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Commentary

TP53 mutations on circulating cell-free DNA

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Detection of cancer by analysis of circulating cell-free DNA (cfDNA) has received enormous attention over the past years (Alix-Panabieres and Pantel, 2016). cfDNA is released into the blood circulation by apoptotic and necrotic cells, and cancer patients have increased concentrations of cfDNA (Schwarzenbach et al., 2011). With the development of novel technologies it is now possible to detect tumor-associated mutations on cfDNA and monitor the evolution of cancer progression in patients (Bettegowda et al., 2014). However, the detection of very low amounts of circulating tumor-derived DNA (ctDNA) in blood samples from early stage cancer patients is still a challenge. ctDNA was revealed in only 48–73% of patients with localized cancers including lung cancer (Bettegowda et al., 2014; Newman et al., 2014).

In this issue of *EBioMedicine*, Fernandez-Cuesta et al. (2016) have assessed cfDNA from patients with small-cell lung cancer (SCLC) for *TP53* mutations known to occur in the majority of SCLC cases (George et al., 2015). The analysis of cfDNA using an assay specifically designed to accurately detect variants at very low allelic fractions revealed *TP53* mutations in 49% of SCLC patients and, most importantly for cancer detection, in 35.7% of early-stage cases. This incidence in early stage SCLC matches those reported for other cancer types and suggests that early-stage tumors might shed only minute amounts of DNA into the circulation. Thus, *TP53* mutations alone have limited sensitivity as marker for detection of early SCLC. So far, it remains to be investigated whether more sensitive technologies or the addition (or combinations) of other cancer-specific mutations will increase the sensitivity of liquid biopsy. Moreover, larger plasma volumes, and across repeated time points, might be required for early cancer detection, far beyond the "one blood drop" promise. The ctDNA concentration in early-stage lung cancer patients can be as low as one genome equivalent in 5 ml blood (Newman et al., 2016). The need for larger blood volumes in early stage cancer patients has been already realized for CTC-based liquid biopsies (Alix-Panabieres and Pantel, 2014).

Besides the demand for higher sensitivity, the specificity of cfDNA measurements faces even more serious challenges. Cancer-associated mutations are thought to be restricted to cancer patients. However, Fernandez-Cuesta et al. observed cfDNA *TP53*-mutated fragments in 11.4% of 123 matched non-cancer controls. Acknowledging the potential for bias in the selection of controls (such as differential performance in QC criteria or cfDNA amount, between cases and controls), they screened a second series of 102 non-cancer controls, and found a comparable proportion of *TP53* mutations (13 *TP53* mutations in 11 controls, 10.8%). Cancer cases and controls were matched for age as well as tobacco and alcohol consumption. The present results are consistent with recent findings of Krimmel et al. (2016), demonstrating very low levels of *TP53* mutations in the peritoneal fluid and peripheral blood of women with benign ovarian lesions. Previously, Genovese et al. (2014) demonstrated that leukemia-associated mutations also occur with increasing age, and although posing a statistically significant risk to develop leukemia, most individuals (>90%) who tested "positive" never developed leukemia during their lifetime. Clonal hematopoiesis with somatic mutations was observed in 10% of persons older than 65 years of age and the absolute risk of conversion from clonal hematopoiesis to hematologic cancer was low (Genovese et al., 2014). Fernandez-Cuesta et al. also observed two TP53 mutations in one SCLC patient, one originating from leukocytes, which adds another layer of complexity.

In conclusion, further improvements are required to reach an acceptable sensitivity and specificity for early cancer detection. The presence of cancer-associated mutations on cfDNA might not necessarily indicate that the individual tested has already cancer or will develop cancer in her/his life time but it might induce substantial anxiety and extensive diagnostic procedures with health risks like radiation in CT scans. Cohort studies focusing on patients at high risk to develop cancer have been successful for CTCs as liquid biomarkers (Alix-Panabieres and Pantel, 2016). Extensive research is required to identify possible combinations of cancer-specific mutations and define quantitative potential thresholds to avoid over-diagnosis. Moreover, the interpretation of DNA screening results of leukocytes (or other normal cells) as "germline" mutations, which is current practice in disease research studies focusing on genetic predisposition, might be corrupted in ageing individuals (e.g., prostate cancer patients) by somatic background mutations in normal cells. Standardization and validation of cfDNA assays are important issues realized by the current EU/IMI consortium CANCER-ID (www.cancer-id.eu).

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Disclosure

The author has no conflicts of interest.

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