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Diagnostic Power of DNA Methylation Classifiers for Early Detection of Cancer

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Abstract

DNA methylation-based epigenetic signatures have become valuable cancer biomarkers. We highlight the advantages of liquid biopsy based DNA-methylation profiling for noninvasive diagnosis of early stage cancers and discuss the advanced analytical approaches developed by commercial and academic partnerships.

The ability to detect cancer early increases the opportunity for effective treatment and appropriate monitoring of the disease. For many types of solid malignancies, symptoms often do not appear until after the primary tumor has metastasized. Therefore, much effort is being put into the development of reliable, noninvasive, and cost-effective early detection methods for common cancers. An increasing understanding of the molecular mechanisms of tumorigenesis and the rapid development of new molecular techniques are widening the scope of early noninvasive cancer detection via tumor-derived molecules from body fluids – through so-called liquid biopsy tests. Circulating cell-free DNA (cfDNA) isolated from various biological fluids such as blood (plasma/serum), urine, bronchoalveolar lavage, mammary aspiration fluids, saliva, or sputum has gained much attention recently. In particular, circulating tumor DNA (ctDNA), the fraction of cfDNA originating from tumor cells, can be used for early diagnostic applications for cancer detection. During tumor progression, ctDNA is released from apoptotic or necrotic tumor cells, and possibly also via active secretion, and can provide information about the genomic make-up of primary and secondary tumors [1].

Both genetic and epigenetic alterations are involved in cancer development [2] and the concept of detecting these alterations via liquid biopsy is transforming into clinical reality. Current methods for detection of ctDNA are often based on sequencing somatic mutations in cfDNA, which is invaluable for monitoring tumor cell clones with actionable mutations. However, the diagnostic sensitivity of these methods may be low among patients with early-stage cancer due to the limited number of recurrent mutations [3,4]. Also, the sensitivity of detecting specific somatic single nucleotide mutations in cfDNA is lowered by the clonality and heterogeneity of the tumor tissue, thereby diluting the signal that is detectable in

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circulation, often requiring ultralow detection sensitivity (at the ‘needle in the haystack’ level). In contrast, DNA methylation is not as molecularly constrained and thus methylation assays have greater ability to detect early stage cancers due to several factors: (i) epigenetic alterations, such as aberrant DNA methylation, happen early in tumorigenesis and can be tissue- and cancer-type specific [2,5,6]; (ii) DNA methylation patterns are widespread across the tumor tissue and across same tumor types, whereas somatic mutations are often limited to subpopulations/clones of tumor cells; and (iii) DNA methylation is consistent across a larger genomic region, thus enabling use of multiple CpG dinucleotides for detection [6,7].

cfDNA from blood or other body fluids can be captured easily to study the status of DNA methylation at genomic locations of interest (see Figure 1). Epigenetic signatures can be identified by various technologies, including enrichment of methylated or unmethylated fragments by use of restriction enzymes or affinity-based approaches, or via bisulfite conversion, which enables differentiation of methylated and unmethylated cytosines. The most widely studied epigenetic alteration to date is the 5-cytosine DNA methylation at CpG dinucleotides, that are highly concentrated in the CpG islands within the promoter region of genes, and promoter hypermethylation in cancers has been linked to the silencing of tumor suppressor genes and subsequent oncogenesis [5,6]. Each DNA methylation analysis technique has its own advantages and limitations but the technological advancements in this fast-paced area of development enable increasingly sensitive and specific assessment of locus-specific DNA methylation. Several studies have already revealed useful diagnostic and prognostic DNA methylation patterns on multiple tumor genes for various cancer types [7–9] and some of the cfDNA methylation-based epigenetic biomarkers, such as VIM and SEPT9 in colorectal cancer; PTGER4/SHOX2 in lung cancer; and GSTP1 in prostate cancer, are already in clinical use and diagnostic kits are commercially available (Table 1).

More recently, advanced techniques have been implemented to capture aberrantly methylated DNA loci genome-wide, in order to identify more comprehensive methylation profiles from different tumor type [4,10,11]. These DNA methylation profiles can be used to create classifiers that can be applied to noninvasively and specifically detect early-stage cancer via liquid biopsy [7,11,12], and the commercial applications now in development have the potential to improve clinical practice of cancer care. Several companies have developed proprietary platforms that facilitate rapid and precise detection of various genomic alterations on ctDNA. Collaboration and partnership are key to realizing the potential of epigenetic applications within the clinical setting; academic experts bring unique and valuable insights into these collaborations, while the commercial partners can provide the necessary investment to package and deliver new technologies. Several companies have now identified and validated epigenetic signatures in the most informative regions of the cancer genomes using both tissues and large numbers of liquid biopsy samples, and by the use of machine-learning algorithms have developed assays that are capable of detecting both the presence of cancer and the tumor’s tissue of origin [4,10,11,13]. Companies at the forefront of the epigenetic discovery and its applications for noninvasive diagnostics around the world include EpiGeneⁱ, Epigenomicsⁱⁱ, Exact Sciencesⁱⁱⁱ, Freenome^{iv}, Genomictree^v, GRAIL^{vi}, Guardant Health^{vii}, Laboratory for Advanced Medicine (LAM)^{viii}, and MDxHealth^{ix}, among many others (Table 1). Identification of cancer-specific epigenetic markers can also shed light on the molecular pathways that are characteristic for

tumorigenesis, and therefore potentially enable application of these biomarkers for new therapies, companion diagnostics, and technologies that support personalized medicine.

Concluding Remarks and Future Perspectives

Approaches based on cfDNA and ctDNA methylation analysis provide new avenues for early detection of various cancer types, and DNA methylation signatures hold a great potential to become routine clinical cancer biomarkers due to their sensitivity, specificity, and ease of analysis. Although the feasibility of cfDNA methylation analysis alone for cancer detection and monitoring has been successfully demonstrated, combining cfDNA mutation markers with classical biochemical cancer markers and/ or imaging techniques [14,15] might be useful for the implementation of liquid biopsy as a standard of care. Epigenetic-based diagnostics of cancer and other diseases that enable detection of early disease will provide opportunities for clinical intervention before the progression of the disease has impacted quality of life, when patients are still fit, and when conditions favor treatment success. These highly specific biomarkers together with other somatic and germline genetic markers can help to personalize disease management; allowing treatment response to be monitored and medications adjusted more precisely, thus giving an opportunity to optimize treatments and to reduce adverse effects. In addition, epigenetic therapies have shown the potential for effective treatment of hematological and solid cancers, and combinations with immunotherapy approaches may greatly benefit cancer treatment [16].

As our understanding of the epigenome and its modifiability, especially its responsiveness to intervention, continues to deepen and evolve, the opportunities for innovation and application of this knowledge in the clinical setting are expanding considerably. In the future, these advances may help to shape our long-term approach to the management of overall human health and well-being; enabling ongoing monitoring of the epigenome, and opportunities to adapt appropriate lifestyles or to implement early clinical interventions, in order to avoid major health issues such as cancer.

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Figure 1.

Origins of Cell Free Tumor DNA (ctDNA) and Various Genomic Alterations Utilized in Liquid Biopsies. As illustrated, peripheral blood is the most common source of liquid biopsy (other body fluids can also be used). ctDNA released by apoptotic or necrotic tumor cells can be isolated from a simple blood draw and the ctDNA can be analyzed to identify multiple common DNA-based alterations observed in tumors, including mutations, copy-number variations, gene fusions, and DNA methylation changes. As shown at the bottom, specialized methods for ctDNA methylation analysis have been developed. Abbreviations: dPCR, digital PCR; Infinium, Infinium Human Methylation BeadChips; MBD, methyl-CpG-binding domain; MC-seq, methyl-capture sequencing; MeDIP, methylated DNA immunoprecipitation; MIRA-seq, methylated-CpG island recovery assay combined with sequencing; Pyro-seq, pyrosequencing; qMSP, quantitative methylation-specific PCR; RRBS, reduced-representation bisulfite-sequencing; SMART, single molecule, real-time sequencing; WGBS, whole-genome bisulfite sequencing.

Table 1.

Selected Noninvasive DNA Methylation Tests for Cancer

Company	Disease	Tests	Technology/biomarkers	Biospecimen	Status
EpiGene	Cervical and oral cancers	Cervi-M assay Oral-M assay	Methylation-specific-qPCR (PAX1 and ZNF582)	Human epithelial cells (cell scrapings from cervical brush for cervical cancer and from oral swabs for oral cancer)	CE marked
Epigenomics	Colon and lung cancers	Epi proColon Epi proLung	MethylLight (SEPT9 for colon; SHOX2 and PTGER4 for lung)	cfDNA from blood	FDA approval 13 April 2016 (Epi proColon) CE-IVD mark in Europe (Epi proLung)
Exact Sciences	Colon cancer	Cologuard stool-DNA-based test	DNA methylation (NDRG4 and BMP3), mutations, total DNA amount, and hemoglobin	Stool	FDA approval 11 August 2014
Freenome	Colon cancer	multianalyte test for colon cancer	'Multi-omic' test (no disclosed markers)	cfDNA, cfRNA, and protein from blood	Early development (clinical trial)
Genomictree	Colon cancer	EarlyTect CRC assay	MethylLight (SDC2)	cfDNA from blood	Early development
GRAIL	Multicancer	No specific test yet	Next generation sequencing (NGS) (no disclosed markers)	cfDNA from blood	FDA breakthrough device designation 13 May 2019
Guardant Health	Lung, breast, colorectal, and ovarian cancers.	Test for detecting minimal residual disease and disease recurrence (Lunar-1); early detection of cancer (Lunar-2)	Mutation and DNA methylation by NGS (no disclosed markers)	cfDNA from blood	FDA trial
Laboratory for Advanced Medicine (LAM)	Liver and other cancers	IvyGene liver Dx	Droplet digital (dd)PCR/NGS (no disclosed markers)	cfDNA from blood	FDA breakthrough device designation 3 September 2019
MDxHealth	Prostate cancer	ConfirmMDx	Methylation-specific-PCR (GSTP1, APC, and RASSF1)	cfDNA from urine	Laboratory developed test (LDT)