 12 of Ki-*ras* and H-*ras* in non-melanoma skin tumours.[1] They could be produced by misreplication of 8-hydroxy-deoxyguanosine lesions induced by oxy-radicals.[2] At least some of the G→A transversions in *P53* in non-melanoma skin tumours could be similarly caused. Skin cancer is related to solar radiation, and near-ultraviolet light (290–380 nm) damages DNA partly by oxidative mechanisms. G→T transversions have also been observed in several *P53* codons in smoking-related lung carcinoma,[3] and specifically in codon 249 in hepatocellular carcinoma cases from southern China[4] and sub-Saharan Africa.[5] These mutations could be due to release of oxy-radicals into the tissue by inflammatory leucocytes. However, the same base-pair change is also induced by bulky carcinogens (eg, benzo(a)pyrene in tobacco smoke, and the food contaminant aflatoxin B_1). Colorectal carcinoma is a third class of tumour in which oxy-radicals might be causal mutagens. Almost 95% of the mutations at *P53* hotspot codon 248 (CGG) in these tumours consist of C→T and G→A transitions at the CpG dinucleotide sequence. These base-pair changes are typically produced by deamination of 5-methyl-cytosine which is enhanced by oxy-radicals, in particular by nitric oxide.[6]

The second wild-type allele of a mutated proto-oncogene or tumour-suppressor gene is often inactivated or eliminated during tumour promotion and progression, which allows full expression of the mutated phenotype. Oxy-radicals are attractive candidates here because they produce gross chromosomal changes as well as point mutations.[7] Indeed, the carcinogenic properties of tumour promoters such as phorbol-esters and peroxides may lie in their capacity to stimulate growth and induce permanent chromosomal damage. Phorbol-esters do produce a cellular pro-oxidant state.[7]

Expression of stress genes

As well as causing permanent genetic changes, oxy-radicals activate cytoplasmic signal transduction pathways that are related to growth, differentiation, and cell death. Indeed, oxy-radicals in many ways mimic the action of polypeptide factors. Calcium-ion mobilisation from mitochondria and endoplasmic reticulum and influx from the extracellular space are some of the earliest events after exposure to oxy-radicals.[8] The consecutive changes in the activities of kinases[9] and phosphatases,[10] which transduce the initial signal to a family of transcription factors, are only beginning to be explored. They are part of a complex network that allows an immediate response to several forms of cellular stress. In some instances oxidative stress may result more directly in the alteration of the activity of transcription factors. For example, nuclear factor kappa-B is separated from its inhibitory subunit after exposure of cells to oxy-radicals, tumour necrosis factor α, or interleukin-1[11] and the oncoproteins Fos and Jun require intact, reduced cysteine residues in strategic positions for dimerisation and full transcriptional activity.[12] The transient activation of these and related transcription factors is quickly followed by their enhanced synthesis and the transactivation of sub-families of effector genes, such as collagenase, metallothionein IIA, and certain viral long-terminal repeats.[13]

Activation of these circuits of gene expression by oxy-radicals can participate in tumour promotion and progression. Although the trigger that initiates these cascades of events is likely to be located at the cell membrane,[14] other pathways may be activated by DNA damage, especially by DNA strand breaks. Several genes that are transcriptionally induced following exposure to DNA-damaging agents, including oxy-radicals, have been cloned.[15] However, it is difficult to exclude the involvement of molecules other than DNA.

The regulation of *P53* expression by radiation and simple alkylating agents is of interest in this regard. Analogous to

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the transcription factors, *P53* binds to specific DNA sequence motifs and transactivates genes (eg, *MDM-2* and *GADD45*) downstream from these sites.[16] Wild-type *P53* protein accumulates in the nuclei after exposure to the above agents, which produce DNA breaks. The fact that DNA with breaks introduced by a restriction enzyme resulted in *P53* accumulation demonstrates that DNA breaks themselves can serve as the initiating signal.[17] Accumulation of *P53* after DNA damage may serve to arrest cells at the G1/S border of the cell cycle to allow repair of DNA lesions before replication.[18] This regulation may be impaired in cells containing mutated *P53*. Oxy-radicals efficiently cause DNA breakage and it remains to be seen whether they regulate *P53* and the cell cycle by mechanisms analogous to radiation. Possibly, as well as these consequences of DNA breakage, direct oxidative inactivation of *P53* has a role in carcinogenesis. The native conformation of P53, which allows its binding to DNA, requires the presence of a reduced cysteine residue in a specific position in the protein.[19]

Antioxidant defences and oxidant carcinogenesis

Epidemiological studies on serum antioxidants and diet suggest that an increased level of vitamin E and β-carotene reduces mortality from cancer in the lung and colon.[20,21] At least for lung cancer in smokers, these results have been questioned by a study in which no protection was observed.[22] The cellular antioxidant defences consist of multiple interdependent components of low and high molecular weight. A fine balance between several antioxidants appears to be more important for the overall protective capacity of the system than the activity of a single enzyme.[23] Therefore it is not surprising that measurements of the levels of antioxidant enzymes in tumours have yielded inconclusive results, with the possible exception of manganese superoxide dismutase, the concentration of which was often below that of normal tissue. An anticarcinogenic function of this enzyme is also suggested by the observation that at least some tumour cells may be hypersensitive to killing by tumour necrosis factor α because they are deficient in the transcriptional induction of this antioxidant enzyme.[24]

In view of the multiple stages in carcinogenesis where antioxidants could act, it is to be expected that they are not anticarcinogenic in every case. Whilst high antioxidant capacity shields DNA from oxidative damage and mutagenesis, it may at the same time protect "initiated" cells from excessive oxidant toxicity and apoptosis, and favour their clonal expansion in tumour promotion.[8] For example, the oncoprotein BCL-2 may owe its oncogenic property to its role in the regulation of a cellular antioxidant pathway. BCL-2 blocks apoptotic cell death induced by hydroperoxides, menadione, and certain cytokines.[25]

No global answer can be given about the role of individual antioxidants in human tumorigenesis. The outcome will depend on the multiple, interacting antioxidant components of the target tissue and the particular carcinogen to which it is exposed.

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