Detection of human DNA–carcinogen adducts

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The role of specific chemicals in the aetiology of human cancer can now be assessed using ^{32}P-postlabelling in combination with corroborative biomonitoring techniques.

THIRTY years after Rachel Carson[1] raised the awareness of the general public to the potential dangers of environmental toxins, the application of multiple biomonitoring techniques has finally provided hard evidence for human exposure to genotoxic chemicals. At a recent International Meeting* focused primarily on ^{32}P-postlabelling methods, the detection of DNA–carcinogen adducts in a variety of human tissues was reported in association with occupational, dietary, tobacco and drug exposures. Most importantly, ^{32}P-postlabelling/thin-layer chromatography methods were corroborated by mass spectrometry, immunoaffinity and high-performance liquid chromatography, and fluorescence techniques and provided better estimates of DNA adduct levels during acute and chronic exposures. These efforts also led to the identification of specific DNA-bound chemicals such as 4-aminobiphenyl and benzo[*a*]pyrene, which are known carcinogenic components of cigarette smoke and other combustion sources. The upshot of these developments may be the beginning of a new approach to human risk assessment based on DNA–carcinogen adducts as molecular biomarkers that are predictive of clinical disease.

DNA–carcinogen adduct formation

DNA–carcinogen adducts are formed during the biotransformation of chemical carcinogens by drug-metabolizing enzymes to reactive intermediates that are electrophilic and bind covalently to DNA[2]. The DNA-bound chemicals or adducts can be removed by DNA repair processes or by cell death, but upon chronic exposures often reach steady-state levels in carcinogen-target tissues. During cell replication, the DNA adducts can result directly in mutations in genes that control cell growth and lead to neoplasia[3]. Although DNA adducts of different carcinogens exhibit widely different mutation efficiencies, the steady-state levels of a specific DNA–carcinogen adduct in target tissues during chronic exposure appear to be dose-related and to be generally predictive of tumour incidence across species[4]. Thus, the accurate estimation and identification of human DNA–carcinogen adducts are expected to be predictive of human disease risk and pilot studies reported at the Meeting provided initial support for this hypothesis.

Methods development

In experimental animal studies, the quantitation of DNA adducts had usually required the use of highly radioactive chemical carcinogens. However, a major breakthrough in detection methods occurred in the early 1980s with the development of the ^{32}P-postlabelling technique[5–8]. The method is based on enzymic hydrolysis of non-radioactive carcinogen-modified DNA to 3′-nucleotides, subsequent [5′-^{32}P]-phosphorylation by ^{32}P-ATP (3,000–7,000 Ci mmol^{-1}) and polynucleotide kinase, and chromatographic separation of nucleotide–carcinogen adducts from normal nucleotides. The result was an assay of remarkable sensitivity, with detection limits of about one adduct per 10^9 normal nucleotides ($\simeq$ 3 adducts per genome).

Quantifying adduct levels

To date, over 60 laboratories have used and customized these methods and most of these practitioners met together for the first time in June to discuss their art. A unique aspect of the Meeting was an international effort by 15 different laboratories to quantify DNA–carcinogen adduct levels in chemically-modified DNA standards and in human tissue samples. Although the results were generally comparable between most laboratories, an unexpected outcome was the apparent underestimation of total adduct levels by this method. The implication, of course, is that DNA adduct levels in human tissues may actually be higher than they are now estimated to be using ^{32}P-postlabelling. Moreover, it became clear that, for quantitative purposes, adduct stability during isolation and storage, adduct recovery in the chromatography systems used, and the efficiency of enzymatic [5′-^{32}P]-phosphorylation must be determined. This will require the further availability of synthetic DNA–carcinogen adduct standards that are representative of different chemical classes and the development of additional corroborative/alternative methods. With these caveats, it is expected that accurate measurements can be achieved, that additional DNA–carcinogen adducts can be identified in human tissues and that environmental monitoring for future health effects can be accomplished. But even now, the combined application of such standardized methods has already provided strong evidence for the presence of DNA adducts derived from aflatoxin, aromatic and heterocyclic amines, polycyclic aromatic hydrocarbons, nitrosamines and chemotherapeutic drugs in humans.

Further developments

Another significant development was the wide application of these methods to the analysis of both structurally simple (low molecular weight) and bulky carcinogens in complex mixtures, as well as DNA adducts derived from irradiation. Aquatic organisms and plants were also sampled and found to contain DNA adducts that could be attributed to environmental pollution. Finally, one of the more exciting findings was that paraffin-embedded tissues, if fixed less than 48 hours in formalin, are quite suitable for ^{32}P-postlabelling analyses. This should allow for many new studies on DNA available for isolation from human tissue archives stored worldwide and should provide a wealth of new information on the role of chemical carcinogens in human cancer. □

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