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Liquid Biopsies for Early Cancer Detection

CANCER

Detection and localization of surgically resectable cancers with a multi-analyte blood test

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Lay summary

Early detection of many cancers is believed to save lives, which is why routine screening for breast and bowel cancer is available to those in the general population who are likely to benefit. This article by a large team of US scientists describes a recently published blood test for multiple cancer types, which while sophisticated, is relatively cheap and accurate. The article discusses some of the features of this test and whether it could one day be modified to screen people for early signs of brain cancer.

Background

The metastatic tumours responsible for most deaths from cancer may have evolved over decades of adult life. This large timeframe provides an opportunity for early diagnosis in the population at large, thus increasing the likelihood of effective therapeutic intervention. This at least, is possible for the most common cancers, like those derived from the lung, colon or breast. Malignant gliomas (hereafter referred to as glioblastomas) are different in that they grow locally and rarely metastasise outside the brain. Given this localisation, it should theoretically be possible, with sensitive imaging technology, to identify and surgically remove the entire tumour mass, thus leading to a cure for at least some patients. The fact that this is not the case, except with some low-grade gliomas, highlights the importance of detecting glioblastomas at a very early stage in their development ¹. Clearly, imaging alone is not the answer; some form of molecular diagnosis is required, using cerebrospinal fluid, (or ideally blood) as source material.

Circulating tumour DNA

The first detection of tumour DNA in the circulation (ctDNA) was reported in 1977 before the technology was available to exploit it ². Since then, the field has expanded enormously, facilitated by technical advances in DNA sequencing, bioinformatics and the availability of cancer mutation databases ³. The diagnostic potential of ctDNA detection received a major boost recently, with a publication in *Science* from a multinational group led by Joshua Cohen ⁴ looking to devise a low cost (~\$500) screening test for cancers of the ovary, liver, stomach, pancreas, oesophagus, colorectum, lung and breast. Their system, called CancerSEEK, combines detection of both ctDNA and selected serum protein markers to increase accuracy and discrimination between patients and normal controls. Although glioblastomas are not included in their list of cancers, the basic principle CancerSEEK could be applied in GBM, since circulating nucleic acid with the characteristic EGFRvIII mutation has been detected in extracellular vesicles (EVs) derived from glioblastoma cells and there is an active research effort in this area ^{5,6}. Its potential application for GBM diagnosis makes CancerSEEK technology appropriate for a *Windows on Glioblastoma* article.

CancerSEEK design and implementation

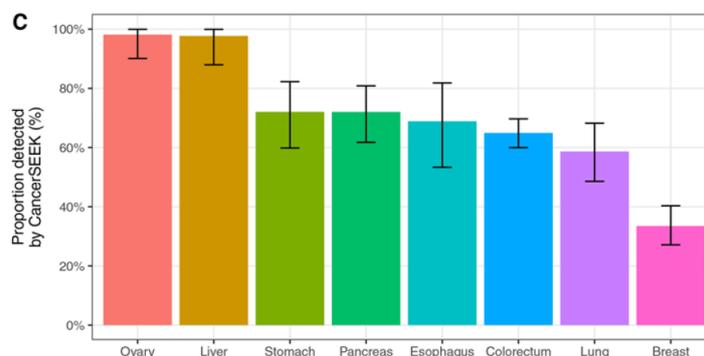
A PCR-based assay was developed by that could simultaneously assess multiple regions of driver genes that are commonly mutated in a variety of cancer types. Based on work to define the minimum number of short amplicons required to detect at least one driver gene mutation in each tumour types evaluated, multiplex-PCR was used to label each original template molecule with a unique DNA barcode to make efficient use of the small amount of

cell-free DNA present in the 1,005 plasma samples tested ⁴. 61 primer pairs were designed to amplify 66 to 80 bp segments containing regions of interest from 16 genes. The amplified DNA was then sequenced using an Illumina MiSeq or HiSeq 4000 instrument. Based on data from the Catalog of Somatic Mutations in Cancer (COSMIC) dataset ⁷, the gene panel should theoretically have detected 41% (liver) to 95% (pancreas) of the cancers. In practice, the panel performed considerably better, detecting at least one mutation in 82%, two mutations in 47%, and more than two mutations in 8% of the 805 cancers evaluated. This is because PCR-based sequencing is more sensitive for detecting mutations than conventional genome-wide sequencing.

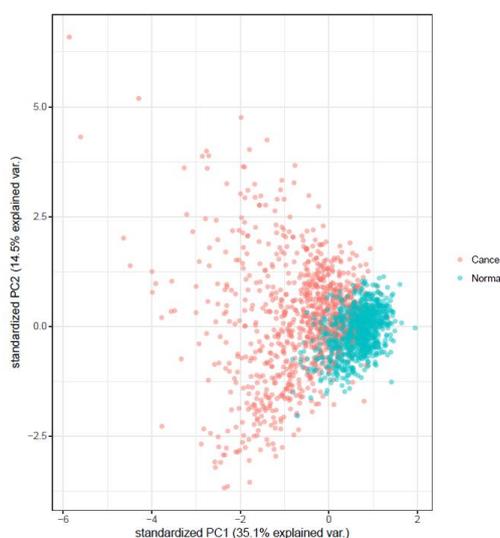
The maximum sensitivity of PCR-based analysis of ctDNA is limited for localised cancers ⁸ but can be enhanced if measurements of protein biomarkers are included, as discovered by the authors in an earlier study with pancreatic cancer ⁹. For the CancerSEEK study, eight proteins out of an initially selected panel of thirty-nine were particularly useful for discriminating cancer patients from healthy controls when used in a Luminex bead-based immunoassay format. These proteins were: CA-125, CA19-9, CEA, HGF (hepatocyte growth factor), myeloperoxidase, OPN (osteopontin), prolactin and TIMP-1 ⁴.

Assay performance

As mentioned above, the ctDNA analysis provided greater sensitivity with liquid biopsy samples when compared with a cancer sequence mutation database. The system was able to detect tumours at distinct levels of sensitivity according to the tissue of origin, with tests for ovarian and liver cancers being the most effective (see Figure below, taken from ⁴).



For a mass screening test to be useful, it must detect cancers at relatively early stage. The median sensitivity of CancerSEEK was 73% for the most common stage evaluated (Stage II), similar (78%) for Stage III cancers, and lower (43%) for Stage I cancers. The sensitivity for the earliest stage cancers (Stage I) was highest for liver cancer (100%) and lowest for oesophageal cancer (20%). Further studies showed that the concordance of mutations between plasma and primary tumours in the same individual was 138 out of 153 cases (i.e. 90%). This ranged from 100% in ovarian and pancreatic cancers to 82% in stomach cancers. Lastly, the possibility of detecting false positives in the normal population was addressed by comparison with a control cohort. Only 7 out of 812 normal individuals tested positive and these could have very early signs of disease or else have elevated blood markers, possibly relating to inflammation. The impressive (>90%) discrimination between disease and normal samples is shown in the figure below where the mutation and protein marker values have been analysed using principle component analysis (taken from Supplementary Information in ⁴).



What does this mean for glioblastoma detection?

The basic principles behind CancerSEEK, namely evaluation of a panel of driver mutations in ctDNA coupled with immunoassays of cancer biomarkers, should be readily applicable to glioblastoma. Candidate mutations for this disease include PTEN, TP53, EGFR, PIK3CA, PIK3R1, NF1, RB1, IDH1, and PDGFRA as reported in a

comprehensive study by Brennan et al in 2013 ¹⁰. Useful protein markers from serum samples could in future be derived from the extracellular vesicles that are known to migrate from the brain into the periphery ¹¹. It would be interesting to see whether the existing CancerSEEK panel of DNA and protein assays is in any way applicable to brain cancer without having to extensively modify the primers and immunoassays used.

Conclusions

The combination of targeted driver mutation and protein biomarker analysis of blood samples from patients with a range of common cancers is very exciting, particularly as the unit cost of such tests makes it feasible to conduct mass screening. Despite the promise of this approach and the enthusiasm in the scientific and lay press generated by its publication, there are inevitably several caveats, including the detection of false positive results in people with chronic inflammatory conditions. This is discussed in a commentary for the Science paper which also includes the reactions of experts in the field of cancer diagnosis ¹². The broad proof of principle has been effectively demonstrated and now needs to be developed further, hopefully including early glioblastoma in the list of tumours for analysis.

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