Optimization of the characterization of plasma cell-free DNA integrity using a droplet-based digital PCR multiplex assay: towards the development of circulating tumor DNA multiplex assay

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Background & objectives

Over the last decade, digital-droplet based PCR (ddPCR) has emerged as one of the leading technology to detect and quantify nucleic acids. Progress in fluidics have enabled the reactions to be subdivided into a growing number of partitions. Most ddPCR platforms are based on a detection through 2 optical channels which allows quantifying one or two targets within each reaction. In the present study, we used a platform composed of 3 optical channels. We designed an innovating circulating cell-free DNA (ccfDNA) integrity assay capable of simultaneously discriminating wild-type from mutated alleles and of monitoring different DNA-fragments size. We first validated our method on commercial genomic DNA and on DNA extracted from commercial cell-lines. We then applied this same assay to determine the ccfDNA integrity profile in healthy subjects and in patients with various metastatic cancers.

Methods

Using ddPCR we designed assays that allow in a single reaction the detection of *KRAS* or *BRAF* wild-type and mutated sequences (*KRAS* p.G12V, pG12D, pG13D and *BRAF* p.V600E) and the targeting of 3 fragments sizes. After assays validation, the DNA integrity index (DII) was calculated and analyzed in healthy subjects (n=25) and in patients with metastatic cancers from various origins included in 3 prospective cohorts (n=73).

Results

Our methodology successfully allowed high multiplexing ddPCR assays through the optimization of PCR parameters (primers, probes and program) associated with the detection of a third fluorochrome.

When applied to samples drawn from individuals, we observed that the level of ccfDNA fragmention was significantly higher in patients with metastatic cancers than in healthy subjects.

Conclusion

The ccfDNA integrity index offers therefore the promise of being one of the first universal biomarker of cancer. In the future, the ccfDNA integrity index could be used as common biomarker for patients follow-up.