

# From single DNA adducts measurement to DNA adductomics in molecular epidemiology of cancer

## ABSTRACT

Environmental carcinogens exert their carcinogenic effects by forming DNA adducts. This type of DNA damage can also be formed endogenously as a result of, e.g., oxidative damage. Unrepaired DNA adducts may induce mutations in critical genes, leading to the initiation of chemical carcinogenesis. Therefore, detection, identification, and quantification of DNA adducts is essential for cancer risk assessment. Over the last 50 years, the major DNA adducts formed by different classes of environmental carcinogens were characterized. With the development of techniques such as  $^{32}\text{P}$ -postlabeling, their measurement was implemented into molecular epidemiology. Advances in liquid chromatography-tandem mass spectrometry (LC-MS) made the measurement of adducts more precise and allowed to gain knowledge about their identity and structures. Therefore, these new techniques opened the way to DNA adductomics, the "omics" approach investigating DNA adducts comprehensively, similarly to proteomics. This review presents the historical perspective of DNA adducts research and the emerging field of adductomics.

## INTRODUCTION

Humans are exposed continuously to different chemicals, often exerting genotoxic effects, which may lead to cancer development. The spectrum of these chemicals is broad and greatly related to lifestyle, such as occupational exposure, diet, or smoking habits. Many of these carcinogenic chemicals are linked to specific types of cancer. For example, smoking results in exposure to polycyclic aromatic hydrocarbons (PAH) linked to lung cancer [1], while occupational exposures to aromatic amines are linked to bladder cancer [2].

All these chemicals exert their carcinogenic effects by forming DNA adducts. This type of DNA damage can also be generated endogenously due to lipid peroxidation, inflammation, and oxidative damage by reactive oxygen species (ROS) [3].

Only a tiny portion of the carcinogenic chemicals can damage DNA directly and initiate the carcinogenesis process. Most require metabolic activation before covalent binding to DNA and forming adducts. The cellular basic DNA repair systems can remove most DNA adducts [4,5]. Those that are not repaired may induce mutations in critical genes such as *H-ras* and *K-ras* (*Rat sarcoma*) oncogenes and the *TP53* tumor suppressor gene [6], leading to the initiation of chemical carcinogenesis. Although the role of DNA adducts at this stage of carcinogenesis is the best documented, evidence pointing out that DNA damage involvement in the later stages also exists [7].

Therefore, detection, identification, and quantitative assessment of DNA adducts is critical for evaluating the potential role of genotoxic factors in cancer etiology and risk assessment. Environmental exposure to diverse chemical carcinogens results in the formation of broad DNA adducts spectrum. Therefore, the need for comprehensive characterization of covalent modification of DNA, screening for all DNA adducts, and gaining information on their chemical structures led to the development of a DNA adductomics approach [8,9].

This paper presents the historical perspectives and new data on DNA adducts and adductomics and their possible applications.

## DNA ADDUCTS - HISTORICAL PERSPECTIVES AND THEIR ROLE IN CANCER RISK ASSESSEMENT

Covalent DNA adducts are formed as a result of the interaction of reactive electrophilic intermediates of carcinogen metabolism and nucleophilic centers in cellular DNA mainly nucleobases, but also deoxyribose residues or phosphates.

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## DEDICATION

This paper is dedicated to memory of Professor Mieczysław Chorąży, who contributed greatly to the field of molecular epidemiology using DNA adducts as a tool. The latter linked him with the author of this review.

**Keywords:** DNA adducts, polycyclic aromatic hydrocarbons, molecular epidemiology, liquid chromatography-mass spectrometry, adductomics

**Abbreviations:** BaP – benzo [a]pyrene; BaPDE – benzo[a]pyrene-7,8-dihydrodiol 9,10-epoxide; DB[a,j]A – dibenzo [a,j]anthracene; DE – diol-epoxide; LC-MS – liquid-chromatography-tandem mass spectrometry; PAH – polycyclic aromatic hydrocarbons

These events are generally considered to lead to an initiation of the carcinogenesis process. The most complete data on metabolism and activation mechanisms have been collected for PAH, particularly benzo[a]pyrene (BaP). These studies provided vital information to understand the mechanism of chemical carcinogenesis. In this regard, the first seminal paper showing the correlation between DNA-binding level and carcinogenicity of selected PAH was published by Brookes and Lawley in 1964 [10]. The following groundbreaking paper was published by Sims and co-workers in 1974 [11], providing evidence that the vicinal "bay-region" 7,8-dihydrodiol 9,10-epoxide (BaPDE) is the ultimate DNA binding metabolite of the aromatic hydrocarbon BaP. Three years later, Jerina and Lehr explained the high reactivity of this metabolite by the bay-region theory. According to this theory, this intermediate's high reactivity, i.e., ease of carbon ion formation, is related to benzyl positions vicinal to the bay region of the tetrahydrobenzo ring [12].

Such position in BaP is C-10, where carbon ion is formed in reaction with DNA. The bay-region vicinal diol-epoxides are generally considered the ultimate carcinogenic reactive species formed from BaP and most PAH. BaPDEs are formed as two diastereoisomers, each comprising a pair of enantiomers. Of these four diol-epoxides, the (+) *anti*-7,8-diol-9,10-epoxide is more biologically active than the other three in most systems tested, including humans [13,14], forming adducts with exocyclic amino group of guanine (N-2) and to much less extent with adenine residue.

Diol-epoxides of BaP and the other PAH are the products of metabolic activation by cytochrome P450 (CYP), mainly CYP1 and CYP2 families [15], initiated by Ah receptor activation [16]. Most PAHs activation mechanisms closely resemble that of BaP, leading to the formation of diol-epoxides of the bay-region type. Generally, for diol-epoxide (DE) derived from the planar PAH, such as BaP, the amino group of guanine is the most exclusive site of reaction in DNA, whereas for *anti*-dihydrodiol epoxides derived from non-planar PAH, such as 7,12-dimethylbenz[a]anthracene or benzo[c]phenanthrene comparable extents of reaction with both adenine and guanine were found [13]. Interestingly, analysis of the mutations spectrum in *c-Ha-ras* oncogene of papillomas formed in mouse skin initiated with dibenz[a,j]anthracene (DB[a,j]A) and other PAH such as 7,12-dimethylbenz[a]anthracene, showed exclusively A<sup>182</sup> → T transversion implicating dAdo adducts in the initiating activity of these PAH [17]. Besides the bay-region diol-epoxides activation, alternative PAH pathways exist. K-region oxides can also form DNA adducts [18]. Moreover, more polar DNA adducts were found than those created by classic bay-region DE. One example is DB[a,j]A, forming up to 23 species of adducts, most of which are polar. However, evaluation of the carcinogenic activity of more polar adducts such as bis-diol-epoxides of DB[a,j]A in experimental models, indicated a lack of tumor-initiating activity [17].

Although PAHs are considered a significant risk of lung cancer, lymphocyte DNA from a case-control study showed that the levels of both PAH and 7,8-dihydro-8-oxo-2'-deoxyguanosine formed as a result of ROS action might help pre-

dict the risk of lung cancer at least in African and Mexican American minority populations [19].

The studies performed, particularly in the second half of the XX century, shortly described above, explained the basic mechanism of the carcinogenic activity of PAH, which also referred to other chemical carcinogens. The results of these studies provided strong arguments that the formation of DNA adducts is critical for the initiation and probably the later stages of the carcinogenesis process.

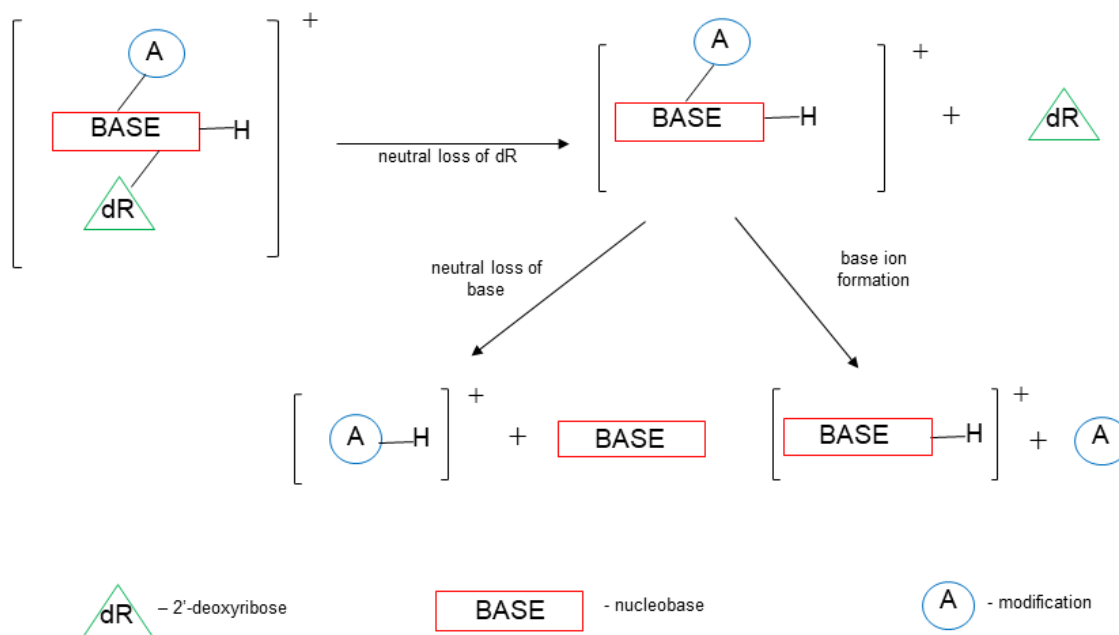
Therefore, DNA adducts can potentially be biomarkers of exposure to chemical carcinogens. These findings paved the way to implement DNA adducts measurement in molecular cancer epidemiology and prevention [20].

## MOLECULAR EPIDEMIOLOGY - BIOMONITORING OF ENVIRONMENTAL, OCCUPATIONAL, AND LIFESTYLE EXPOSURES

The observed correlation between the level of chemical carcinogens binding to DNA and their carcinogenic activity makes them good candidates for internal biomarkers of exposure.

Thus, the measurement of DNA adducts became a helpful tool in molecular epidemiology monitoring external and endogenous exposure to hazardous agents. The application of DNA adducts as biomarkers was possible when sensitive enough and noninvasive methods of DNA adducts detection were developed. Those included fluorescence and immunological assays and <sup>32</sup>P-postlabeling. The latter was crucial and widely used to measure DNA adducts level in blood cells and relatively easily available tissues such as the placenta [21]. Based on this technique, several studies evaluated the human exposure to PAH. Among them, those carried out by the Kari Hemminki and Mieczysław Choraży groups assessing occupational, environmental, and tobacco exposure are worth noting. The study populations included coke workers in Upper Silesia, Poland, in which higher levels of PAH adducts in blood cells than the local controls were found [22]. Upper Silesia region was a heavily industrialized area at the beginning of this century and still is. The studies on the Silesian population showed an elevated level of adducts in total white blood cells of the area residents, in some cases only slightly lower than that observed in coke workers [22,23], but significantly higher than in the agricultural population from the Biała Podlaska region of Poland [24]. In both populations, seasonal variations in adduct levels were observed [25,26]. Individual variations in adduct level such as those noted in coke workers may be partly explained through the analysis of genetic factors, which play a role in individual susceptibility toward specific pollutants. In this regard, adduct levels in white blood cells of chimney sweeps significantly increased in comparison with the control population only after adjustment for the expression of cytochrome P450-CYP1A1 and glutathione S-transferase GSTM1 genes. The enzymes encoded by these genes are involved in PAH activation and detoxification [27].

The Krzysztof Szyfter group performed analyses of DNA adducts in laryngeal tissue in cooperation with Kari Hem-



**Figure 1.** Three fragmentation pathways of nucleoside adducts (modified from [9]).

minki. These studies showed a relationship of adducts level to smoking, the known risk factor for laryngeal cancers, but most clearly in the tumor tissue [28]. All the studies using DNA adducts as biomarkers, performed mainly in the nineties of the XX century, confirmed the usefulness of DNA adducts measurement for biomonitoring exposure to chemicals of different origins. However, the interpretation of the data obtained was sometimes difficult. One reason was the methodological differences, e.g., between immunological assays such as Ultrasensitive Enzymatic Radio-Immuno Assay (USERIA) and Enzyme-Linked Immunosorbent Assay (ELISA) *vs.*  $^{32}\text{P}$ -postlabeling. The identification of DNA adducts was also challenging to assess and was limited to broad estimates like PAH. Methods such as immunoaffinity chromatography provided more precise information, but usually limited to detection a single or one type of adduct.

Implementing liquid chromatography-tandem mass spectrometry (LC-MS) resulted in measuring adduct levels more precisely and allowed us to learn about their identity and structures. This was the onset of the adductomics approach.

## NEW TECHNIQUES ALLOW ADDUCTOMIC ANALYSIS

Adductomics means the totality of cellular adducts and refers to both DNA and proteins, which nucleophilic sites are susceptible to modifications by electrophiles to yield various adducts [29,30]. In the emerging field of DNA adductomics, the “omics” approach investigates DNA adducts comprehensively, similarly to proteomics. Therefore, screening, identification, and quantification simultaneously of a large number of adducts of interest are possible using this approach [31]. The term “adductomics” first appeared in a paper published in 2006 [29].

Development of such an approach was possible with the advances in mass spectrometry. Liquid chromatography-tandem mass spectrometry (LC-MS<sup>2</sup>) sensitivity surpassed that of  $^{32}\text{P}$ -postlabeling due to high selectivity. Moreover, using this technique, accurate quantitation using stable isotope dilution can be performed. Both exogenous and endogenous adducts, resulting from nucleobase alkylation, oxidation, deamination, and cross-linking due to various exposures, may be measured [32-35].

Traditional LC-MS<sup>2</sup> has become the gold standard for targeted adductomics analysis [30,35-38], which refers to a search for covalent adducts formed upon exposure to a specific chemical agent and usually targets one or a few DNA adducts per assay. Such analysis fails to provide a global picture of DNA adductomics [39].

Liquid chromatography coupled with multistage accurate mass spectrometry (LC-MS<sup>n</sup>)-based DNA adductomics intends to screen the totality of DNA adducts in biological samples comprehensively. This approach is named untargeted adductomics and is now the mainstream of adductomics analysis. It responds to a constant need for discovering and screening DNA adducts for prophylaxis purposes in risk of environmental pollution evaluation, but also in case of chemotherapeutics forming adducts for precision medicine [31,39].

As shown in Fig. 1, (LC-MS<sup>n</sup>)-based DNA adductomics investigations take advantage of the structure of deoxyribonucleosides, and basically, the neutral loss of the deoxyribose group upon fragmentation (MS/MS) of the positive ion precursor is used for screening DNA adducts. More recent approaches combine the neutral loss of the bases, a typical ion fragmentation pathway of base adducts, with the conventional neutral loss of dR, allowing for the simultane-



ous screening of nucleoside adducts and aglycone base adducts. Another approach complements the neutral loss bases. These three fragmentation pathways may be combined and allow comprehensive DNA adductomics analysis.

Biological samples used for adductomics estimation may include tissues, blood, oral cells and urine, and require 1–200 µg of DNA for quantitative analysis, depending mainly on the MS instrumentation applied [8].

Rapidly evolving technological capabilities of mass spectrometers, such as high resolution mass spectrometry (HRMS), combined with ultra-high performance LC (UHPLC), may overcome these limitations and promise more accessible and easier screening of known and unknown adducts at the very low level [40,41]. However, the application of DNA adductomics is impeded by the lack of available databases, mass spectral libraries, and software for identifying DNA adducts [39].

A recent paper by La Barbera *et al.* [39] presents the establishment of a database that might be used for screening DNA adducts in biological samples with the application of untargeted HRMS. This database might be a resource for chemical annotation of the DNA adductome.

Adductomics analyses performed using different versions of LC-MS techniques were the subjects of many studies and included investigations of the genotoxic effects of chemical carcinogens and endogenous DNA damaging agents. The list of these studies is provided in recent reviews [9,42]. An example of such investigation presents the paper of Carra *et al.* [43], who screened lung DNA for endogenous DNA adducts resulting from oxidative stress and LPS-induced lipid peroxidation along with that induced by nitrosamine exposure in a mouse model. The authors demonstrated a general workflow for the analysis of endogenous DNA adducts based on high resolution data-dependent scanning, an extensive MS<sup>2</sup> fragmentation and neutral loss MS<sup>3</sup>.

One of the interesting applications of adductomics is the European Union Project EXPOSOMICS, which aims to comprehensively evaluate all dimensions of exposures, both exogenous coming from environment pollution, life-style, radiation, etc., and endogenous, such as lipid peroxidation, inflammation, and oxidative damage, measuring a wide range of biomarkers. Therefore, the project requires all omics technologies, including adductomics [44]. The project has been launched in 2020 and until now the members of consortium concentrated on methodological aspects of exposomics studies and forming communication network. Therefore, the actual results of this project are not available yet.

## CONCLUSIONS

DNA adductomics started to be successfully applied in screening of unknown adducts using untargeted approaches followed by more sensitive targeted approaches. However, till now, most DNA adductomics analyses have been performed *in vitro*. The ultimate goal should be to analyze *in vivo* systems and human samples. One of the significant

factors limiting the sensitivity for screening DNA adducts in human samples is the amount of DNA available for analysis. Other challenges include selectivity, quantitation, and, most importantly, ease of data analysis [9]. With the increasing availability of most developed LC-MS instrumentations, as costs decrease and data analysis becomes more straightforward, adductomics may become a more routine procedure. Ideally, DNA adductomics should be combined with genomic-wide sequencing to correlate DNA adduct formation with biologically essential mutations. Advances in both adductomics analysis and molecular biology techniques will help the analysis of specific sequences, and targeting particular ones will be possible. A long way was made from the measurement of single or unidentified adducts to the perspective of measuring the totality of DNA adducts present in biological samples.

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