COMMENTARY

Next-generation liquid biopsy elucidates cfDNA origins from organ delineation to cellular population granularity

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Circulating cell-free DNA (cfDNA) has emerged as a promising noninvasive biomarker derived from dying cells of various organs, offering insights into abnormal cell death in numerous clinical scenarios. Specifically, the advent of next-generation sequencing of fetal DNA circulating through maternal blood has revolutionized non-invasive prenatal testing (NIPT), facilitating the detection of fetal chromosomal anomalies. Similarly, detecting donor-derived DNA in the circulatory milieu of organ transplant recipients provides an early avenue for the discernment of graft rejection. Evaluation of circulating tumor DNA (ctDNA) allows for the detection, genotyping, and monitoring of cancer, while identifying pathogens in the bloodstream expedites the diagnosis of infectious maladies. These technologies excel when genetic differences exist between the DNA sequence of the target tissue (fetus, tumor, graft, or pathogens) and that of the host. However, this is not always the case in many diseases affecting a single or multiple organ systems within the same genetic background.

Recently, studies highlight the distinct methylation patterns specific to each cell type, which remain conserved and highly stable under physiologic or pathologic conditions.[1] This discovery allows the tracing of cell-specific DNA methylation patterns, shedding light on the origins of circulating cfDNA and cell death dynamics in health and disease conditions.

THE BASIS FOR NEXT-GENERATION LIQUID BIOPSY

Technologies developed for the detection of cfDNA methylation can be broadly classified into two categories: bisulfite conversion-based and non-bisulfite conversion methods. The former can be divided into genomic-wide detection such as whole-genome bisulfite sequencing (WGBS), and targeted bisulfite sequencing, which can effectively reduce cost and improve coverage and sensitivity of candidate regions by multiplex library construction and high-depth sequencing. Non-bisulfite conversion methods mainly include antibody enrichment methods such as methylated DNA immunoprecipitation sequencing (MeDIP-seq), restriction enzyme-based methods, and TET-assisted pyridine borane sequencing *et al.*

By conducting WGBS on 77 primary cell types from 205 healthy individuals, Loyfer and colleagues pioneered the creation of a comprehensive and high-resolution atlas of the human methylome, together with an extensive set of cell-type-specific markers of 39 cell types and computation tools for fragment-level analysis of mixed cell type samples.[1] In a subsequent study, the same team further refined the vascular endothelial cells (VEC)-specific methylation patterns in general and organotypic contexts for the kidney, lung, pancreatic islets, and liver.

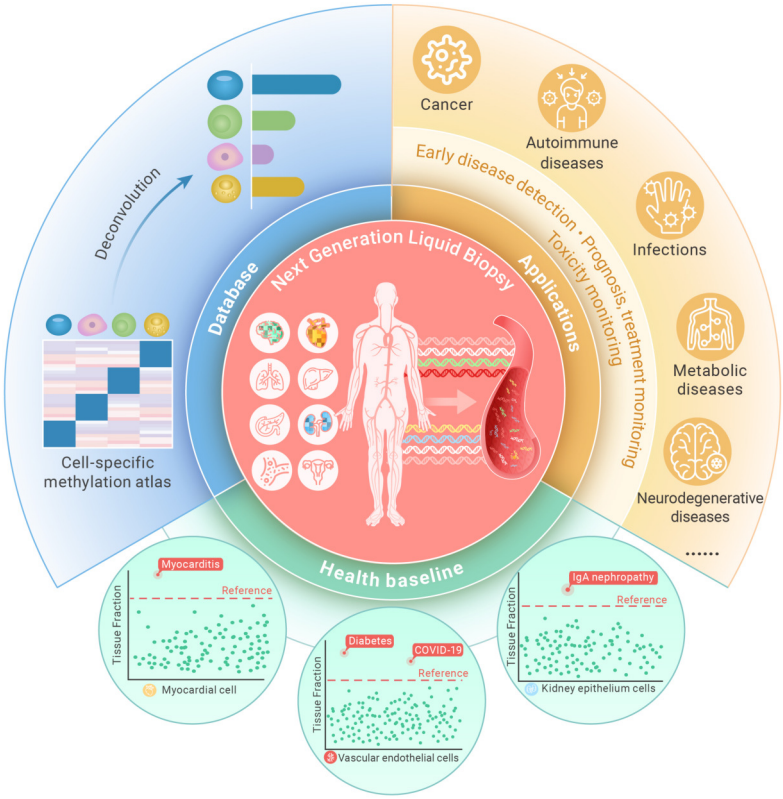

Figure 1. Perspectives in next-generation liquid biopsy High-throughput sequencing of cfDNA offers an opportunity to rapidly characterize and monitor changes in a noninvasive and real-time manner. However, several key issues need to be addressed before implementing cfDNA methylation assays into clinical practice. These issues include the low initial amount of cfDNA, selection of high sensitivity technology, expansion of a comprehensive cell-specific methylation atlas, and establishing a robust deconvolution method. By addressing these key issues and applying them in various clinical scenarios, distinct cell death dynamics would be simultaneously observed using a single blood biopsy. This advancement could revolutionize the field of noninvasive monitoring and provide valuable insights into disease progression, treatment response, and personalized medicine.

This cell-specific methylation atlas enhances the resolution to the cellular level and provides a wealth of data for further exploration. Though encompassing major organs and cells associated with common diseases, certain cell types such as osteoblasts, cholangiocytes, and hematopoietic stem cells, along with many subpopula

tions of cell types, are not included in the atlas due to the constraint of accessible material. Thus, this cell-specific methylation atlas remains open to expansion and elaboration in the future.

Leveraging cell-specific methylation patterns and deconvolution methods, researchers have explored cfDNA composition in healthy donors, which could serve as a reference or baseline for comparing and interpreting the cell turnover from individuals with diseases or organ dysfunction. It appeared that in the absence of disease, cfDNA was mainly derived from leukocytes, megakaryocytes, and erythrocyte progenitor cells, followed (in abundance) by vascular endothelial cells and hepatocytes, with minor contributions from all solid tissues. This benchmarking data aids in detecting abnormal cellular turnover under pathogenic conditions, also offering potential for further refinement as the methylome atlas advances.

APPLICATIONS OF NEXT-GENERATION LIQUID BIOPSY

Cell-specific methylation differences enable the detection of cell death and monitoring of organ or tissue damage through simple cfDNA blood tests. The decomposition of cell types in cfDNA has found applications in various fields, including cancer, infection, and complex diseases. Consideration of DNA shedding rate into the bloodstream is crucial for clinical applications. Diseases like cancer and cardiovascular diseases tend to shed DNA more readily into blood than pulmonary or neurodegenerative diseases. Moreover, transient cellular damage may be missed due to cfDNA's short half-time (less than 2 hours), while chronic diseases with persistent, gradual, and accumulative damages are suitable for clinical translation.

Utilization of cfDNA to simultaneously detect and localize multiple cancer types is currently the leading diagnostic and monitoring strategy in the cancer field. Of notes, cancer-specific markers are the major choice in this scenario, as the occurrence and metastasis of cancer may cause damage to normal tissues thus the increase in cell-derived cfDNA in multiple non-cancer organs may greatly interfere with cancer tissue origin prediction with cell-specific markers.[2] In a pilot study of breast cancer patients after radical surgery, the analysis of cfDNA methylation revealed distinct dose-dependent and tissue-specific epithelial and endothelial responses to radiation across multiple organs. Thus, both cancer specific and tissue- or cell-specific methylation markers are required to monitor both desired cancer cell death and undesired toxicity in healthy tissues.

COVID-19 infection is another systemic disease with multi-organ involvement and a common cause of sepsis. It was recently reported that severe COVID-19 is associated with injury to the lung and liver with involvement of red blood cell progenitors or vascular endothelial cells. By analyzing the cfDNA methylation profile at admission, researchers have been able to identify patients who are at a higher risk of requiring intensive care or death during hospitalization. In the setting of long COVID or post-acute sequelae of SARS-CoV-2 infection (PASC), the vascular pathology of COVID-19 is a topic of great interest, since persistent microvascular endotheliopathy associated with cryptic SARS-CoV-2 tissue reservoirs has been implicated in PASC pathology. As VEC are an essential component of each tissue, these cells contribute to multiple pathologies and are often targeted by important drugs. The abnormal VEC turnover within a particular organ or tissue measured in cfDNA may shed light on the process of tissue damage that is currently undetectable without an invasive biopsy.

Likewise, cfDNA methylation analysis holds great potential for early detection and monitoring of autoimmune diseases such as systemic lupus erythematosus, in which the immune system attacks its own tissues, causing widespread inflammation and tissue damage in the affected organs including joints, skins, brains, lungs, kidneys, and blood vessels.

In the cardiovascular field, cfDNA methylation was used to predict survival and map specific sources of injury in pulmonary arterial hypertension (PAH).[3] Patients with PAH exhibit elevated levels of circulating cfDNA, and the cfDNA derived from erythrocyte progenitor cells, cardiac myocytes, and vascular endothelium correlates with disease severity and predicts worse survival outcomes. Further investigation is warranted to determine if abnormal turnover of vascular endothelial cells could serve as an earlier sign of organ or tissue damage in these acute-onset detrimental diseases. As vascular endothelial cell dysfunction also contributes significantly to metabolic diseases in a more chronic manner, further exploration is justified.

With regard to chronic neurodegenerative diseases (Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis), proof-of-concept studies indicate that cfDNA methylation patterns could be useful biomarkers.[4] Extended cell-type-specific methylation markers of neural system may offer a minimally invasive window for diagnosing and monitoring these challenging diseases.

In the pulmonary diseases, a panel of lung-specific methylation markers, targeting alveolar and bronchial epithelial cells of lung was applied to assess lung-derived cfDNA in plasma samples from healthy individuals, patients with lung cancer, and patients with chronic obstructive pulmonary disease (COPD). The study revealed that normal lung cell turnover likely releases cfDNA into the air spaces, rather than to the bloodstream.[5] Lung-derived cfDNA is observed in the plasma when there is a pathological disruption of lung tissue architecture, as seen in lung cancer and to a lesser extent in other lung diseases.[5] These findings suggest that monitoring lung damage using lung cell cfDNA may have potential applications in other settings, warranting future investigation.

PERSPECTIVES

With an evolving, comprehensive methylation atlas of different cell types in the body, a general assay characterizing the full cell-type spectrum of cfDNA is within reach. Such an assay could illuminate normal tissue dynamics, and disease pathogenesis paradigms, serving as a valuable noninvasive resource for early detection and monitoring across various human pathologies such as cancer, autoimmune diseases, infections, metabolic diseases, and neurodegenerative diseases (Figure 1).

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DECLARATION OF INTERESTS

The authors declare no competing interests.