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Adductomics: Characterizing exposures to reactive electrophiles

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ABSTRACT

To understand environmental causes of disease, unbiased methods are needed to characterize the human exposome, which represents all toxicants to which people are exposed from both exogenous and endogenous sources. Because they directly modify DNA and important proteins, reactive electrophiles are probably the most important constituents of the exposome. Exposures to reactive electrophiles can be characterized by measuring adducts from reactions between circulating electrophiles and blood nucleophiles. We define an 'adductome' as the totality of such adducts with a given nucleophilic target. Because of their greater abundance and residence times in human blood, adducts of hemoglobin (Hb) and human serum albumin (HSA) are preferable to those of DNA and glutathione for characterizing adductomes. In fact, the nucleophilic hotspot represented by the only free sulfhydryl group in HSA (HSA-Cys^{34}) offers particular advantages for adductomic experiments. Although targeted adducts of HSA-Cys^{34} have been monitored for decades, an unbiased method has only recently been reported for visualizing the HSA-Cys^{34} 'subadductome'. The method relies upon a novel mass spectrometry application, termed fixed-step selected reaction monitoring (FS-SRM), to profile Cys^{34} adducts in tryptic digests of HSA. Here, we selectively review the literature regarding the potential of adductomics to partially elucidate the human exposome, with particular attention to the HSA-Cys^{34} subadductome.

1. Environmental exposures and disease

Investigations of human twins and results from more than 400 genome-wide association studies (GWAS)[1] indicate that genetic factors contribute about 10–30% of the risks of cancer and cardiovascular diseases (Lichtenstein et al., 2000; Manolio et al., 2009). Thus, it appears that non-genetic factors (i.e. 'the environment') are the major causes of chronic diseases in human populations. Yet the things that people generally associate with the environment, namely, air and water pollution, hygiene and sanitation, smoke from fuel combustion, and occupation, collectively contribute only about 7–10% to the burden of chronic diseases (Rodgers et al., 2004; Saracci and Vineis, 2007). Rather, the major environmental risk factors identified thus far are smoking, overweight, and the diet (Peto, 2001; Willett, 2002) all of which represent the combined effects of many toxicants. For example, cigarette smoke contains a multitude of likely human carcinogens (Hecht, 2003; Smith et al., 2003) as do many foods (Ames, 1983). However, with few exceptions, the identities of major environmental toxicants and their roles in causing chronic diseases have not been addressed.

Given the poor state of knowledge about health-impairing environmental exposures, epidemiologists pursue narrow hypotheses that largely skirt disease etiology in favor of known environmental risk factors, even when the attributable risks are small. Although such hypothesis-driven studies confirm some environmental sources of disease, they offer only fragments to our understanding of the major causes and mechanisms of chronic diseases. In fact, the current state of environmental epidemiology is reminiscent of genetic epidemiology 30 years ago, when an investigator would test for association between a single genetic polymorphism and a particular health outcome. With completion of the human genome project and subsequent technological advances leading to GWAS, such an approach would not be used today.

1.1. The exposome and biomonitoring

It has recently been argued that unbiased approaches, analogous to GWAS, are needed to investigate health effects arising

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[1] Abbreviations: BO-Alb, HSA adduct of benzene oxide; 1,4-BQ-Alb; HSA adduct of 1,4-benzoquinone; DTT, dithiothreitol; ESI, electrospray ionization; FS-SRM, fixed-step SRM; GWAS, genome-wide association studies; Hb, hemoglobin; HNE, 4-hydroxy-2(*E*)nonenal; HSA, human serum albumin; HPLC, high-performance liquid chromatography; IAA, iodoacetamide; IAA-iT3, internal standard consisting of the adduct of IAA with isotopically labeled T3; MS, mass spectrometry; NHANES, National Health and Nutrition Examination Survey; PCBs, polychlorinated biphenyls; PTM, post-translational modification; RAL, relative adduct level; SRM, selected reaction monitoring; T3, the third largest tryptic peptide of HSA.

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from all environmental exposures, known collectively as the 'exposome' (Rappaport, 2011; Rappaport and Smith, 2010; Smith and Rappaport, 2009; Wild, 2005). In developing 'environment-wide association studies', it is essential to recognize that disease pathologies are mediated through chemicals that affect critical molecules, cells, and systems *inside* the body. Thus, exposures cannot be restricted to xenobiotic chemicals arising from air, water, smoking, etc., but must also include dietary constituents as well as toxicants produced endogenously by inflammation, stress, lipid peroxidation, infections, etc. In fact, blood concentrations of some toxic natural products and endogenous chemicals are much greater than those arising from polluted air and water, and are probably important contributors to cancer and cardiovascular disease (Ames, 1983; Dalle-Donne et al., 2006; Liebler, 2008). The need to include both exogenous and endogenous toxicants highlights the importance of a top-down strategy based upon biomonitoring to characterize exposures (using blood, say) rather than a bottom-up strategy using samples of air, water and food (Rappaport, 2011; Rappaport and Smith, 2010).

Another important consideration is the variability of the exposome in space and time. Indeed, knowledge that immigrant populations adopt the disease patterns of their host countries (Armstrong and Doll, 1975; Kato et al., 1973) emphasizes the importance of varying diets and lifestyles on health risks. Furthermore, whereas a person's genome is essentially fixed at conception, his or her internal chemical environment varies during life due to changes in exogenous and endogenous exposures, age, exercise, infections, lifestyle and psychosocial factors. This variability of toxicant sources and levels places a premium upon obtaining repeated biospecimens to generate 'snapshots' of the exposome during critical stages of individuals' lives, notably during gestation, early childhood, puberty, and the reproductive years (Rappaport, 2011). By investigating series of archived biospecimens from longitudinal cohort studies, it should be possible to organize such snapshots into individual exposomes, each highlighting the particular mix of environmental factors that a person experienced during life.

1.2. Candidate exposure studies

Because it is not currently feasible to measure all chemicals in the blood, we should focus upon the classes of toxicants that are likely to contribute to disease processes, namely reactive electrophiles, hormones and hormone-like substances, receptor-binding agents, and metals (Rappaport, 2011; Rappaport and Smith, 2010). Methods are currently available for measuring many such agents in human biospecimens. For example, several hundred analytes, including metals, hormone-like substances, and persistent organic compounds, have been detected in random samples of blood and urine from the U.S. population as part of the National Health and Nutrition Examination Survey (NHANES) (CDC, 2009). Using an initial list of 266 candidate exposures from the NHANES database, Patel et al. (2010) recently reported strong associations between the risk of type-2 diabetes and blood or urine levels of heptachlor epoxide, γ-tocopherol, β-carotenes, and polychlorinated biphenyls (PCBs). Note that these environmental factors include two exogenous toxicants (heptachlor epoxide and PCBs), both of which increased the risk of type-2 diabetes, as well as a vitamin (γ-tocopherol) which increased disease risk and a class of micronutrients (β-carotenes) which decreased disease risk. Interestingly, effect sizes from this candidate exposure study were comparable to the strongest loci ever reported in GWAS for any health endpoint. Although Patel *et al.* identified possible environmental causes of type-2 diabetes, their study was biased in favor of a set of exogenous chemicals, vitamins, and micronutrients that had been selected *a priori* by NHANES investigators for various reasons. Thus, it is important for future studies to apply unbiased approaches to detect a larger cross section of toxic chemicals arising from all sources (Rappaport, 2011; Rappaport et al., 2010).

2. Reactive electrophiles and their adducts

Electrophiles, including reactive oxygen and nitrogen species, aldehydes, oxiranes and quinones, have long been suspected of causing cancer and other chronic diseases because they directly damage DNA and proteins (Brodie et al., 1971; Dalle-Donne et al., 2006; Liebler, 2008; Miller and Miller, 1966). Electrophiles enter the blood from absorption in the lungs or gut (e.g., inhalation of ethylene oxide) or, more typically, via metabolism of xenobiotics in the liver or other tissues (e.g., production of benzene oxide and acetaldehyde from metabolism of benzene and ethanol, respectively), from oxidation of lipids and other natural molecules [producing acrolein, 4-hydroxy-2(*E*)nonenal (HNE) and other aldehydes] and from inflammation (producing reactive oxygen and nitrogen species) associated with ionizing radiation, infections and preexisting diseases. Once in the blood, electrophiles react with all available nucleophiles to form adducts via S_N1 and S_N2 substitution, 1,4-addition, Schiff-base formation, and radical-mediated reactions (Liebler, 2008; Tornqvist et al., 2002).

Because they are reactive, it is difficult to measure electrophiles directly in blood. However, one can measure adducts of electrophiles resulting from reactions with DNA (in nucleated cells), reactions with prominent blood proteins, such as hemoglobin (Hb) and human serum albumin (HSA), and reactions with the ubiquitous tripeptide antioxidant, glutathione [reviewed by Blair, 2006; Rubino et al., 2009; Tornqvist et al., 2002]. Reactions of electrophiles with DNA occur primarily at nucleophilic nitrogen atoms of particular bases (especially guanine); reactions with Hb and HSA are observed primarily at the free thiol groups of Cys and amine groups of His, Trp, Lys and the *N*-termini; and reactions with glutathione occur primarily at the free Cys thiol. Since protein adducts are not repaired and are much more abundant than DNA adducts in blood (1 ml of blood contains about 150 mg Hb, 30 mg of HSA, and 0.003–0.008 mg of DNA) (Tornqvist et al., 2002), they are more useful measures of internal dose than DNA adducts, which have paradoxically received far more attention in this regard. And because protein adducts are also much longer lived than glutathione adducts—mean residence times are 28 d and 63 d for adducts of HSA and Hb, respectively, in humans (Furne et al., 2003; Granath et al., 1992; Troester et al., 2002), compared to hours for glutathione adducts (Wagner et al., 2007)—they provide more stable measures of exposure to electrophilic precursors. Archived blood and serum or plasma, which are biospecimens of choice in cohort studies such as NHANES or the U.K. Biobank (Ollier et al., 2005) are important sources of Hb and/or HSA for investigating adducts.

2.1. Targeted protein adducts

As indicated in Section 2, adducts of Hb and HSA are the most promising candidates for investigating exposures to reactive electrophiles in human populations. Levels of *targeted* Hb and/or HSA adducts have been studied in humans for several toxicants, notably acrylamide, aflatoxin B1, aldehydes, aminobiphenyl and other aromatic amines, benzene, 1,3-butadiene, ethylene oxide, and polycyclic aromatic hydrocarbons (reviewed in Rubino et al., 2009). Most of these studies relied upon older gas chromatography–mass spectrometry techniques to selectively cleave adducts from Cys residues of Hb and HSA or from the *N*-terminal Val residue of Hb, while newer studies have employed liquid-chromatography tandem mass spectrometry (LC–MS/MS) to investigate adducted peptides in protein digests.