International Agency for Research on Cancer

World Health Organization

CROSS-CUTTING SCIENTIFIC THEME:

Circulating tumour DNA: application to population-based studies

Introduction

Cancers are characterized by genetic and epigenetic alterations and the analysis of cancer-specific changes in DNA is increasingly used for diagnosis, prognosis and therapeutic decisions. Genetic and epigenetic profiles of cancers are typically obtained from surgical samples or biopsies. However, there are many difficulties in obtaining tissue biopsies stemming from the invasive nature, the inherent clinical risk for the patients and cost considerations.

Circulating free DNA (cfDNA) refers to DNA found in body fluids (such as plasma and serum) as a result of passive or active release from healthy or cancerous tissues. The fraction of cfDNA that originates from cancer cells is known as circulating-tumour DNA (ctDNA). ctDNA harbours genetic and epigenetic alterations that are found in the tumour. These changes, identified with sensitive and robust techniques (such as 'omics'), can now be used as tumour biomarkers in the blood. Therefore, assessment of somatic genetic changes (somatic mutations, copy number aberrations, loss of heterozygosity) and epigenetic alterations (DNA methylation) in cfDNA ("liquid biopsy") may represent a powerful approach that overcomes fundamental limitations of the current tools and molecular analyses that rely on tissue biopsies.

The observation of ctDNA in early stage tumours also implies there may be some potential in early detection of cancer. Most of the studies conducted so far may be considered "convenience studies", in which already available samples were interrogated retrospectively. Large-scale molecular epidemiology studies are necessary to determine the utility of these ctDNA in the context of early detection.

Although assessment of ctDNA has a tremendous potential, important challenges need to be overcome before full application. The amount and quality of ctDNA in plasma is generally well below that used in omics techniques, complicating the assessment of the utility of ctDNA in molecular epidemiology studies. In addition, to be applicable in early detection, mutations/methylation changes must be identified without prior knowledge of the tumour's (epi)genotypes, and these alterations are found at frequencies well below what is detected routinely by omics techniques. Nevertheless, there is now a major opportunity to apply in population-based studies the ctDNA techniques developed in clinical translational research.

Over the last few years, IARC has placed considerable emphasis on optimizing the molecular techniques to be able to consider the potential of ctDNA in the context of early detection. First, the emphasis was on optimizing the techniques applicable to ctDNA, and then applying these methods to the exceptional biobank resources at IARC. These generally included assessing the performance of the methods in retrospective case-control studies, followed by, where applicable, testing their performance in pre-diagnostic material collected in large prospective cohorts such as the European Prospective Investigation into Cancer and Nutrition (EPIC) study.

Detection of mutations and epigenetic changes in ctDNA in blood samples as a pre-diagnostic biomarker and as a measure of disease progression

The participating Sections/Groups are: GEN/GCS, GEN/GEP, MCA/EGE, MCA/MMB, NME/NEP and INF/ICB[1]

The description of the genomic and epigenomic (DNA methylation) landscapes of most human cancers has uncovered several important characteristics conducive to ctDNA-based analysis, suggesting a particular opportunity for early detection using ctDNA for specific cancer types. The proposal is to investigate, for a series of cancers for which extensive diagnostic and pre-diagnostic samples are available, to what extent gene mutations and DNA methylation changes identified in the tumour are also identifiable in ctDNA from blood samples (i) collected at the time of diagnosis, and (ii) collected before diagnosis. Also, if specific (epi)genetic changes in ctDNA are identifiable before diagnosis, how many months/years prior to diagnosis are they present in liquid biopsies? Current proposals are being developed for cancers of lung, pancreatic, breast, and liver, although studies involving other cancer types are also being considered.

The initial focus has been on the development of assays that allow assessment of mutations and DNA methylation in specific genes in ctDNA. These include the genes that are found to be frequently mutated (such as *TP53*, *RB1*, and *KRAS)* and or aberrantly methylated (including CDKN2A/p16, MLH1, RASSF1A, VIM, and FBLN1). Since some oncogenic viruses integrate into the genome of cancer cells, the evaluation of viral ctDNA as pre-diagnostic marker for cancers associated with human papillomavirus infection will also be considered. Specific aims of this study are: (1) to develop and test the detection limits of assays capable of simultaneously assessing specific gene mutations and DNA methylation changes in the challenging technical environment of ctDNA. This involves optimization of laboratory techniques and the development of associated bioinformatics tools to identify sequence variants and methylation patterns within a larger genomic region of the target genes; (2) to assess the presence of gene mutations and DNA methylation changes in the plasma DNA of a retrospective series of cases and non-cancer controls. The selection of cancer-specific mutations and DNA methylome changes will be based on cross-comparison between the data of tumour profiling at IARC, datasets from the literature and publicly

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[1] GEN/GCS = Section of Genetics/Genetic Cancer Susceptibility Group
GEN/GEP = Section of Genetics/Genetic Epidemiology Group
MCA/EGE = Section of Mechanisms of Carcinogenesis/Epigenetics Group
MCA/MMB = Section of Mechanisms of Carcinogenesis /Molecular Mechanisms and Biomarkers Group
NME/NEP = Section of Nutrition and Metabolism/Nutritional Epidemiology Group
INF/ICB = Section of Infections/Infections and Cancer Biology Group

available "The Cancer Genome Atlas (TCGA)" data; and (3) to conduct ctDNA analysis for the presence of specific mutations and DNA methylation changes in pre-diagnostic samples from case-control studies nested within a prospective cohort. To this end, we will take advantage of the EPIC cohort with large sub-cohorts for the cancer types under study. This will allow for an estimation of the prevalence of ctDNA mutations and aberrant methylation in biological samples from individuals in pre-diagnosis through the follow-up period.

Together, these approaches should allow a careful evaluation of the diagnostic and predictive value of individual genetic and epigenetic markers in ctDNA and combinations of these markers at diagnosis, as well as precise time windows for their potential application in pre-diagnosis. It should provide proof-of-principle that ctDNA has the potential to assist in the early detection of specific human cancers and it will be applicable to several other forms of cancer.

Questions or areas of advice to be addressed by the Scientific Council (SC)

1. Should IARC take advantage of recent advances in sequencing technology and bioinformatics tools and the availability of large cohorts to explore liquid biopsies in molecular epidemiology settings?

2. Does the SC consider the general design of the proposed studies on ctDNA suitable and what additional actions could be taken at IARC for a greater impact of these studies (including competitiveness)?

3. Does the SC identify significant methodological challenges and issues with compatibility of ctDNA analysis with the current bio-repositories at IARC?

4. What criteria should IARC apply in selecting cancer types to focus on in the context of ctDNA studies?