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Scanning for Cancer Genomic Changes in Plasma: Toward an Era of Personalized Blood-Based Tumor Markers

K.C. Allen Chan^{1,2,3*}

The analysis of circulating cell-free tumor DNA has remarkable potential for the detection and monitoring of cancers. Genetic alterations are present in virtually all types of cancers, allowing broad applicability of this approach regardless of the primary organ and histologic type of the cancer. To date only a few specific proteins and glycoproteins have been discovered that can be reliably used as tumor markers. Consequently, extensive efforts have been made over the last decade to identify cancer-associated genetic changes that might be suitable for use as new tumor markers. However, only a few of these genetic markers have been translated into clinical use. One major reason is that the genetic changes in cancer are very heterogeneous even within a single type of cancer. Therefore, it is difficult to achieve a high clinical sensitivity for cancer detection by targeting only one or even a few genetic markers. In a recent study by Dawson et al., an approach has been proposed for developing individualized tumor markers (1). As the first step of this approach, the tumor tissue of each cancer patient was sequenced by either targeted amplicon sequencing or whole-genome sequencing to identify cancer-specific single nucleotide changes and structural variants. The alterations identified were detected and quantified in the plasma of the corresponding patient with the use of digital PCR or targeted deep sequencing. Tumor samples from a total of 52 patients with metastatic breast cancer were analyzed using targeted sequencing to identify point mutations in the phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (*PIK3CA*)⁴ and tumor protein p53 (*TP53*) genes (1). Twenty-five (48%) of the patients had at least one mutation detected using this method. Whole-genome sequencing was performed on tumor

tissue samples from 9 of these individuals. Additional structural variants and/or single-nucleotide mutations were identified in all of the tumors from these 9 patients. Overall, cancer-associated alterations were identified in 30 patients. In 29 of the 30 patients, the tumor-related alterations were detected in at least 1 plasma sample and the concentrations of the alterations appeared to correlate with the clinical status of the patients. The detection rate for circulating tumor DNA was superior to that for detecting circulating tumor cells and the analysis of CA 15–3 (1).

The ability to accurately identify cancer-associated genetic alterations is highly dependent on the platform used for tumor tissue analysis. In the study conducted by Dawson et al., patient-specific changes were identified in each of the 9 tumor tissues analyzed by whole-genome sequencing. In contrast, mutations were detected in fewer than half of the tumor tissues tested by using targeted sequencing for the 2 genes that are frequently mutated in breast cancers. In addition, the number of somatic mutations that can be identified by whole-genome sequencing is much larger than the number that can be identified by any assay targeting a specific set of genes. The sensitivity of detecting the cancer-associated alterations in plasma will be dependent on the number of targets used, the amount of plasma DNA analyzed, and the fractional concentration of tumor-derived DNA in the plasma sample (2). The latter factor will be significantly affected by the overall tumor burden (2).

Apart from its applications in monitoring of disease progression and early detection of recurrence, circulating tumor DNA analysis can also be used for determination of patient prognosis, prediction of treatment response, and detection or screening of early cancers. Almost all patients who have received curative-intent cancer treatment will ask the question, “Will the cancer come back?” Identification of cancer-specific DNA alterations offers a powerful tool to aid in answering this important question. The presence of detectable cancer-specific changes in plasma following treatment could potentially indicate the presence of residual tumor cells in the body. However, a few practical points will need to be addressed before this approach can be translated into routine clinical use. First, muta-

¹ Department of Chemical Pathology, ² State Key Laboratory of Oncology in South China, ³ Li Ka Shing Institute of Health Sciences, the Chinese University of Hong Kong, China.

* Address correspondence to the author at: Department of Chemical Pathology, Rm. 34134, 1/F Clinical Sciences Building, Prince of Wales Hospital, Shatin, Hong Kong, China. Fax +852-2636-5090; e-mail allen@cuhk.edu.hk.

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⁴ Human genes: *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; *TP53*, tumor protein p53; *EGFR*, epidermal growth factor receptor.

tions or structural variants detected in tumor tissues may also be present in premalignant tissues arising from the same progenitor clone of cells as the tumor. Therefore, prospective clinical studies involving a substantial number of patients will be required to determine what plasma level of cancer-related changes is associated with a significant risk of clinical recurrence. The marker used within individual cancer patients will frequently be unique, and a platform that can accurately quantify concentrations of individual markers without a calibration system will be required. In the study by Dawson et al., digital PCR and targeted amplicon sequencing were used to quantify the cancer-associated changes in the plasma. Both methods achieved very low detection limits, although digital PCR was able to directly measure the absolute amounts of the genetic alterations, as opposed to amplicon sequencing, which determined only the relative abundance of the changes relative to the wild-type sequences. In the latter approach, estimates of the absolute mutation concentration can be made on the basis of the total concentration of DNA in the plasma sample. Although the mutant concentrations estimated by the 2 methods correlated well, the absolute concentrations could differ by more than 100-fold.

The number of therapeutic agents that target specific molecular pathways involved in carcinogenesis is growing. For many of these targeted therapies, the treatment response can be predicted by analyzing specific mutations in the targeted proteins that control cell growth or differentiation. For example, the presence of activating mutations in the epidermal growth factor receptor (*EGFR*) gene is predictive of response to the *EGFR* tyrosine kinase inhibitor in patients with non-small cell lung cancer. However, patients with inoperable cancers often may not have tumor tissues available of acceptable or good quality for molecular analysis. In these circumstances and within this subset of patients, analysis of circulating tumor DNA would be very useful for predicting the treatment response.

The next frontier for plasma DNA-based cancer detection is the use of this approach for cancer screening. It has recently been shown that the analysis of tumor-derived Epstein-Barr virus DNA in plasma is useful for the detection of early asymptomatic nasopharyngeal carcinoma (3). One patient identified through this type of screening had a tumor only 1 mm in diameter. This finding suggests that even very small tumors can release a substantial amount of DNA into the circulation. The application of individualized somatic changes for the screening of early cancer is also technically challenging because tumor tissues are not available for assessing the patient-specific changes in this situation. Theoretically, cancer-associated somatic alterations can be deduced directly from the cancer pa-

tient's plasma sample. Mutations which are detectable in plasma but not in the constitutional genome are potentially derived from cancer, but the ability to detect single-nucleotide mutations and structural variants directly in plasma is limited by sequencing errors. Theoretically, sequencing errors can be differentiated from real cancer mutations when the candidate region is covered by many sequenced reads. Cancer-associated mutations should be detectable on multiple plasma DNA fragments, and their frequency should reflect the fractional concentration of tumor-derived DNA in the plasma sample. In contrast, sequencing errors are likely to occur randomly, and the frequency of such errors would follow the Poisson distribution. Owing to the low fractional concentration of cancer DNA in the plasma of early cancer patients, very high sequencing depth would be required to differentiate real mutations from background sequencing errors. To improve the cost-effectiveness of this approach, the search for the mutations can be restricted to specific regions, for example, the exome. In this regard, Murtaza et al. (4) used exome capture sequencing to compare the mutational profiles of the plasma DNA of cancer patients determined before and after chemotherapy or targeted therapy and identified mutations that may be associated with acquired resistance to these treatments. In another approach, cancer-associated chromosomal aberrations, including gains and losses, can be targeted as markers for cancers. Chromosomal regions with amplification and deletion in the tumor tissue would be relatively over- and underrepresented, respectively, in the plasma of the cancer patient (2, 5). These changes can be detected by massively parallel sequencing of plasma DNA through the comparison of the densities of sequenced reads between different chromosomal regions. Because the presence of an aberration is not determined directly by the sequence context, this approach is less susceptible to the effect of sequencing errors. In addition to indicating the presence of an occult cancer, the patterns of chromosomal aberrations, single-nucleotide mutations, and structural variants identified in plasma may also be useful to determine what type of cancer is present.

In summary, the large number of genetic alterations in cancer may potentially allow utilization of genomic changes in circulating cell-free DNA as personalized tumor markers for the screening, monitoring, and prognostication of cancers. With the rapid reduction in the cost of massively parallel sequencing, this approach may soon be adapted for routine clinical care.

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