Molecular Biomarkers in Environmental Health Research: Advances and Public Health Applications

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Abstract. Environmental factors pose significant public health risks. Traditional epidemiological studies face limitations in accurately assessing individual exposure, elucidating underlying mechanisms, and identifying susceptible populations. Utilizing specific biomarkers, molecular epidemiology provides a powerful tool to unravel the "black box" between environmental exposure and health outcomes. This article systematically reviews the advances and applications of molecular biomarkers in environmental health research. Firstly, based on the "exposure-disease continuum" theory, biomarkers are classified into three categories: biomarkers of exposure (e.g., cotinine for tobacco smoke exposure), biomarkers of effect (e.g., 8-OHdG indicating oxidative damage), and biomarkers of susceptibility (e.g., genetic polymorphisms), alongside an overview of their detection technologies. Subsequently, through analysis of typical cases involving ambient $PM_{2.5}$, heavy metals, and environmental endocrine disruptors (EDCs), the specific applications of various biomarkers in revealing exposure levels, deciphering pathogenic mechanisms, and assessing health risks are analyzed, demonstrating their core value in advancing environmental health research from macroscopic associations to microscopic mechanisms. Finally, the article discusses challenges and limitations concerning technical standardization, biological interpretation, translational application, and assessment of complex mixture exposures. It also offers perspectives on future directions, including promoting the integration of multi-omics technologies, strengthening interdisciplinary collaboration, and facilitating the translation of research findings into public health practice, aiming to provide a scientific basis for building a precise environmental health risk assessment and early warning system.

Keywords: Molecular Epidemiology; Biomarkers; Environmental Exposure; Public Health; Precision Prevention.

1. Introduction

The impact of environmental factors on population health has long been a crucial area of epidemiological research. Traditional epidemiological studies have provided essential evidence for public health policy formulation by establishing statistical associations between environmental exposures and disease occurrence. However, these studies exhibit significant limitations in accurately assessing individual actual exposure levels, elucidating biological mechanisms, and identifying susceptible populations, making it challenging to meet the demands of precision public health prevention and control.

The rise of molecular epidemiology has brought methodological innovation to environmental health research. Researchers can bridge the gap from macroscopic associations to microscopic mechanisms by detecting specific biomarkers. Biomarkers of internal exposure (e.g., urinary tobacco metabolite cotinine, blood heavy metal concentrations) directly reflect the actual absorbed dose by the organism, addressing the significant errors in traditional exposure assessment [1]. Biomarkers of effect (e.g., DNA adducts, oxidative stress product 8-OHdG, epigenetic changes) provide mechanistic evidence for exposure-disease associations [2]. Biomarkers of susceptibility (e.g., metabolic enzyme gene polymorphisms) help explain individual differences within populations. The application of these biomarkers enables environmental epidemiology research to delve deeply into the "black box" mechanism.

Currently, a major challenge in this field lies in translating laboratory-discovered biomarkers into tools suitable for large-scale population studies and ultimately applying them in public health practice.

From the molecular epidemiology perspective, this article systematically reviews the progress in biomarkers for environmental health research, focusing on their applications in exposure assessment, health risk early warning, and precision prevention, to provide a scientific basis for advancing the precise prevention and control of environment-related diseases.

2. Molecular Biomarkers Technology

Molecular biomarkers are core tools in modern environmental health research. As a critical bridge connecting environmental exposure and health effects, their scientific classification and precise detection methods are the cornerstones of molecular epidemiological research. Based on their indicated biological stage and functional significance in the "exposure-disease continuum," molecular biomarkers can be divided into three major categories: biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility. These biomarkers help us understand the biological mechanisms between environmental exposure and health outcomes and provide scientific evidence for public health interventions.

2.1 Biomarkers of Exposure

Biomarkers of exposure refer to biological indicators that can directly and specifically reflect an organism's level of contact with environmental harmful factors. These biomarkers include the exogenous substances themselves, their metabolites, or adducts formed with biological macromolecules. Compared to traditional environmental monitoring methods, biomarkers of exposure provide direct evidence of an individual's exposure dose, avoiding potential error sources in external exposure assessment such as geographical differences, temporal variations, and personal behavioral differences.

Many biomarkers of exposure have already been applied in public health research. Cotinine, as the primary metabolite of nicotine in the human body, is the most specific and reliable biomarker of internal exposure for assessing tobacco smoke exposure. Its biological properties make it an ideal biomarker of exposure: a moderate half-life (16-20 hours) allows it to reflect an individual's tobacco smoke exposure over the past few days; stable chemical properties make it easy to detect in biological samples; and its concentration shows a good dose-response relationship with exposure dose.

The detection of cotinine primarily relies on immunological methods (e.g., Enzyme-Linked Immunosorbent Assay, ELISA) [3] and chromatographic techniques. Nowadays, high-sensitivity and high-specificity analytical techniques such as Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) [4] and Gas Chromatography-Mass Spectrometry (GC-MS) [5] are widely used. The development of these technologies has greatly improved detection accuracy and throughput, enabling large-scale biomonitoring studies at the population level.

LC-MS/MS technology is commonly used for cotinine detection in practical applications. This technique features high sensitivity (detection limit can reach 0.1 ng/mL) and high specificity, allowing accurate determination of cotinine concentration in urine, blood, or saliva [6]. The detection of cotinine provides a precise exposure assessment foundation for studying the health effects of tobacco smoke, enabling researchers to establish more accurate dose-response relationship models.

2.2 Biomarkers of Effect

Effect biomarkers refer to identifiable biochemical, physiological, behavioral, or other internal changes that can reflect the early biological effects of a chemical or its metabolites, changes in body structure or function, or disease [7]. These biomarkers reside in the intermediate process of the exposure-disease continuum, being neither exposure indicators nor clinical disease endpoints, but rather the biological bridge connecting the two. The core value of effective biomarkers lies in their ability to provide early warning signals of exposure-related health damage, offering a time window for preventive intervention. Due to their high sensitivity, molecular effect biomarkers can provide more objective and reliable evaluation indicators, particularly suitable for situations involving low-

dose exposure to most environmental chemicals in humans [8]. Biomarkers of early biological effects can detect reversible and subclinical lesions early, ensuring effective rehabilitation [9].

From a biological mechanism perspective, effect biomarkers reflect biological systems' initial disturbance and adaptive response to environmental exposure. For example, when the body encounters oxidative stress, it activates antioxidant defense mechanisms while producing oxidative damage products. These dynamic changes can be captured by measuring specific biomarkers. In recent years, with the development of omics technologies, research on effect biomarkers has evolved from single indicators to integrated multi-omics analysis, including epigenomics, transcriptomics, proteomics, and metabolomics.

Regarding technical methods, the detection of effect biomarkers encompasses various platforms from traditional enzymatic analysis and immunoassays to modern high-throughput sequencing and mass spectrometry imaging. Particularly, advances in mass spectrometry have enabled the simultaneous detection of hundreds of metabolic products and protein modifications, providing a powerful tool for systematically understanding the biological effects of environmental exposure. Furthermore, the emergence of single-cell sequencing technology allows researchers to study the effects of environmental exposure at the single-cell level, offering new perspectives for understanding cellular heterogeneity and microenvironment effects.

2.3 Biomarkers of Susceptibility

Biomarkers of susceptibility refer to biological indicators that can reflect individual differences in response to environmental exposure, including genetic factors, epigenetic modifications, physiological status, and other aspects. These biomarkers explain why different individuals exhibit varying health effects under the same environmental exposure conditions, providing a molecular basis for understanding individual differences in environmental health issues.

Genetic susceptibility biomarkers primarily involve the study of gene polymorphisms, especially those encoding metabolic enzymes, DNA repair enzymes, cell receptors, and key components of signaling pathways. Genome-Wide Association Studies (GWAS) have identified hundreds of genetic variants associated with the risk of environment-related diseases, greatly enhancing our understanding of complex phenotypes and achieving fruitful results in studying human complex diseases [10]. However, the current research trend is moving from single genetic variants to polygenic risk scores, integrating information from multiple genetic variants to predict an individual's disease susceptibility better.

Epigenetic modifications, another important category of susceptibility biomarkers, provide a molecular mechanism linking environmental exposure to gene expression regulation. Environmental factors influence epigenetic marks such as DNA methylation, histone modification, and non-coding RNA, which may affect an individual's sensitivity to environmental exposure. This bidirectional regulatory mechanism gives epigenetic biomarkers exceptional value in environmental health research.

Advances in detection technology have significantly propelled research on susceptibility biomarkers. From early PCR-based methods to current whole-genome sequencing, epigenome-wide association studies (EWAS), and metagenomic sequencing [11], we can study the molecular basis of individual susceptibility with higher resolution and a more comprehensive perspective. The development of single-cell multi-omics technologies enables researchers to simultaneously analyze genetic variations, gene expression, and epigenetic states at the single-cell level, providing new tools for understanding the cellular heterogeneity of environmental health effects.

3. Application of Biomarkers in Health

Applying molecular biomarkers in environmental health research has significantly expanded our understanding of the relationship between environmental exposure and health effects. By detecting specific biomarkers, we can assess exposure levels more accurately and deeply reveal the biological

mechanisms through which environmental factors cause health damage. This chapter will focus on applying molecular biomarkers in three typical environmental exposure areas, combined with specific case analyses.

3.1 Ambient Fine Particulate Matter ($PM_{2.5}$) Exposure and Cardiopulmonary Diseases

Ambient $PM_{2.5}$ pollution is one of the most important global environmental health risk factors. Biomarker-based studies have provided crucial evidence for understanding the health hazard mechanisms of $PM_{2.5}$.

CpG is a specific biomarker that can detect $PM_{2.5}$. DNA methylation is one of the most common epigenetic modifications, mainly occurring at the CG dinucleotide sequence of CpG sites. It is regarded as an early molecular biomarker for predicting adverse health responses related to environmental exposure, reflecting the changes in cell states caused by environmental exposure and revealing the interaction between genes and the environment more deeply. In the methylation modification of specific genes caused by exposure to exogenous chemicals, certain specific CpG sites, especially those located in the promoter region of genes, have specific regulatory roles and play a decisive role in the activation and inhibition of gene transcription. Specific CpG sites are termed "CpG hotspots." Identifying the specific regulation of CpG hotspots helps assess the biological effects of gene expression more accurately [12]. Therefore, the methylation of specific CpG sites can be regarded as a potential effect biomarker for $PM_{2.5}$ exposure.

Regarding exposure assessment, urinary 1-hydroxypyrene (1-OHP), a specific biomarker for polycyclic aromatic hydrocarbon (PAH) exposure, can effectively reflect the internal exposure level of organic components in $PM_{2.5}$. Studies have shown that 1-OHP concentration exhibits a good dose-response relationship with $PM_{2.5}$ exposure concentration and is significantly correlated with respiratory inflammation indicators. At the level of effect biomarkers, multiple studies have found significant associations between $PM_{2.5}$-borne PAHs and 8-hydroxy-2'-deoxyguanosine (8-OHdG), a common human metabolite indicator of DNA oxidation [13].

3.2 Heavy Metal Exposure and Health Risks

Heavy metal exposure is another important environmental health issue. Blood lead and urinary cadmium, as classic biomarkers of internal exposure, play an irreplaceable role in the health risk assessment of heavy metals. Blood lead levels accurately reflect recent lead exposure, while urinary cadmium concentration is closely related to the body's cadmium burden. Large-scale epidemiological studies based on these biomarkers have provided a scientific basis for establishing safety thresholds for heavy metals.

Regarding effect biomarkers, studies have found that lead exposure is closely related to inhibiting aminolevulinic acid dehydratase (ALAD) activity. ALAD is a key enzyme in the heme synthesis pathway; its inhibition can lead to the accumulation of aminolevulinic acid (ALA), subsequently causing oxidative stress and neurotoxicity [14]. This biomarker reflects the biological effect of lead and explains part of the molecular mechanism of lead toxicity.

Research on effective biomarkers for cadmium exposure has also made significant progress. Elevated urinary β2-microglobulin and N-acetyl-β-D-glucosaminidase (NAG) activity are sensitive indicators of cadmium-induced renal tubular damage. Studies suggest that urinary NAG >17 U/g Cr or β2-MG >1000 μg/g Cr can be considered renal injury [15].

3.3 Environmental Endocrine Disruptors and Reproductive Health

Environmental endocrine disruptors (EDCs) are a class of exogenous substances that can interfere with the function of the endocrine system, including bisphenol A (BPA), phthalates (PAEs), etc. [16]. These substances are widely present in daily consumer products and may adversely affect reproductive health. Urinary BPA and phthalate metabolites (such as MEHP and MEHHP) serve as reliable biomarkers of internal exposure for assessing EDC exposure. In terms of impacts on reproductive health, EDC exposure is associated with changes in semen quality parameters. Studies

have found that urinary BPA concentration negatively correlates with sperm density, motility, and morphological abnormalities. BPA may interfere with spermatogenesis by affecting estrogen receptor signaling pathways and epigenetic regulation [17].

Of particular concern is that EDC exposure may have long-term effects on embryonic development and child health. Detection of EDC biomarkers in umbilical cord blood shows that prenatal exposure may be associated with birth outcomes and child neurodevelopment. Epigenetic studies indicate that prenatal EDC exposure may lead to changes in the methylation status of imprinted genes, which could be one mechanism for its long-term health effects [18].

3.4 Case Summary

Analyzing these typical cases shows that molecular biomarkers are irreplaceable in environmental health research. They help us better assess exposure levels and health risks and provide important clues for revealing the health damage mechanisms of environmental factors. With the continuous advancement of detection technologies and the discovery of new biomarkers, we are expected to establish a more precise environmental health risk assessment and early warning system, providing a scientific basis for formulating effective preventive measures. Future research needs to strengthen the integrated application of multi-omics technologies further and promote the translation of molecular biomarkers from research to public health practice.

4. Challenges and Limitations

Although molecular biomarkers show great potential in environmental health research, their application still faces many challenges and limitations.

4.1 Technical Standardization Challenges

Different laboratories use varying detection methods, instrument platforms, and operating procedures, making comparing and integrating results directly difficult. For example, slight differences in pre-processing steps, such as the collection, transport, and storage conditions of biological samples, can significantly affect the stability of biomarkers and the detection results.

4.2 Complexity of Biological Interpretation

A single biomarker often lacks specificity; multiple exposure factors can cause the same biomarker, and conversely, the same exposure can lead to changes in multiple biomarkers. This many-to-many relationship makes causal inference difficult. Furthermore, genetic background, age, gender, and lifestyle among individuals can influence biomarkers' baseline levels and response, posing challenges for result interpretation.

4.3 Barriers to Translational Application

Translating laboratory research findings into public health practice requires overcoming numerous bottlenecks. Issues such as the cost-effectiveness of large-scale population screening, the standardization and quality control of biomarker detection, and how to establish health risk evaluation standards based on biomarkers all need urgent solutions. Meanwhile, biomarker research also involves ethical issues, such as individual privacy protection, the responsibility of reporting results, and potential genetic discrimination.

4.4 Limited Ability to Assess Complex Mixture Exposures

Environmental exposure is a complex mixture of multiple coexisting pollutants. Current research primarily focuses on single or a few biomarkers, making it difficult to reflect the effects of mixed exposures and their interactions comprehensively. Although omics technologies provide a panoramic view, methodological breakthroughs are still needed to filter out key biomarkers with public health significance from massive amounts of data and verify their utility.

5. Conclusion

The core problem currently facing research is how to effectively translate the findings of molecular biomarker research into public health practice. Solutions should focus on three aspects: Promote the integrated application of multi-omics technologies and establish standardized detection processes and data interpretation norms to address technical heterogeneity. Secondly, interdisciplinary collaboration should be strengthened, uniting epidemiology, biostatistics, and public health experts to jointly develop health risk assessment systems and early warning models based on biomarkers. Finally, promote policy translation research, explore practical application pathways for biomarkers in setting environmental health standards, identifying high-risk populations, and evaluating intervention effects, ultimately achieving the leap from basic research to public health action.

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