Conclusion

There exist several promising immunologic approaches to vaccine therapy of cancer. The challenge of immunotherapy research is to determine which combination of approaches leads to a favorable clinical response and outcome. Several studies have shown enhanced survival of patients receiving vaccines; however, a randomized phase III clinical trial has yet to show a statistically significant improvement in the survival of such patients.

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Cap

Definition

A cap is a structure at the 5′ end of eukaryotic mRNA, introduced after transcription by linking the terminal phosphate of 5′-GTP to the terminal base of the mRNA. The added G (and sometimes some other bases) are methylated, giving a structure of the form 7MeG5′ppp5′Np.

Carbohydrate Antigen 19-9

Definition

Carbohydrate antigen 19-9 is a cell surface antigen consisting of a sialylated lacto-N-fucopentose that is frequently found in increased levels in the serum of patients with various types of malignancy.

Carcinoembryonic Antigen

Definition

Carcinoembryonic antigen (CEA) is a cell surface glycoprotein detectable immunohistochemically in limited amounts in benign cells and more heavily expressed in fetal and some types of neoplastic cells.

Carcinogen

Definition

A carcinogen is any agent, chemical, physical or viral that causes cancer or increases the incidence of cancer.

Carcinogen Macromolecular Adducts

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Definition

Carcinogen-macromolecular adducts are chemical modifications (‘addition products’) of nucleic acids and proteins that form in tissues and cells exposed to reactive chemical species.

Characteristics

Endogenous and exogenous agents produce macromolecular adducts

Chemical carcinogens that induce macromolecular damage may be endogenous or exogenous. The endogenous formation of → reactive oxygen species and other free radicals may cause both appropriate and inappropriate chemical modification of nucleic acids. Exogenous chemicals, including environmental pollutants or

drugs, require activation to reactive species through metabolism. Following covalent binding of reactive chemical species to DNA, a mutation can result if the DNA is not correctly repaired. When reactive chemical species bind covalently to a protein, the resulting adducts persist for the lifetime of the protein. Formation of → DNA adducts is considered necessary for carcinogenesis, while protein modification is considered an indicator of exposure and a surrogate for DNA adduct formation.

Some normal physiological (endogenous) processes result in chemical modification of nucleic acids. For example, selective 5-methylation of cytosine in DNA regulates normal gene expression, and 7-methylation of guanosine in 5′ cap structures of mRNA is necessary for efficient protein synthesis in eukaryotes. Normal endogenous metabolic processes, including lipid peroxidation, nitric oxide metabolism and endogenous nitrosation can produce oxygen free radicals, oxidative-DNA adducts, etheno-adducts and nitrosamine adducts.

Exogenous carcinogenic agents that form macromolecular adducts can be direct-acting if they are highly reactive. Examples are the nitrosoureas, some nitrosamines, ethylene oxide and ozone. However, most are inert, like the polycyclic aromatic hydrocarbons (PAHs), and require metabolic activation. Exogenous carcinogens include some plant and fungal products (aflatoxins, ochratoxins, hydrazines), pyrolysis products from cooking (heterocyclic amines, PAHs), industrial combustion products (aromatic amines, PAHs, nitro-PAHs, benzene, vinyl chloride, nitrosamines, ethylene oxide), urban pollution contaminants (PAHs, nitro-PAHs, aromatic amines) and contents of tobacco smoke (PAHs, nitosamines and aromatic amines).

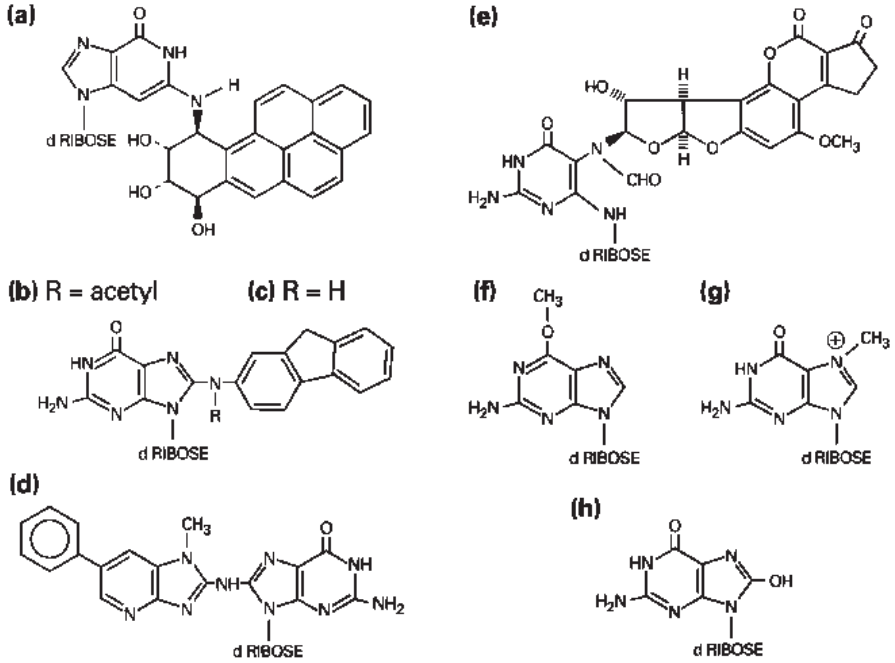
Carcinogen metabolism leading to macromolecular adduct formation

In order to form macromolecular adducts, exogenous agents that can be inhaled, ingested or absorbed through the skin, are altered metabolically by families of enzymes. These enzymes convert a small fraction of the initial dose to highly reactive intermediate metabolites that become bound covalently to specific bases in DNA or amino acids in protein.

Polycyclic aromatic hydrocarbons, such as benzo[a]pyrene (BP) are composed of variable numbers of fused benzene rings and are chemically unreactive and insoluble in water. These compounds are ubiquitous environmental contaminants, contained in cigarette smoke and produced by many industrial processes (→ tobacco carcinogenesis). To form macromolecular adducts in the body they are metabolized to simple epoxides by cytochrome P450, hydrated through the action of epoxide hydrolase and subjected to epoxidation (cytochrome P450) to form unstable dihydrodiol-epoxides. The unstable metabolites spontaneously convert to a positively charged, highly reactive free radical called a carbocation (the ultimate carcinogen), which binds covalently to DNA and protein. The structure of the major BP DNA adduct with deoxyguanosine is shown in Fig. 1a, and this compound also forms adducts with protein.

Aromatic amines are characterized by the presence of benzene rings and an exocyclic nitrogen. A prototypical aromatic amine, 4-aminobiphenyl (4-ABP), is implicated in human bladder cancer. In addition, another class of environmental contaminants, nitrated polycyclic aromatic hydrocarbons are related to aromatic amines by nitroreduction. The presence of the amino-group, which can be either acetylated or non-acetylated, contributes to the complexity of aromatic amine metabolism. Activation of aromatic amines proceeds by N-oxidation with sulfotransferase catalysis that results in the formation of acetylated and non-acetylated guanine adducts as shown in Fig. 1(b and c) for the carcinogen N-2-acetylaminofluorene. Aromatic amines also readily form adducts with protein.

Heterocyclic amines, such as 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PHIP), are formed from the pyrolysis ($>150^{\circ}C$) of amino acids, creatinine and glucose that occurs during cooking. They have a similar structure to aromatic amines and undergo N-hydroxylation (cytochrome P450) and enzymic O-esterification. The major guanine adduct of PHIP is shown in Fig. 1d.



Carcinogen Macromolecular Adducts. Fig. – DNA Adduct Structures.

a) (7R)-N^2-{10-[r-7, t8, t-9-trihydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene]-yl}-deoxyguanosine
b) N-deoxyguanosin-(8-yl)-2-acetylaminofluorene
c) N-deoxyguanosin-(8-yl)-2-aminofluorene
d) 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
e) aflatoxin B_1-N^7-deoxyguanosine
f) O^6-methyl-deoxyguanosine
g) N^7-methyl-deoxyguanosine
h) 8-hydroxydeoxyguanosine

Fungal micotoxins, like aflatoxin B1 derived from *Aspergillus flavus*, contaminate cereals, grain and nuts. Aflatoxin B1 ingestion is correlated with a high incidence of liver cancer. Aflatoxins are hetercyclic and contain several endocyclic oxygen molecules. They are activated by simple epoxidation (cytochrome P450) across the olefinic double bond at the 8,9-position giving rise to a carbocation. However, some addition products with DNA are unstable and lead to non-mutagenic depurination. The major → aflatoxin-guanine adduct is shown in Fig. 1e. Serum and albumin adducts of aflatoxin have been characterized and used frequently as human biomarkers.

Carcinogenic N-nitrosamines can be found in many substances including food, alcoholic beverages and tobacco. N-nitrosodimethylamine is activated (cytochrome P450) through α-hydroxylation to form an unstable α-hydroxy-nitrosamine, which forms formaldehyde and methyl diazohydroxide. The methyl diazohydroxide becomes a free radical and powerful methylating agent, which produces multiple DNA modifications (Fig. 1 f and g). Some nitrosamines such as the tobacco-specific nitrosamine, 4-[methylnitrosoamino]-1-[3-pyridyl]-1-butanone (NNK), are assymetrical and give rise to the formation of bulky DNA adducts. Nitrosamines are also known to form protein adducts.

Oxygen radical damage produced by both endogenous and exogenous events can result in the formation of macromolecular adducts. Pathways that lead to the formation of oxygen radicals include degradation of organic perox-

ides (catechol, hydroquinone, and 4-nitroquinoline-N-oxide), hydrogen peroxide, lipid peroxidation and the catalytic cycling of some enzymes. Treatment with certain drugs or exposure to plasticizers can stimulate peroxisome proliferation, also giving rise to oxyradicals. Exposure to tumor promoters indirectly increases oxyradical formation, for example through the action of phorbol esters, mediated by protein kinase C, and in inflammation mediated by nitric oxide. Oxygen free radicals produce multiple DNA adducts including 8-hydroxydeoxyguanosine (Fig. 1 h).

Stuctural modification of DNA to form carcinogen-DNA adducts

The structure of DNA can be modified through various mechanisms. These include oxidation, alkylation, dimerization, deamination and reaction with large, bulky aromatic-type carbocations. Endogenous and exogenous pathways lead to the formation of oxygen free radicals and the formation of oxidative DNA damage. Examples of oxidative DNA adducts include thymine glycol, 8-hydroxydeoxyguanosine, uracil glycol, 5-hydroxyuracil, 5-hydroxy-methyluracil and 6-hydroxy-5,6-dihydrocytidine.

Alkyl-radicals form during the metabolic activation of certain N-nitrosamines or spontaneously in the case of N-alkylureas (N-methyl-N-nitrosourea) or N-nitrosoguanidines. Protonated alkyl-functional groups, which become available to modify DNA, attack nucleophilic centers. There are ten of these: N1, N3, and N7 of adenine; N3 of cytosine; N2, O6 and N7 of guanosine; O2, N3, and O4 of thymidine. Repair of some of these lesions is correlated with mutagenicity. For example, O^6-methyldeoxyguanosine (Fig. 1f) can be repaired and is a promutagenic lesion, whereas N^7-methyldeoxyguanosine (Fig. 1g) is neither repaired nor mutagenic.

Larger, bulky aromatic-type adducts bind to DNA producing three-dimensional structures that reside either in the minor or the major groove of the DNA helix. Activated BP binds preferentially to the exocyclic (N2) amino group of deoxyguanosine (Fig. 1a). While guanine is a preferred site for PAH modification, covalent binding to deoxyadenosine and deoxycytosine are also possible. Aromatic amines form adducts at the C8, N2, and O6 positions of deoxyguanosine and deoxyadenosine but the major aromatic amine adducts form at the C8 of deoxyguanosine (Fig. 1b and c). Evidence suggests that activation of aflatoxin B1 produces adduction primarily at the N7-position of deoxyguanosine (Fig. 1e).

Methods to measure carcinogen-DNA adducts

Single methods currently in use for carcinogen-DNA adduct detection include radiolabeling, immunoassays, immunohistochemistry, ^{32}P-postlabeling, fluorescence and phosphorescence spectroscopy, mass spectrometry, atomic absorbance spectrometry and electrochemical conductance. Single methods are typically not able to chemically characterize specific adducts in human tissues, although they work well for animal models exposed to one agent. Using human samples, greater success in DNA adduct characterization has typically been obtained by combining preparative methods (immunoaffinity chromatography, high performance liquid chromatography or gas chromatography) with immunoassays, ^{32}P-postlabeling, synchronous fluorescence spectrometry or mass spectrometry. These assays are typically able to detect as little as 1 adduct in 10^9 nucleotides using ~5-100 μg of DNA. A relatively recent method, accelerator mass spectrometry, can detect 1 adduct in 10^{12} nucleotides but requires administration of exceedingly low levels of radioactively-labeled compounds.

Importance of DNA adducts in chemical carcinogenesis

The presence of a DNA adduct in a critical gene provides the potential for occurrence of a mutagenic event, resulting in subsequent alterations in gene expression and a loss of growth control. A substantial period of time is required for a tumor to become evident, and DNA damage is considered to be necessary but not sufficient for tumorigenesis since other events, such as mutagenesis and cell proliferation, must also take place. DNA adduct levels, mea-

sured at any point in time, reflect tissue-specific rates of adduct formation and removal, which depend upon carcinogen activation, DNA repair, adduct instability and tissue turnover. In experimental models dose-response associations have been observed for DNA adduct formation, mutagenesis, and tumorigenesis induced by chemicals, while reductions in DNA adduct levels have been associated with chemoprevention. However, some adducts are highly-mutagenic and associated with carcinogenesis, while others are not. Studies in animal models have demonstrated an association between mutation 'hot-spots' in proto-oncogenes and tumor suppressor genes, and specific adducts. Mutations considered potentially carcinogen-specific have been observed in p53, ras, and other reporter genes in humans. Molecular epidemiologic studies involving DNA adduct measurements have the potential to elucidate the role of DNA adduct formation in human cancer risk.

Structural modification of proteins to form carcinogen-protein adducts

In the same way that DNA can be modified by reactive chemical species, endogenous or exogenous carcinogens can become bound to proteins after direct interaction or metabolic activation. Typically, a reactive cation binds covalently at a nucleophilic amino acid. Alkylating agents most commonly attack the amino acid cysteine, however, aspartate, histidine, valine, tryptophan, glutamate, and lysine residues are also targets.

Because protein adducts are not repaired, protein adduct measurements are considered to reflect carcinogen dosimetry. Chemically-stable adducts are thought to provide a measure of dose integrated over the life time of a given protein. The blood proteins hemoglobin and serum albumin have been most studied as human biomarkers because they are readily accessible and have known rates of turnover. However, histone and collagen adducts have been explored as indicators of longer-term exposures.

Methods to measure carcinogen-protein adducts

The earliest animal model studies that examined hemoglobin or serum albumin adducts involved the use of a radiolabeled carcinogen. More recent approaches have included immunoassays, HPLC with fluorescence detection and various mass spectrometry approaches. These methods are particularly powerful because of the ability to determine the specific chemical structure of the purified protein adduct. In addition, sensitivity can be typically as low as ~0.1 fmole of adduct in mg quantities of protein. The strengths of this approach are the specificity of the methods and the availability of large quantities of sample material.

Significance of protein-adducts

The utility of proteins for human dosimetry in environmental and occupational chemical exposures was first demonstrated by the kinetic relationship between protein adduct persistence and protein lifetime. This important principle was established for hemoglobin modified by ethylene oxide or alkylating agents. It provided the basis for subsequent studies that investigated associations between carcinogen-protein adduct levels and carcinogen exposures. Protein adduct formation is a valuable surrogate for DNA adduct formation since many chemical carcinogens bind to both DNA and protein in blood with similar dose-response kinetics. In addition, blood proteins are available in large quantities, enhancing the feasibility of measuring carcinogen-protein adducts in human biomonitoring studies.

Many protein adduct studies have considered exposures to different chemicals including ethylene, methylmethane sulfonate, BP, aflatoxin B1, 2-amino-3-methylimidazo[4,5-f]quinoline, dimethylnitrosamine, ethylene and propylene oxide, NNK and styrene. However, hemoglobin adducts formed through the metabolic activation of aromatic amines have proven to be excellent indicators of tobacco smoking. Tobacco smokers are readily distinguished from non-smokers, and a dose response has been observed between smokers of black tobac-

co containing high levels of 4-ABP and blonde tobacco containing low levels of 4-ABP. Protein adducts have been measured for twenty aromatic amines contained in cigarette smoke. The 3-aminobiphenyl-hemoglobin is a unique marker for passive smoking because 3-aminobiphenyl is present in side-stream but not main stream tobacco smoke. Hydroxyethylvaline in hemoglobin is also a dosimeter of tobacco smoking, but it is less specific because ethylene oxide has other environmental origins in addition to tobacco smoke. Although questions remain concerning the relationship between protein adduct levels and disease risk, measurement of protein adducts has been, and will continue to be, a valuable tool in molecular epidemiology studies.

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Carcinogen Metabolism

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Synonyms

- → Detoxification

Definition

The transformation of chemicals is important in carcinogenesis both in terms of bioactivation as well as detoxication. Most chemical carcinogens need to be activated within the body. Such reactive forms can then cause biological damage (Fig. 1). As an example for competing processes urethane was chosen (Fig. 2). Exactly what proportion in human cancers are the result of chemical exposure is not clear. However, in most countries at least 1/3 of cancer cases are due to tobacco carcinogens. A significant number of cancer cases may be related to diet, although it is unknown exactly which chemicals in food cause or influence cancer. These days however, the number of cases due to industrial exposure seems to be very low.

Characteristics

History

In 1761, the London physician J. Hill made the observation that the use of snuff was associated with nasal cancers. More than hundred years later in 1895, Rehn and others, in Germany and Switzerland, reported a link of large-scale arylamine exposure of workers in the aniline dye industry to bladder cancer. In Japan, Yamagiwa and Ichikawa were in 1915 the first to demonstrate the formation of tumors in rabbits exposed to coal tar, a mixture of polycyclic hydrocarbons. The concept that metabolic processes are a necessity for the bioactivation of chemical carcinogens was primarily developed by J. A. and E. C. Miller at the University of Wisconsin in the early 1940's. Over the next few decades, they and others provided further insight, defining metabolically derived carcinogenic products that react with DNA ('ultimate carcinogens'). However, although the relationship between carcinogens and mutagenesis had been considered it was not clearly defined. It was only after B. N. Ames developed a (still widely used) bacterial mutation system in which rat liver extracts are able to transform carcinogens into mutagens that the correlation between carcinogenesis and mutagenesis became obvious. Advances in enzymology and re-

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