Molecular Mechanisms in Chemically Induced Cancer*

P. J. O'Connor, Ph. D.

About one third of the agents established as carcinogenic to man have affinities for lung. These include defined compounds and the complex mixtures found in tar, oils, soot, car exhausts, and tobacco smoke.¹ To understand mechanisms of initiation of these cancers, detailed studies of the biologic actions of specific classes of chemicals have been made. These have implicated DNA as a critical target molecule and highlighted the role of metabolism in the generation of the chemically reactive form of the carcinogen which can bind to macromolecules.² Benzo(a)pyrene, for example, which occurs in engine exhausts, cigarette smoke, and generally as a product of incomplete combustion, has been used as a classic polycyclic aromatic hydrocarbon to study metabolism and binding to DNA in cells from individuals from defined family groups. These data indicate that such parameters are genetically controlled, thereby implicating an inherited predisposition as a potential factor in the development of lung cancer.³

Benzo(a)pyrene is, however, one of over 100 chemicals identified in cigarette smoke. More than 40 are carcinogenic in animals, and several are N-nitroso compounds, a highly potent group of carcinogens producing tumors at diverse sites in animals.¹ Exposure to these agents can also arise from foodstuffs, beverages, industrial atmospheres, and from endogenous sources due to the N-nitrosation of ingested precursors.⁴ Many of the N-nitroso compounds require metabolism to form chemically reactive intermediates and those which transfer small alkyl (eg, methyl and ethyl) groups to DNA show affinities for respiratory tissues. The methylating agent 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, for example, is one of the tobacco-specific nitrosamines and the most potent lung carcinogen known in laboratory animals.⁵

Mechanisms of carcinogenesis induced by alkylating agents have been reviewed extensively.⁶ Factors that determine the organ specificity of tumor induction include dose, route of administration, metabolism, DNA alkylation, and the capacity for the repair of specific DNA lesions. Of the 12 lesions produced in DNA by these agents, only 2 are highly promutagenic (ie, O⁰-alkylthymine and O⁰-alkylguanine). They are implicated in mechanisms of cancer initiation, since during DNA synthesis, due either to normal cell replacement or arising from carcinogen induced tissue toxicity, replication over these modified bases gives rise to GC→AT or AT→GC transitions, thereby conferring a heritable change on the cell. The presence of DNA adducts during cell division may also lead to clastogenic events, which could place a gene under the influence of different controlling elements, thereby leading to aberrant expression. These random, mutational changes may affect any gene, but of particular relevance for carcinogenesis are the so-called oncogenes, which are capable of diverting cells toward the malignant phenotype if they are affected by changes of the kind described above.⁶

Protection against the biologic effects of environmental alkylating agents is afforded by specific DNA repair mechanisms capable of restoring the integrity of the genetic message and is best illustrated by reference to the well characterized Escherichia coli repair protein encoded by the ada gene. This 37 kDa methyltransferase (MT) removes the methyl group from the O⁰-position of guanine or the O⁰-position of thymine in DNA and binds it covalently to a cysteine residue within the protein itself. A second cysteine residue within the same protein removes the alkyl group from the phosphate moiety of the sugar-phosphate DNA backbone. This autoactivating process is expensive to the cell's economy as synthesis of new repair protein is required.⁶ Its value to the cell has been demonstrated by transfection of the cloned ada gene into repair-deficient Chinese hamster ovary cells in culture. Such studies have shown that this prokaryotic gene can function in a mammalian environment and that it can effectively protect against the toxic, mutagenic, and SCE-induced effects of treatment with MNU.⁷ These observations confirm the hazardous nature of specific DNA alkylation products, particularly of O⁰-methylguanine (O⁰-MeG), and similar studies with transgenic animals⁸ should eventually demonstrate their role in carcinogenesis.

The nuclei and mitochondria of mammalian cells possess a smaller (24 kDa) MT protein capable only of repairing O⁰-MeG.⁸ Repair of O⁰-methylthymine is via a separate protein, and methylphosphotriesters are repaired slowly, if at all, in mammalian cells. The repair of O⁰-MeG from individual nuclei can be followed in situ by immunohistochemical procedures.⁹ In rats treated with the classic methylating agent N-nitrosodimethylamine (NDMA; 40 mg/kg), O⁰-MeG-positive nuclei are distributed sporadically throughout the lung tissue. In some bronchioles, for example, the nuclei of the lining cells are intensely positive, but 12 days later the O⁰-MeG has been removed from most of the cells. Some, however, still show highly positive nuclei and are clearly repair-deficient. Lung tissue evidently contains cells which are both metabolically proficient and repair deficient, characteristics which are genetically determined, represent a double risk factor and could be affected by genetic predisposition.

We are now attempting to relate these high-dose animal studies to the human situation by using low-doses of NDMA (ie, 1–100 μg/kg). Although the O⁰-MeG introduced into hepatic DNA by such doses is rapidly repaired, the efficiency of repair is markedly increased in rats previously exposed to the inducing agent phenobarbital.¹¹ Thus, at environmentally relevant doses O⁰-MeG does not escape the repair process, it is not sequestered in DNA, and prospects for increasing repair efficiency using membrane active agents are now indicated. More sensitive, immunohistochemical assays of O⁰-MeG for use with human tissue (eg, smoker’s lung) are also being developed.

Sensitive radioligand DNA footprinting techniques already permit direct measurements of the exposure of human tissues to environmental alkylating agents.¹²¹³ The tissue DNA of patients undergoing surgery for cancers of the esophagus in northern China¹⁰ and Southeast Asia¹³ or GI problems in Manchester¹³ contain O⁰-MeG in the range 0.01–0.2
μmol/mol parent base. The O-MeG levels in samples from the East suggest a general environmental exposure while the skewed distribution, with many negative samples in the Manchester group, suggest an association with lifestyle and/or medication. A possible example of the latter is indicated by isoniazid, which, although not an alkylating agent as such, leads to the methylation of DNA in liver and lung at low levels when administered to rats and mice. While such exposures may not present a significant risk for man they are presumably additive with other environmental exposures. Certain individuals may have greater capacities for the generation of alkylating intermediates via this and other endogenous routes and consequently may be more at risk from the toxic and carcinogenic properties of such compounds.

Prospects for cancer prevention therefore should include not only the prevention of exposure to exogenous and endogenous alkylating agents themselves, but also an improved knowledge of ways in which DNA repair activity may be optimized and the deleterious effects of alkylation averted.

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Oncogenes and Genetic Abnormalities in Lung Cancer
Desmond N. Carney, M.D., Ph.D.

In spite of recent advances in the diagnosis, staging, treatment, and identification of prognostic factors for lung cancer, few advances have been made in overall patient survival over the past decade. Thus, lung cancer, which accounts for 35% of all cancer deaths in males and 19% in females, remains a major medical dilemma. While elimination of cigarette smoking would significantly reduce the number of cases, it is clear that this is an unrealistic option. Thus, there is a need for the identification and application of new therapeutic strategies.

The major advances over the past 5-10 years in lung cancer have been the greater understanding of the biological properties of both small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC); the recognition of the extreme heterogeneity that exists within these tumors with respect to these properties; and, finally, the better understanding of the molecular events which take place in this disease including both specific and nonspecific chromosomal abnormalities, and alteration in the expression of a range of oncogenes. These changes include chromosomal rearrangements and deletions, point mutations, gene amplification, and altered gene expression. These recently recognized events will be discussed as they pertain to lung cancer.

Proto-Oncogene Amplification and Expression

Much of the information relating to oncogene derangements in lung cancer initially came from the study of well-characterized cell lines of both SCLC and NSCLC. Detailed biologic characterization of SCLC cells revealed two major classes, namely: classic and variant SCLC cell lines. The latter cell lines were associated with a more aggressive growth behavior both in vivo and in vitro; a selective loss of certain neuroendocrine markers; a relative resistance to radiotherapy; and cytogenetic abnormalities including the presence of double minute chromosomes or one or more homogeneously staining regions (HSRs).

Initial studies of these variant cell lines revealed up to 76-fold amplification of c-myc compared to classic SCLC lines. Overexpression of c-myc was noted in most amplified cell lines. Patients whose cell lines were amplified for c-myc had a shorter survival than those who were not. These and other in vitro transfection studies suggest that in SCLC, c-myc is important in growth regulation and in the expression of the variant morphology.

Further studies on larger panels of both SCLC and NSCLC cell lines have revealed that other members of the myc-family of oncogenes, namely N-myc and L-myc, may be important in the biology of SCLC. As with c-myc, in most specimens where N-myc amplification has been demonstrated, overexpression of N-myc mRNA has been noted. In contrast, expression of L-myc has been found to occur in

*Department of Medical Oncology, Mater Misericordiae Hospital, Dublin, Ireland.
Reprint requests: Dr. Carney, Mater Hospital, Dublin 7, Ireland