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doi:10.1093/annonc/mdy153
Published online 24 April 2018

Methylation in cell-free DNA for early cancer detection

Liquid biopsies and, in particular, analysis of cell-free DNA (cfDNA), have emerged as a promising and potentially transformative non-invasive diagnostic approach in oncology [1, 2]. cfDNA is composed of fragmented DNA released by cells into the circulation, typically as a result of cell death. cfDNA found in the plasma of healthy patients is composed of germline DNA released by normal cells. In cancer patients, some component of the overall cfDNA is composed of DNA released by tumor cells, often termed circulating tumor DNA (ctDNA). The fraction of ctDNA amidst the background of overall cfDNA is highly variable and often low, particularly in patients with early stage cancers [3]. Thus, the detection and analysis of tumor-derived cfDNA poses several challenges and has required the development of specialized technologies with high analytical sensitivity and specificity.

While recent studies have demonstrated the clinical potential of cfDNA for tumor genotyping and blood-based tracking of therapeutic response and resistance [4–7], one of the most transformative potential applications of cfDNA analysis is to detect the presence of cancer in patients without clinical evidence of disease. This potential has been demonstrated most clearly through the detection of residual disease following curative intent cancer surgery [8–11]. By screening post-operative cfDNA for the presence of specific mutations identified in the patient's resected tumor, detection of these tumor-specific mutations using highly sensitive techniques (often capable of detecting mutant alleles present at a frequency of 0.01%–0.1% or less) can accurately identify those patients who will eventually recur.

Similarly, the potential to detect nascent cancers in asymptomatic individuals with a simple screening blood test when they are still curable could revolutionize cancer medicine. Most studies to date utilizing cfDNA for cancer detection have focused on the detection of mutations in cancer-related genes. However, this approach for early cancer detection poses several key challenges. First, cfDNA levels in patients with early-stage cancers are often much lower than with advanced disease [3]. Second, unlike the residual disease setting discussed above, there is no prior knowledge of what specific mutations might be present in an individual patient's tumor. Third, benign lesions may harbor some of the same mutations commonly seen in certain tumors, leading to potential false positives. For example, *BRAF V600* mutations, which are present in nearly 7% of advances cancers, are often observed in benign nevi [12]. Many mutations detectable in cfDNA can also originate from the bone marrow through a process known as clonal hematopoiesis of indeterminate potential (CHIP), which increases exponentially with age [13]. Indeed, evidence of CHIP is observed in 10%–15% of patients over the age of 70 years. Finally, since many cancers share common mutations in genes such as *TP53* and *KRAS*, which are mutated in ∼50% and ∼20% of all cancers, respectively, localizing an early cancer to a specific organ site following the detection of mutations in cfDNA poses a significant challenge.

In this study, Liu et al. evaluate an alternative approach for early cancer detection based on assessing the methylation status of thousands of CpG sites in cfDNA [14]. Indeed, widespread methylation changes are commonly observed across multiple cancer types, with tumors of certain tissue origin displaying specific methylation patterns [15]. Thus, there are several potential advantages of assessing methylation. As mutation detection in cfDNA focuses on changes in a finite number of genes, these techniques are limited not just by analytical sensitivity and specificity, but also by the absolute number of cancer genomes present in a single tube of blood. If no DNA fragment from a specific mutated locus is present in single blood draw, no technique, no matter how perfect, can identify the presence of cancer. However, given the widespread methylation changes present in most cancers, assessing thousands of CpG sites increases the chances that tumor-derived DNA may be detectable in a given blood sample. Another key advantage is that methylation patterns often reflect the epigenetic origin of specific cancers and have been used to unmask the tissue of origin for cancers of unknown primary [16]. Thus, if evidence of cancer is detected, this approach offers the potential to interpolate tumor origin from these data to guide clinical efforts to localize and intervene.

To develop this approach, the authors mined the Cancer Genome Atlas database to identify 10 888 CpG sites frequently found to be hypermethylated in 32 tumor types. CpG sites also methylated in control cfDNA isolated from healthy individuals were excluded, resulting in 9322 individual CpG sites for analysis. In brief, whole-genome amplification was carried out on

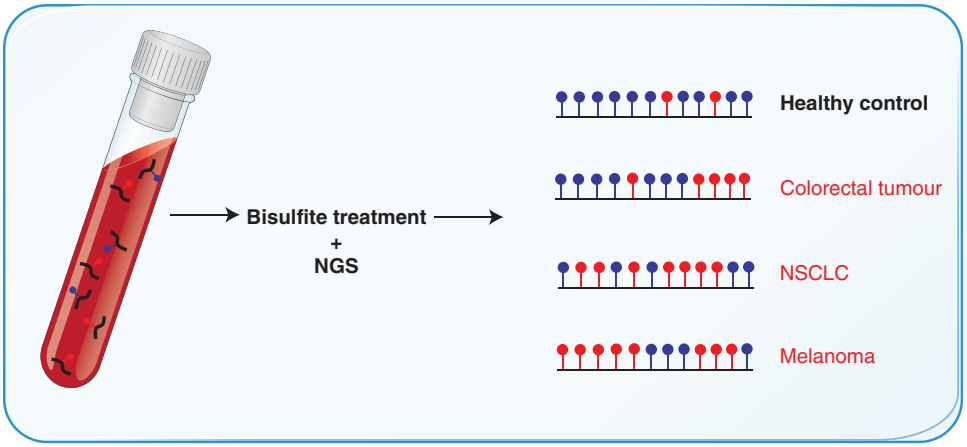

Figure 1. Targeted methylation profiling of plasma cfDNA for cancer detection and classification. Next-generation sequencing (NGS) of 9322 CpG sites in bisulfite-treated cfDNA was used to identify methylation changes in cfDNA for cancer detection. The pattern of methylation changes detected was also used to classify the tissue origin of detected cancers.

bisulfite-treated cfDNA before hybrid capture to preserve molecular diversity from the limited amount of cfDNA input. Sequencing data across CpG sites was then analyzed with a novel algorithm to determine a single methylation score for each sample (Figure 1).

The authors evaluated this pan-cancer methylation assay in 68 patients with advanced cancer (including colorectal, breast, melanoma and non-small-cell lung cancer) who were not receiving systemic therapy at the time of the blood draw, as well as 66 healthy control individuals. The methylation score correctly detected the presence of cancer in 86.8% of cancer patients and 0% of the 25 healthy patients from the validation set. Importantly, using a tissue classification algorithm based on methylation patterns, the authors correctly identified the tissue origin of each cancer in 76.3% of patients. The ability to infer tissue origin from cfDNA highlights the potential advantages of incorporating methylation analysis into blood-based early detection strategies.

A key limitation of this study is that it involved patients with advanced cancers, rather than patients with early stage and potentially curable cancers, which is the point at which screening tests would need to be effective in order to provide curative treatment options. Further evaluation will be necessary in larger numbers of patients with early cancers and ultimately in asymptomatic individuals to better assess the potential of this approach.

Still, this study is an important proof-of-concept for how incorporating DNA methylation changes into early detection strategies may be beneficial. Recently, two blood-based early detection studies were reported that highlight the potential benefit of looking beyond mutation detection alone in cfDNA. Chan et al. used detection of Epstein–Barr Virus DNA in cfDNA from 20 174 Chinese patients to screen for nasopharyngeal carcinoma, with a sensitivity of 97.1% and a specificity of 98.6% [17]. The CancerSEEK assay assessed secreted tumor-related protein biomarkers in addition to detection of key cancer mutations in cfDNA in 1005 patients with non-metastatic cancer and 812 healthy controls, with sensitivities ranging from 69% to 98% by tumor type, and a sensitivity of 99% [18]. This present study suggests that DNA methylation could also be a useful tool that should be further evaluated, either alone, or in conjunction with mutation detection, in cfDNA for improved early cancer detection strategies.

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Funding

NIH/NCI (R01CA208437), NIH/NCI Gastrointestinal Cancer SPORE (P50CA127003), and a Stand Up To Cancer Colorectal Dream Team Award (all to RBC).

Disclosure

RBC has received consulting fees from Amgen, Astex, Avidity, BMS, FOG Pharma, Genentech, LOXO, Merrimack, N-of-one, Roche, Roivant, Shire and Taiho, as well as research support from Astra Zeneca and Sanofi. The remaining author has declared no conflict of interest.

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doi:10.1093/annonc/mdy134
Published online 14 April 2018

Divergent adaptation in thyroid cancers

Thyroid cancer can be divided into several histological subtypes, the most common one being Papillary Thyroid Cancer (PTC). PTC has a good prognosis, whereas other subtypes such as Anaplastic Thyroid Cancer (ATC) have a dismal survival.

Some patients with ATC have a previous history of PTC and in rare cases two tumours with distinct histology can coexist at the same time. This led to the hypothesis that ATC is the result of progression from PTC, generally through the accumulation of additional malignant traits driven by clonal selection, hence linear evolution from PTC to ATC.

The hypothesis was sound and motivated by good histological data. However, as it happened with the advent of ancient genomics in archaeology [1], DNA data are often bound to shake previous assumptions.

The field of cancer genomics is often driven by big numbers and large cohorts, but it is the analysis of rare and unusual cases that is often key to grasp the underlying cancer biology. Many cancer genes have indeed been identified by studying rare germline predispositions to cancer, from *APC* [2] to *NF1* [3].

In a study led by Ana Vivancos and Joan Seoane, from the Vall d'Hebron Institute of Oncology in Barcelona, Spain, Capdevila et al. [4] analyse the genomic profiles of a cohort of ATC and PTC tumours. Interestingly, they also examine a small set of rare cases with concomitant ATC and PTC neoplasms.

The data are clear and indicate that ATC is unlikely to have linearly evolved from PTC. Instead, the two lesions seem to develop in parallel and are characterised by early evolutionary divergence. Hence, divergent adaptation, rather than linear evolution, drives tumourigenesis in these thyroid cancers, with the two phenotypes co-existing side by side.

Although a larger study and additional data will be necessary to confirm this pattern, this study demonstrates how branched evolution is pervasive in cancer, supporting the idea that some tumours are born to be bad.

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Funding

AS is supported by the Wellcome Trust (202778/B/16/Z), Cancer Research UK (A22909) and the Chris Rokos Fellowship in Evolution and Cancer. We also acknowledge funding from the Wellcome Trust to the Centre for Evolution and Cancer (105104/Z/14/Z).

Disclosure

The author has declared no conflicts of interest.

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doi:10.1093/annonc/mdy170
Published online 2 May 2018