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New horizons of DNA adductome for exploring environmental causes of cancer

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Abstract

Chemical carcinogenesis is focused on the formation of DNA adducts, a form of DNA damage caused by covalent binding of a chemical moiety to DNA. The detection of carcinogen-DNA adducts in human tissues, along with demonstration of mutagenicity/carcinogenicity in experimental systems, and validation of adducts as biomarkers of environmental exposure and indicators of cancer risk in molecular epidemiological studies suggests a pivotal role of DNA adducts in cancer development. However, accurate measurement of DNA adducts in varied biological samples is challenging. Advances in mass spectrometry have prompted the development of DNA adductome analysis, an emerging method that simultaneously screens for multiple DNA adducts and provides relevant structural information. In this review, we summarize the basic principle and applications of DNA adductome analysis that would contribute to the elucidation of the environmental causes of cancer. Based on parallel developments in several fields, including next-generation sequencing, we describe a new approach used to explore cancer etiology, which integrates analyses of DNA adductome data and mutational signatures derived from whole-genome/exome sequencing.

KEYWORDS
cancer, DNA adduct, environmental carcinogen, mass spectrometry, next-generation sequencing

1 | INTRODUCTION

Environmental factors contribute substantially to cancer development.[1] Exposure to mutagenic or carcinogenic compounds in the environment occurs from multiple sources, including environmental pollution, occupation, lifestyle choices, and pharmaceutical use. Once absorbed in the body, chemical substances interact with DNA bases, leading to the formation of DNA adducts. Most of the DNA adducts are repaired by cellular processes, but the remaining, unrepaired adducts can cause genomic mutations during DNA replication, which results in genomic instability and could promote carcinogenesis.[1,2]

DNA adducts play a critical role in early stages of carcinogenesis by initiating mutagenesis,[2] and serving as markers of exposure to environmental carcinogens.[3] Moreover, they indicate the etiology of human cancer, as exemplified by AFB1.[4-6] Detection of the AFB1-DNA adduct, coupled with identification of distinct mutation patterns in *TP53* induced by this adduct, has provided convincing

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evidence on the causal role of exposure to AFB1 in the development of liver cancer in regions with high exposure of AFB1.[7-9]

Assessment of DNA adducts has remained challenging in several biological samples. With advances in techniques, the sensitivity and specificity for evaluating the levels of DNA adducts have significantly improved.[10,11] Notably, advances in MS have prompted the development of DNA adductome analysis that allows comprehensive analysis of both targeted and untargeted DNA adducts and provides relevant structural information. The integration of DNA adductome data with mutational signatures derived from next-generation sequencing has inspired newer avenues in delineating the chemical-induced DNA damages.

In this review, we describe the basic principle of DNA adductome analysis and discuss its applications in cancer etiologic research. We also describe an integrated analysis of DNA adductome and whole-genome/exome sequencing data to elucidate the environmental causes of cancer.

2 | OVERVIEW OF COMPREHENSIVE ANALYSIS OF DNA ADDUCTS

2.1 | Timeline of DNA adduct measurement

The field of DNA adduct measurement is rapidly evolving. We undertook an electronic search on the PubMed database using "DNA adducts" plus "radiolabeled compound", "^{32}P-postlabel", "immunoassay/immunohistochemistry", "LC-MS", or "DNA adductome/adductomics" as keywords. Known for its high sensitivity, the ^{32}P-postlabelling assay has been the primary method for analyzing

DNA adducts, with the highest number of publications in the 1990s (Figure 1). However, the ^{32}P-postlabelling assay has several limitations: it is labor-intensive, needs large amounts of radioactive phosphorus, and fails to provide structural information on identified DNA adducts. In contrast, immunoassay/immunohistochemistry analysis offers high specificity, but it cannot measure all DNA adducts because specific Abs against individual DNA adducts are required. Furthermore, since the 1990s, LC-MS has been increasingly used to analyze DNA adducts. Thus, the number publications related to LC-MS has surpassed those using the ^{32}P-postlabelling assay. DNA adductome analysis, which is the next-generation comprehensive analysis of DNA adducts that depends on LC-MS instruments, has shown recent developments after the first report in 2006.[10] DNA adductome analysis allows comprehensive characterization of multiple DNA adducts in terms of amount and type, and provides structural information of those adducts.[11-14] As DNA adductome analysis can provide information on both individual exposure levels and broad landscape of total exposures, it can play an important role in the characterization of the exposome, a concept proposed to capture the total exposure during an individual's lifetime.

2.2 | Principle of DNA adductome analysis

The principle of adductome analysis is based on the monitoring of neutral loss of DNA bases and deoxyribose moieties. In the LC-MS analysis, neutral loss of 2′-deoxyribose moiety is the dominant fragmentation pathway for nucleoside adducts.[10,14] Loss of 116 amu corresponds to a deoxyribose moiety and thus serves as a marker of deoxyribonucleosides. This "constant neutral loss" increases the
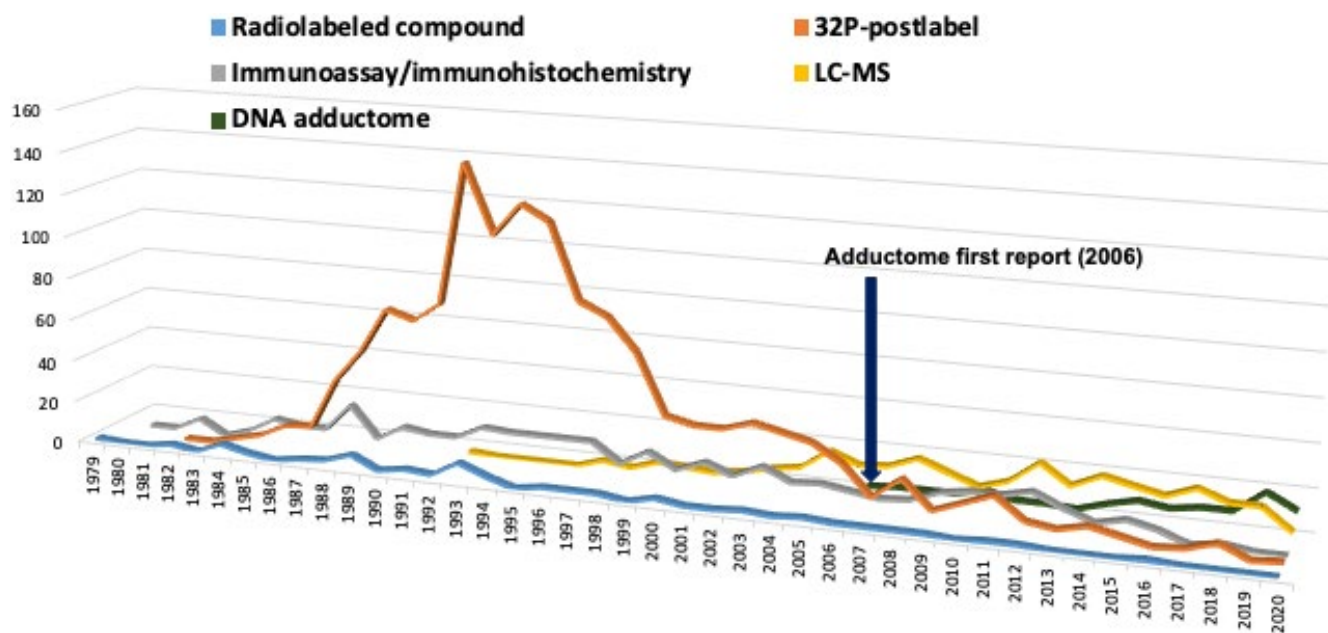

FIGURE 1 Timeline of DNA adduct measurement, with the number of published papers per year shown for each method. The search of published reports was undertaken with PubMed using the following keywords: "DNA adducts" plus "radiolabeled compound", "^{32}P-postlabel", "immunoassay/immunohistochemistry", "LC-MS" or "DNA adductome/adductomics"

specificity of screening DNA adducts. Unstable nucleoside adducts easily lose the deoxyribose moiety following enzymatic/thermal hydrolysis of DNA, resulting in aglycone base adducts. These aglycone base adducts indicate loss of base moiety in the MS analysis.

2.3 | Applications of DNA adductome analysis

DNA adductome analyses using HRAM instruments, such as Q-TOF and Orbitrap, have been carried out to screen for multiple DNA adducts.[15,16] High resolution accurate mass spectrometry can acquire spectral data with precise mass measurement in the order of 0.001 amu. This precision is sufficient to determine the molecular formula of an ion. Moreover, MS/MS and/or MS/MS/MS fragmentation data obtained under these conditions can be used to detect a DNA adduct and provide structural information or confirmation. Therefore, the HRAM approach is suitable for both targeted analyses of the previously reported DNA adducts

with known chemical structures, and untargeted analyses investigating unknown DNA adducts. In this section, we highlight several studies that used untargeted DNA adductome analyses for varied research purposes.

In a previous study, our group examined MGT-derived DNA adducts using the HRAM approach.[15] As shown in Figure 2, multiscale entropy oscillated the collision energy between high and low energy levels at regular intervals. Under these conditions, both full scan and fragmentation data can be simultaneously obtained. Furthermore, specific DNA adducts observed in the MGT-treated group were screened using bioinformatics analyses. We compared their m/z values with those of the authentic DNA adducts that were available from an in-house DNA adducts database. The results confirmed several DNA adducts to be "major contributors" of the MGT status. As most of these major contributors coincided with oxidative stress and inflammation-related DNA adducts,[15] inflammation responses might contribute to the increased prevalence of mutations in the MGT-treated group.
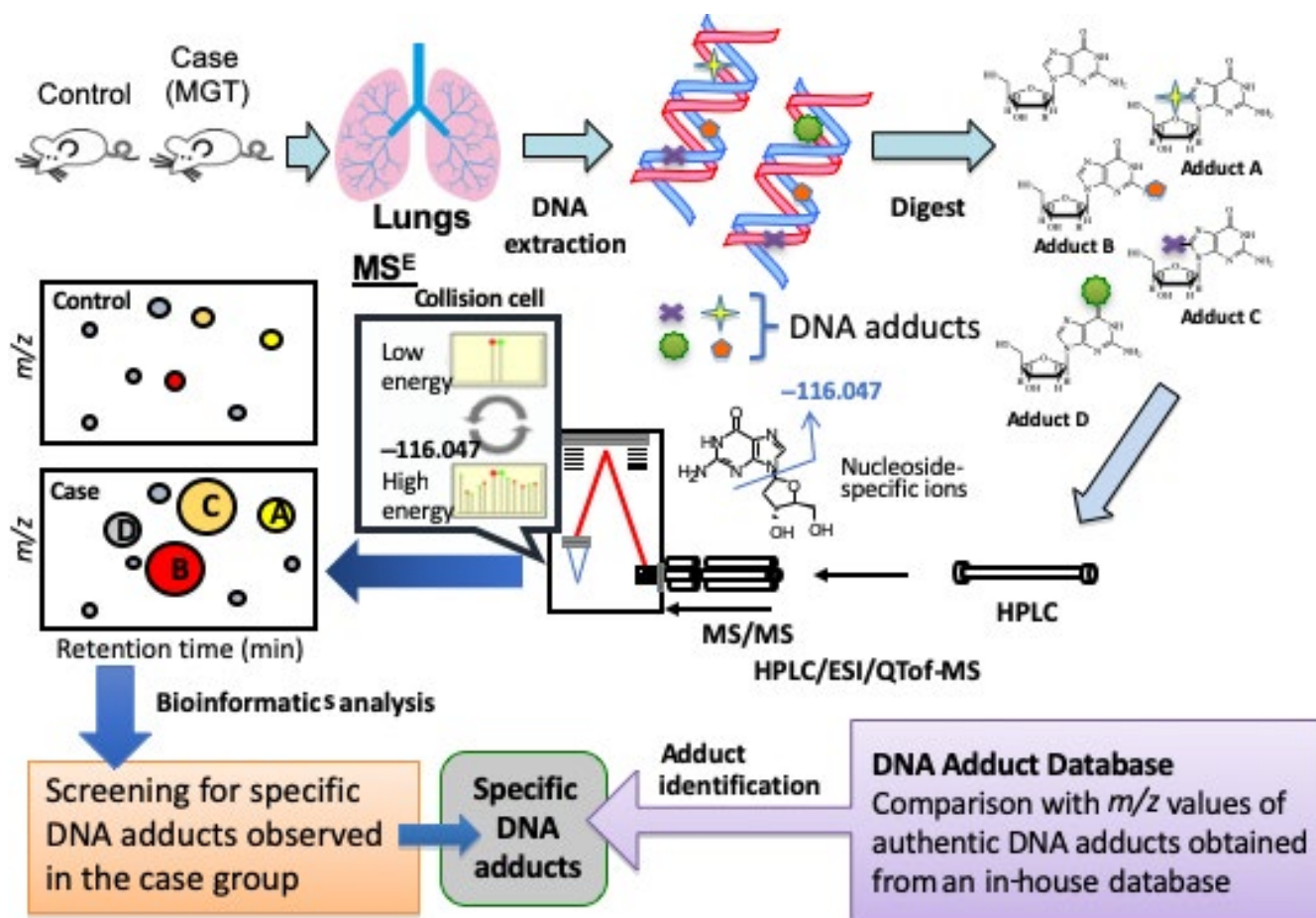

FIGURE 2 Concept of DNA adductome analysis of mice lungs using the high resolution accurate mass-multiscale entropy (MS^E) approach. DNA extracted from the lungs of mice treated with or without nanosized magnetite (MGT) is enzymatically digested to mononucleotides, and subjected to HPLC-quadrupole time of flight (QTof)-mass spectrometry (MS). The MS^E approach is a method that changes the collision energy to high and low levels at regular intervals; under these conditions, we can simultaneously obtain both full scan and fragmentation data. Loss of 116.047 amu, which corresponds to a deoxyribose moiety, is a hallmark for deoxyribonucleosides. Finally, data are mapped by their m/z values, retention time, and intensity. By using bioinformatics analysis, we can screen specific DNA adducts observed in the MGT-treated group. To identify each of the specific DNA adducts, their m/z values are compared with the authentic DNA adducts listed in an in-house DNA adducts database. ESI, electrospray ionization

In another untargeted DNA adductome study, we examined DNA adduct formation in 1,4-dioxane-treated rat livers.[17] Treatment with 1,4-dioxane significantly induced A:T to G:C transitions and A:T to T:A transversions in the liver of *gpt* transgenic mice, although the mechanisms of its genotoxicity remain to be elucidated. We undertook a PCA-discriminant analysis, and identified three candidate adducts as characteristic of 1,4-dioxane treatment. Of these, two adducts contained thymine or cytidine/uracil moieties, and the third was identified as 8-oxo-dG, based on mass fragmentation and HRAM spectrometry data. These findings suggested that oxidative stress responses could account for the increased frequency of mutations resulting from 1,4-dioxane treatment.

Untargeted DNA adductome-HRAM analyses can provide mechanistic insights into the association between dietary exposures and risk of cancer. For example, a positive association between consumption of red meat and risk of colorectal cancer has been reported in several epidemiologic studies.[18] A hypothesis suggests that heme iron in the red meat could facilitate formation of genotoxic NOCs and LPOs. These promutagenic compounds form DNA adducts such as O^6-carboxymethyl-dG, thereby leading to increased susceptibility to colorectal cancer. To detect the contributing DNA adducts, Hemeryck et al[19] carried out in vitro DNA adduct profiling using the adductome-HRAM analysis. Red or white meat digests were incubated with colonic microbiota obtained from 10 healthy volunteers and subjected to DNA adductome analysis. The analysis identified 90 alkylation and lipid peroxidation-induced DNA adducts with reference to the in-house DNA adduct database. Interestingly, red and white meat displayed different DNA adduct profiles. Of these, 12 NOC- and/or LPO-related DNA adducts, such as hydroxymethylthymine, guanidinohydantoin, O^6-methylguanine, O^6-carboxymethylguanine, malondialdehyde-x3-cytidine, and 3,N^4-ethenocytidine were identified as potential markers for heme-rich meat digestion. Therefore, formation of these genotoxic adducts might underlie the association between red meat consumption and increased risk of colorectal cancer.

The gut bacteria are implicated in colorectal carcinogenesis, although the mechanism remains unclear. Strains of *Escherichia coli* residing in the human gut are known to produce colibactin, a small genotoxic molecule with unknown structure. A recent study by Wilson et al[20] identified colibactin-dA adducts using an untargeted LC-MS[3] adductome system. They identified two specific adenine adducts in mammalian cells exposed to the colibactin-producing *E. coli*, but not in cells exposed to mutant strains lacking the colibactin-producing genes. Based on mass fragmentation, HRAM spectrometry data, chemical synthesis, and NMR spectral analyses, they characterized the adducts as a mixture of two diastereomeric adducts containing a 5-hydroxypyrrolidine-2-one ring system with an attached N3-substituted adenine ring. Moreover, these adducts were detected in the colonic epithelial cells of germ-free mice inoculated with colibactin-producing *E. coli*. As DNA double-strand breaks and interstrand cross-links were observed in both human cell lines and animals infected with colibactin-producing *E. coli* strains,[21] it can be considered that these gut bacteria could contribute to the development of colorectal cancer.

Fewer HRAM-adductome analyses are undertaken using human biological samples than those using cell lines or animal models. Thus, we carried out an HRAM-adductome analysis to identify the environmental factors causing esophageal cancer in Cixian, an area well-known for high incidence of this cancer type in China.[22] Nontumorous tissues were collected from esophageal cancer patients who underwent surgical procedures in the high- and low-incidence (for comparison) areas. Two-dimensional PCA indicated clear clustering in both areas, with several DNA adducts showing a greater contribution in the high-incidence area. Based on the in-house DNA adduct database, we specifically identified THP-dG adduct in samples from the high-incidence area.

2.4 | Limitations of DNA adductome analysis and solutions

One limitation of cell-based DNA adductome analysis is the requirement of a significant amount of DNA (~100 µg), which depends on the abundance of adduct and sensitivity of the instrument.[11,12] As alternatives for tissue samples, blood and urine have been used in DNA adductome analyses. In particular, urine DNA adductome has attracted attention because it is noninvasive and samples can be easily collected, transported, and stored with low biological hazard. The major source of DNA adducts excreted in the urine is DNA repair processes, such as base excision and nucleotide excision repair, and sanitization of the 2′-deoxyribonucleotide pools.[23] Using urine samples, Chang et al[24] detected significantly increased levels of DNA adducts in N-nitrosamine-treated mice than in control mice. Additionally, Cooke et al[25] presented human urinary adductome maps, which detected both 2′-deoxyribonucleoside and nucleobase adducts, but nucleobase adducts were found to be the major adducts. Although these studies indicate the utility of the urinary adductome approach, further studies are warranted to validate the technique.

Another limitation is that the structure of DNA adducts has to be elucidated by referring to an existing DNA adduct database. However, Guo et al[26] recently developed a DNA adductome database that included DNA adduct information and MS[n] spectral data obtained from high-resolution Orbitrap/Q-TOF MS. Such databases can serve as useful resources for identification of DNA adducts.

3 | DNA ADDUCTS AND MUTATIONAL SIGNATURES

Cancer arises from the sequential accumulation of mutations in driver genes. Next-generation sequencing data has transformed our understanding of the special and temporal patterns of somatic mutations underlying cancer development. Characteristic mutational signatures can be obtained on the basis of footprints left

by different mutations in the genome.[27] For instance, a total of 49 SBS signatures have been identified based on approximately 84 million somatic mutations from whole-genome and exome sequences.[27] Approximately one-third of these 49 signatures have unknown etiology; the remaining two-thirds are attributed to DNA repair deficiency, environmental exposure to chemical compounds, or cancer chemotherapy. Of these, four signatures, SBS11, SBS22, SBS24, and SBS42, are well-characterized and the underlying etiology has been clearly linked to environmental exposure or cancer treatment (Figure 3).

The SBS11 signature is associated with temozolomide, an alkylating agent used in cancer treatment. This signature is dominated by the C:G to T:A transition, and prominent trinucleotide signatures are ACC, CCC, CCT, GCC, TCC, and TCT. Moreover, this signature shows a strong transcriptional strand bias for C to T substitutions, indicating that mutations at guanine base and alkyl-adducts are selectively removed by the transcription-coupled nucleotide excision repair. Whole-genome sequencing of *Salmonella typhimurium* TA100 strain exposed to alkylating agents, such as ethylnitrosourea, methylnitrosourea, and ethyl
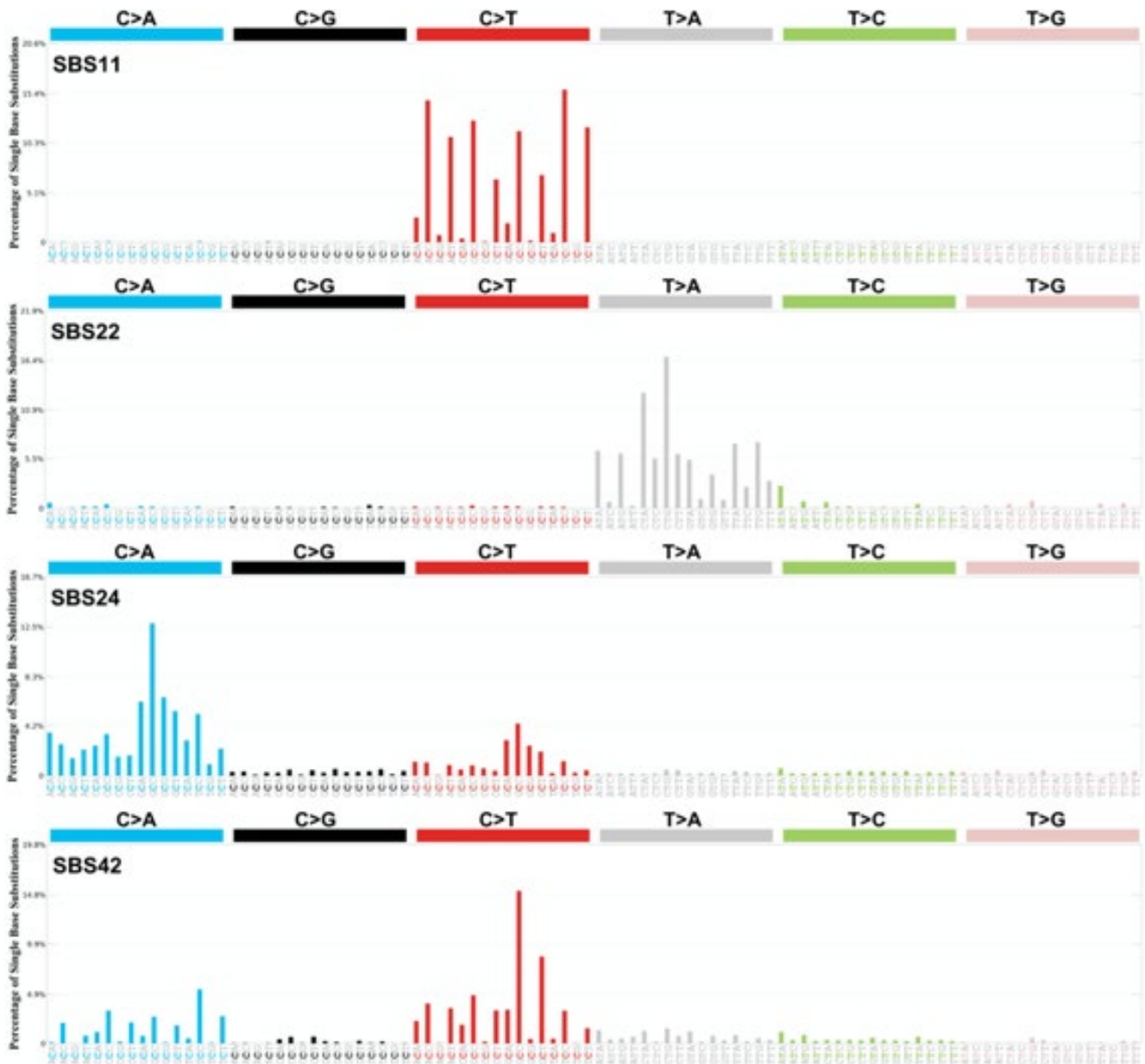

FIGURE 3   Single-base substitution (SBS) signatures that have been clearly linked to environmental exposure or cancer treatment: SBS11, alkylating agent (temozolomide); SBS22, aristolochic acids; SBS24, aflatoxin B1; SBS42, haloalkanes. The illustration was quoted from the COSMIC database (https://cancer.sanger.ac.uk/cosmic/signatures). The horizontal axis represents the mutational types and the vertical axis indicates the percentage of mutations attributed to a specific SBS. Each mutational signature is displayed on the basis of the trinucleotide frequency of the human genome. The trinucleotide refers to the mutated base and sequence context immediately 5′ and 3′ to the mutated base
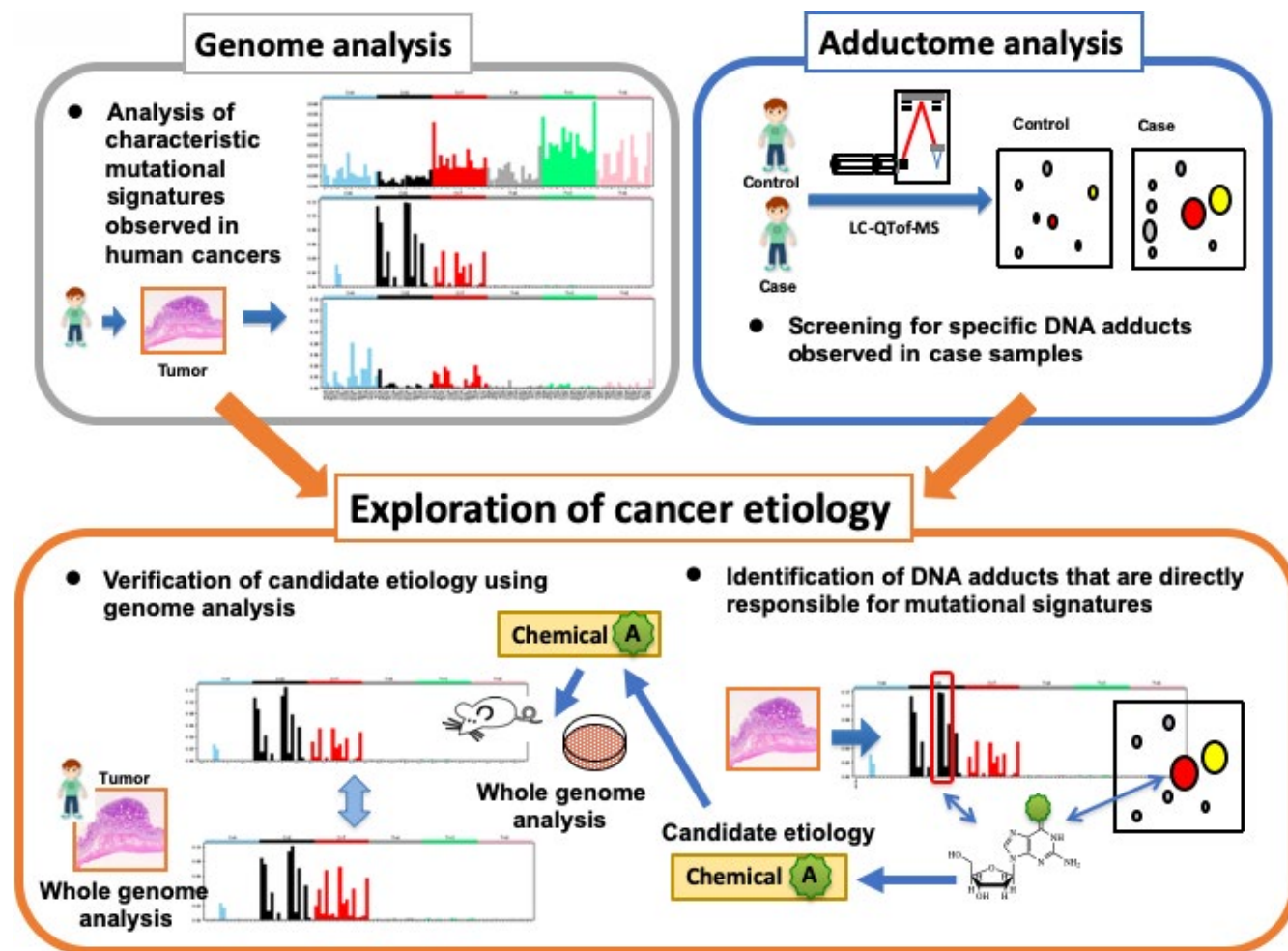
FIGURE 4 A new approach for exploring environmental causes of cancer. Mutagenic compounds are screened using liquid chromatography (LC)-mass spectrometry (MS)-based DNA adductome. Experimental animal models are also used to validate biological activities of the newly identified compounds, including mutational signatures. Finally, these data are compared with human mutational signature data. QTof, quadrupole time of flight

methanesulphonate, indicated frequent C:G to T:A transitions in groups treated with all alkylating agents.[28] Moreover, the prominent trinucleotide signature is the NCY consensus context, which is similar to that of human cancers associated with alkylating agents (SBS11).

The SBS22 signature is found in aristolochic acid-associated urothelial cancers. This signature features A:T to T:A transversions, with CTG as the prominent trinucleotide signature context. Studies in which the mouse embryonic fibroblasts and *gpt* delta mice were exposed to aristolochic acids, under in vitro and in vivo conditions, respectively, noted an almost identical A:T to T:A transversion signature with SBS22.[29,30] Moreover, a strong strand bias was observed, with mutations exclusively occurring on the nontranscribed strand. This indicates that the selective removal of AL-dA adducts stemmed from transcription-coupled nucleotide excision repair. Furthermore, AL-dA adducts were detected in renal tissues.[31] Taken together, these findings provide strong evidence for the involvement of aristolochic acids in the etiology of human urothelial cancers.

The SBS24 signature is exclusive to liver cancer, and its underlying etiology is attributed to exposure to aflatoxin B1. This signature is dominated by G:C to T:A transversions, with prominent trinucleotide signatures being GCC, CCA, TCC, and GCA. Similar mutational signatures were observed in aflatoxin B1-exposed human cell lines and aflatoxin B1-induced mice liver tumors.[32] Furthermore, similar signatures were observed in the newly sequenced hepatocellular carcinoma samples from Qidong, China, where exposure to aflatoxin B1 is well documented.[32] Additionally, extreme transcription-strand bias for genes with high expression levels suggests that AFB1-dG adduct is involved in mutation induction at those sites.

The SBS42 signature is associated with occupational cholangiocarcinoma in employees of the printing industry in Japan. The causative agents were haloalkanes, such as 1,2-DCP.[33] This signature is dominated by the C:G to T:A transition, and shows a strong transcriptional strand bias. The prominent trinucleotide signatures are GCC and GCT, and secondary contexts are ACC, CCC, and TCC. As similar mutational signatures with secondary

FIGURE 5 Mutational signatures of esophageal cancer in China. Mutational signatures were extracted from the trinucleotide mutational pattern of esophageal cancer collected from patients living in high- and low-incidence areas in China. Four mutational signatures were identified (A-D). The illustration has been reproduced from Totsuka et al.22 The horizontal axis represents the mutational types and the vertical axis indicates the percentage of mutations attributed to a specific signature. Each mutational signature is displayed on the basis of the trinucleotide frequency of the human genome. The trinucleotide refers to the mutated base and sequence context immediately 5′ and 3′ to the mutated base
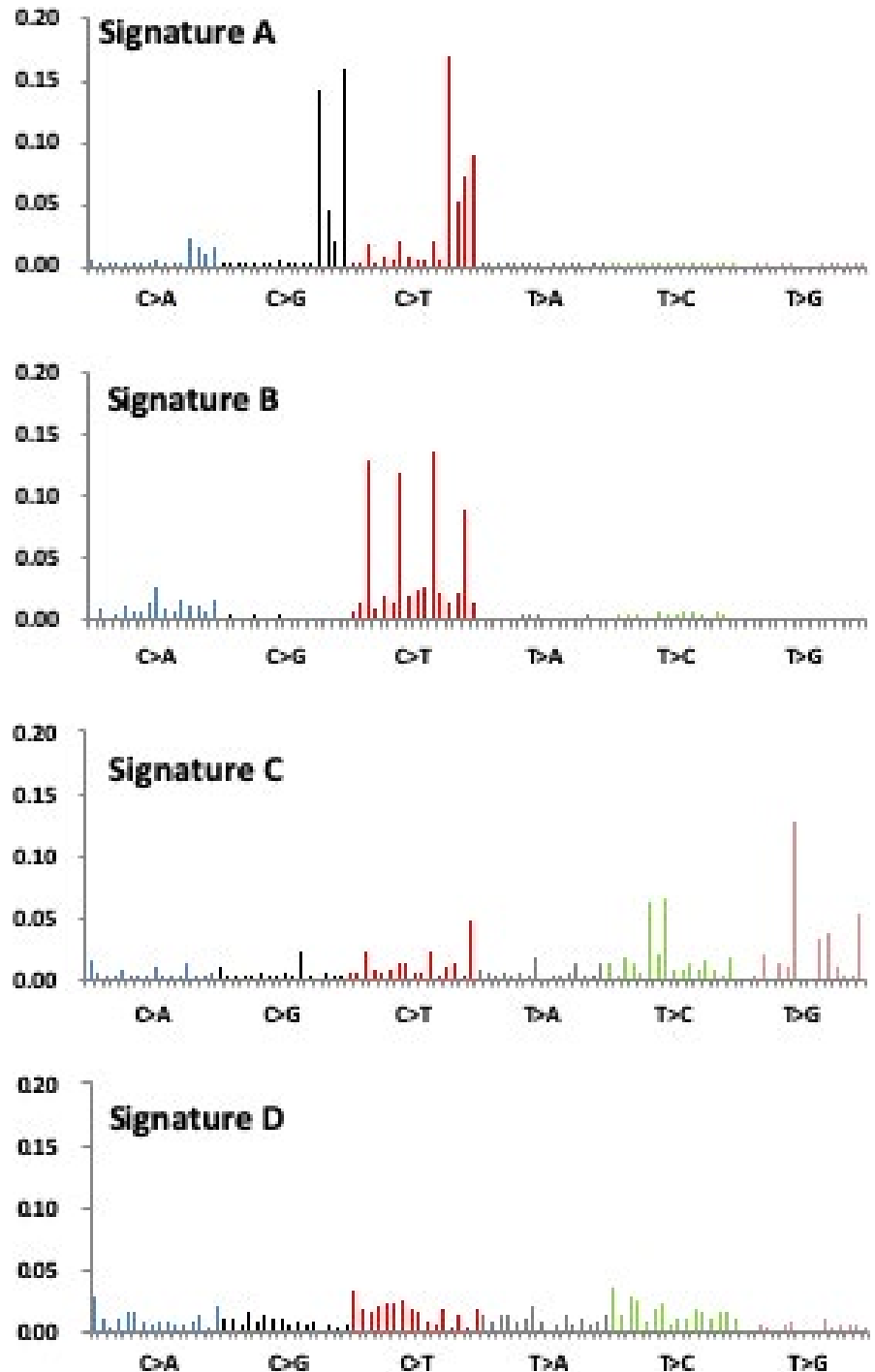
contexts were observed in 1,2-DCP-exposed *S. typhimurium* TA100 strain, 1,2-DCP could be involved in the etiology of occupational cholangiocarcinoma.

4 | INTEGRATION OF DNA ADDUCTOME AND GENOMIC DATA

The identification of characteristic mutational signatures for AFB1 and aristolochic acids provided strong evidence for the contribution of these compounds to human cancer etiology. However, the

majority of the chemical compounds lack such a unique mutational signature, making it difficult to conclude the causal relationship between mutational signatures and human cancer development. With advances in DNA adductome analyses and increasing availability of whole-genome/exome sequencing data, we adopted a new approach for exploring the environmental causes of cancer (Figure 4). First, mutagenic compounds are screened using LC-MS-based DNA adductome analysis. Second, experimental animal models are used to validate biological activities of the newly identified compounds, including mutational signatures; these data are compared with human data. Finally, prospective cohort studies or nested case-control

studies are undertaken to examine the association between DNA adduct levels in blood or tissue samples and cancer risk in human subjects.

This approach has provided promising results in our untargeted DNA adductome analysis that aimed to identify the environmental causes of esophageal cancer in high-incidence areas of China.[22] The LC-MS-based DNA adductome analysis identified THP-dG as a major DNA adduct. The THP-dG adduct is derived from NPIP, a potent esophageal carcinogen in rats. The mutagenicity analysis indicated that NPIP significantly increased mutation frequency in both the liver and esophagus of *gpt* delta rats in a dose-dependent manner. The predominant mutations were A:T to C:G transversions, followed by G:C to A:T and A:T to G:C transitions. Furthermore, we undertook whole-exome sequencing using samples from esophageal cancer patients living in the high- and low-incidence areas.[22] A nonnegative matrix factorization algorithm identified four types of mutational signatures (Figure 5, A-D); however, based on the contribution of each of these mutation signatures in individual samples, a clear separation was not observed between the high- and low-incidence areas. This result suggested that the etiology of esophageal cancer might not differ between these areas. However, one of the mutational signatures (C) was weakly associated with THP-dG levels, suggesting that exposure to NPIP is partially involved in the development of esophageal cancer. Additionally, the study indicated that the THP-dG adduct levels in the peripheral blood samples were significantly elevated in the high-incidence area compared to the low-incidence area. Furthermore, efforts are ongoing to examine the association between levels of THP-dG in the blood and risk of esophageal cancer in a nested case-control study. Integration of these data could establish the causal role of exposure to NPIP in the development of esophageal cancer in high-incidence areas of China.

5 | CONCLUSION

DNA adduct formation and mutation induction play a critical role in cancer etiology, as established by decades of research. The rapidly evolving techniques in MS have prompted the development of DNA adductome analyses that could elucidate genotoxicity mechanisms and cancer etiology. An integrated approach that combines data from epidemiologic studies, experimental models, next-generation sequencing, and mathematical analysis of mutations would be crucial to identify and quantitate the causative agents of mutational signatures of unknown etiology. The esophageal cancer study by our group provides an example of such an integrated approach, and integration of these data could provide strong evidence on the environmental causes of cancer.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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