記念特別講演論文

The Role of Oxygen Free Radicals in Carcinogenesis

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Carcinogenesis is a complex process resulting from DNA damage, altered gene expression, *etc.* A precise understanding of the molecular events leading to the initiation, promotion, and progression of cancer is not available but advances in biochemistry and molecular biology have greatly increased the pace of our progress. In particular, research in recent years has implicated oxygen radicals and their products as important endogenous contributors to carcinogenesis in human beings. Oxygen radicals and products of oxygen radical metabolism have been demonstrated to damage DNA leading to mutations in oncogenes and growth suppressor genes. Oxygen radicals have also been implicated in the process of tumor promotion. This account will review recent findings related to the involvement of oxygen radicals in gene mutation and tumor promotion from a qualitative and quantitative perspective.

DNA Damage by Oxygen Radicals

Oxygen free radicals are generated in humans by reduction of molecular oxygen and during lipid peroxidation. Certain oxygen radicals, such as the hydroxyl radical, attack DNA directly and produce strand breaks and DNA adducts. DNA adducts can be miscoding and lead to the introduction of mutations at

the site of adduction. For example, 8-oxodeoxyguanosine (eq 1) produces G → T transversions at the site of adduction.¹⁾ These types of mutations are frequently seen in oncogenes and growth suppressor genes and may reflect DNA damage by endogenously generated oxygen radicals. DNA repair proteins have been characterized that protect against oxygen radical-dependent mutations by excising the adducted base.²⁾ Quantitation of the levels of excised bases in plasma and urine provides strong evidence that oxygen radical damage to DNA occurs at a significant level on a continuous basis in human beings.³⁾

DNA Damage by Malondialdehyde

Damage to other biomolecules by oxygen radicals may also contribute to carcinogenesis. Peroxidation of the unsaturated fatty acid residues and cholesterol molecules of membranes produces derivatives that lead to DNA damage and mutation. For example, bifunctional carbonyl compounds such as malondialdehyde (MDA) and 4-hydroxynonenal are mutagenic in bacterial and mammalian cells and produce a number of adducts when reacted with DNA.^{4,5)} MDA forms adducts to guanine, adenine, and cytosine bases (Fig. 1) and induces G → T

Fig. 1 Malondialdehyde Adducts to Guanine, Adenine, and Cytosine Nucleosides

transversions, A → G transitions, and C → T transitions.⁶⁾ MDA also induces frameshift mutations, mainly additions, which may be caused by a pyrimidopurinone adduct to guanine.⁷⁾ Efforts are underway to develop highly sensitive analytical methods with which to quantitate the formation of MDA-DNA adducts in tissues samples and biological fluids from human beings. Such methods should be useful dosimeters with which to evaluate the hypothesis that MDA generation by oxygen radicals contributes to human carcinogenesis.

Oxygen Radicals and Tumor Promotion

In experimental animal studies, DNA damaging agents are termed tumor initiators. Several non-tumorigenic compounds enhance the response to initiators and are termed tumor promoters. The process of tumor promotion is poorly understood but results in a decreased latency period and an increased number of tumors. The classic examples of tumor promoters are phorbol esters isolated from plant sources. More recently, a number of potent tumor promoters have been isolated from marine sources. Many of these compounds appear to act by mechanisms similar to phorbol esters in that they bind to protein kinase C in an important early step leading to tumor promotion.

The results of *in vitro* experiments performed with freshly isolated cells or cultured cells suggest that oxygen radicals may con-

tribute to tumor promotion by phorbol esters and certain non-phorbol ester tumor promoters.⁸⁾ The role of oxygen radicals in tumor promotion is not understood but it may involve direct or indirect DNA damage or altered gene expression. A key piece of experimental evidence to support the involvement of oxygen radicals in tumor promotion is the demonstration that they are formed in intact animals treated with phorbol or non-phorbol ester tumor promoters. This is a challenging task because oxygen radicals have very short half-lives that prevents their direct detection in animals or their tissues.⁹⁾ Our laboratory has used (7*S*,8*S*)-dihydroxy-7,8-dihydrobenzo [*a*]pyrene ((+)-BP-7,8-diol) as a diagnostic probe for oxygen radical-dependent oxidation.¹⁰⁾ (+)-BP-7,8-diol is epoxidized to (7*S*,8*R*)-dihydroxy-(9*S*,10*R*)-epoxy-7,8,9,10-tetrahydrobenzo [*a*]pyrene ((+)-*syn*-BPDE) by cytochrome P-450 enzymes and to (7*S*,8*R*)-dihydroxy-(9*R*,10*S*)-epoxy-7,8,9,10-tetrahydrobenzo [*a*]pyrene ((-)-*anti*-BPDE) by peroxyl free radicals (Fig. 2).

[³²P]-Postlabeling analysis of the diastereomeric BPDE-DNA adducts was used to investigate the pathways of (+)-BP-7,8-diol oxidation in mouse skin *in vivo*.¹¹⁾ The pattern of deoxynucleoside-3',5'-bis-phosphate adducts in epidermal scrapings from female CD-1 mice indicated that cytochrome P-450 was the major oxidant. Similar results were obtained when the tumor-promoting phorbol

Fig. 2 Stereochemistry of Epoxidation of (+)-BP-7,8-diol by Cytochrome P-450 and Peroxyl Free Radicals

ester tetradecanoylphorbolacetate (TPA) was coadministered with (+)-BP-7,8-diol. However, when animals were pretreated with TPA 24 h before coadministration of TPA and (+)-BP-7,8-diol, the pattern of BPDE-DNA adducts indicated that peroxyl radicals made a major contribution to (+)-BP-7,8-diol epoxidation.¹²⁾ Peroxyl radical-dependent epoxidation was maximal when the time between the two TPA administrations was 24–72 h. No increase in (–)-*anti*-BPDE-DNA was observed when the non-tumor-promoting phorbol ester 4-*O*-methyl-TPA was substituted for TPA. The calcium ionophore A23187 stimulated peroxyl radical generation when substituted for the first, but not the second, TPA treatment. The antiinflammatory steroid fluocinolone acetonide inhibited (–)-*anti*-BPDE-DNA adduct formation when coadministered with the first but not the second TPA treatment. These findings demonstrate the existence of two independent pathways of

oxidant generation in mouse epidermis, one dependent on cytochrome P-450 and the other dependent on peroxyl free radicals. The results also suggest that repetitive topical administration of tumor-promoting phorbol esters remodels epidermal metabolism leading to a significant increase in oxygen radical generation.

References

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