A simple blood test for cancer detection has been the quest of many researchers. Thus, the finding that cell-free circulating DNA in the plasma of cancer patients has characteristics of tumor DNA has produced much interest. In fact, in the last few years, more than 200 publications on this topic have appeared in the literature.

In the 1970s, early studies showed that increased quantities of DNA could be found in the plasma of patients suffering from different malignancies (1). Later it was reported that this circulating extracellular DNA exhibits tumor-related alterations, such as decreased strand stability (2), Ras or p53 mutations, microsatellite alterations, aberrant promoter hypermethylation of tumor suppressor genes, rearranged immunoglobulin heavy-chain DNA, mitochondrial DNA mutations, and tumor-related viral DNA (3).

The success of viral DNA as a tumor marker in plasma/serum has been rapid because of the development by the group headed by Dennis Lo in Hong Kong of a method of accurately quantifying viral DNA in the circulation (4). This very active group has been able, for example, to quantify the amount of Epstein–Barr virus DNA in the serum of patients with nasopharyngeal cancer. It is possible to predict by measuring the amount of free Epstein–Barr virus DNA the patients who are likely to relapse several months before the clinical appearance of a tumor.

The results obtained with plasma/serum DNA in many cancers have opened a new research area, indicating that plasma/serum will eventually become a suitable source for the development of noninvasive diagnostic, prognostic, and follow-up tests for cancer. A recent development in this new field is the finding of tumor-related RNA in the plasma or serum of cancer patients (5). These features include tyrosinase mRNA (5), telomerase components (6, 7), the mRNAs encoded by different tumor-related genes (8–12), and viral RNA (13). The work on circulating RNA markers is particularly promising because the percentage of detectable tumors seems higher than that found with DNA markers. In the case of breast cancer, for example, investigators in one study had to use six microsatellite markers, p53 mutations, and the methylation patterns of the first exon of p16 to detect tumor-related DNA alterations in the plasma of 66% of the breast cancer patients studied (14). In a study in which RNA markers were used, however, two telomerase markers yielded positive results for 44% of the plasmas (6). Telomerase RNA seems to be a promising marker because it can be found in the serum of patients with small, still-undifferentiated breast tumors without any metastasized nodes. Using real-time reverse transcription-PCR (RT-PCR), Dasi et al. (7) showed that circulating telomerase RNA is a sensitive marker. In their study, 8 of 9 plasma samples from colorectal cancer patients and 9 of 9 plasmas from patients with lymphoma were positive for human telomerase reverse transcriptase, whereas all 10 healthy controls were negative. In another study on breast cancer, of the 45 patients analyzed, 27 cases (60%) were positive for mammaglobin and 22 were positive for CK19. Moreover, tumor size and proliferative index were associated with the presence of mammaglobin, CK19, or both RNAs in plasma (8).

The article by Ng et al. (15) in this issue of Clinical Chemistry represents an important advance in the understanding of circulating nucleic acids. Dennis Lo’s team has used RT-PCR to measure circulating RNA in cancer patients and healthy individuals. They found that glycerolaldehyde 3-phosphate dehydrogenase (GAPDH) mRNA concentrations were significantly higher in patients with hepatocellular carcinoma than in healthy controls. Although there was some overlap between a subset of patients with low GAPDH mRNA concentrations and healthy controls with higher concentrations, it seems that the majority of the patients could have been detected by measuring the quantity of mRNA in the plasma. Nevertheless, this simple and robust method might be used as a very easy and useful assay for follow-up after surgery or therapy.

This article also brings new insights on the biological problem concerning the origin of circulating nucleic acids. Indeed, before RNA extraction, the authors submitted the plasma samples to different centrifugation or filtration techniques (15). Plasma samples from each individual were either filtered through different-sized filters (5–0.22 μm) or ultracentrifuged. Plasma RNA was measured by real-time RT-PCR for GAPDH and plasma DNA by real-time PCR for the β-globin gene. The concentrations of plasma GAPDH mRNA decreased by a median of 15-fold in the 0.22 μm-filtered and ultracentrifuged samples compared with paired unfiltered samples, indicating that an important fraction of plasma RNA is particle associated. These particles are of different sizes and could be apoptotic bodies, which would explain why the circulating RNA is protected in the plasma. In contrast, there was no significant difference in β-globin DNA concentrations among plasma samples filtered through different-sized pores, showing that circulating DNA either obeys a different mechanism of release or that the DNA-containing particles are destroyed for some reason.

The field of circulating nucleic acids is becoming more exciting. The use of plasma DNA and RNA may provide opportunities to establish noninvasive tests for detecting cancer, in addition to their use in prenatal detection (16), and may open new and largely unexplored biological pathways.
References


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