Opinion

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Dpcr Opening More Specific Possibilities for Oncological Diagnosis

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ABSTRACT

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Cancerous tissue is highly heterogeneous and cancer biomarkers vary across types ofdisease and stages of disease`s progression, this complicates cancer detection andidentification at early stages [1]. The cancer identification of mutations present in anindividual tumor often rely on analysis of biopsy or cytology samples, where only asmall fraction of the tumor is analyzed, and may not provide a complete representation oftumor heterogeneity, these has significant disadvantages, because low abundancemutations relative to wild type DNA [2]. In addition, the sub clonal populations of cellswithin a tumor may contain a mutation that differs from the primary mutation, and thesub clonal mutation could be correlated to a prognosis and/or a response to personalizedtreatments [1]. Sequencing is still the gold standard for mutations identification, its costis steadily decreasing as much effort is made to reduce the cost and also to improve thedata interpretation in downstream analysis of next-generation sequencing [3], despitethis, its use remains limited for the diagnostic in a routine laboratory. The detection ofmutations is performed mainly by real-time quantitative PCR (qPCR) [4], but thistechnique presents limitations including preferential amplification of small fragments,production of chimeric sequences, the amplify all alleles with approximately equalefficiency comparable to their initial concentrations, and difficulty in detecting lowabundance or poorly represented sequences [5].

In the last years, a technology calleddigital PCR (dPCR) has become commercialized. As in qPCR, fluorescent dyes areincluded in the DNA amplification reaction. However, unlike qPCR the amplificationreaction in dPCR is divided into thousands of individual reactions prior to amplification.These partitioning can be achieved by using microwell plates, capillaries, oil emulsions,or arrays. Ideally, partitioning occurs such that each individual reaction mixturecontains either a single target molecule or none at all[4]. The acquisition of data atreaction end point, and the number of positive (fluorescent) and negative partitions iscounted, the target copy number in the sample is calculated based on the number ofpositive and negative partitions [6]. The Poisson's Law is used to accurately calculate the number of DNA targets per partition and the copy number in the original sample.These offers the advantage of quantify directly the absolute concentration of targetspresent in a DNA sample without the need for external calibrators [7], it is lesssusceptible to PCR inhibition and high background DNA levels in samples such asDNA isolated from FFPE biopsies [8]. The sensitivity is significantly higher thanqPCR, the accuracy and precision of the assay improves by counting larger numbers ofmolecules individually.

These detection limits facilitate the detection of minor alleles,such as in circulating tumor DNA, with a relatively simple and non-invasive approachto monitoring disease recurrence, which requires a high sensitivity of mutation detectionto provide effective therapies at the earliest stage of progression. Cases such as thedetection of the BRAFV600E mutation as well as for follow-up monitoring todetermine the treatment response in patients with malignant melanomas [9,10], thedetection of mutated genes in liquid biopsies for metastatic colorectal cancer [11,12],the detection mutant KRAS and TP53 in circulating exosomes for pancreatic cancer[13],thedetectionofminimalresidualdiseasewith

BCR-ABLtranslocationsforlymphoproliferative disorders [14], all of them reflect the need tools to mutationdetection highly sensitives, the which is relevant to for determine the treatmentresponse. The dPCR technology, being promissory for the detection of mutations in therange of 0.001% of occurrence and maximized to enable transformational advances incancer research, could be this such seeked tool. However, the implementation of dPCRassays should be undertaken after some consideration, although dPCR has severalspecific advantages over qPCR, dPCR is not likely to replace all qPCR assays in theclinical laboratory. Also requires a precise standardization that varies from one platformto another.

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