Analytical validity of ddPCR as a method for the detection and quantification of somatic mutations on ctDNA of ovarian cancer patients.

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Background: Somatic mutations in ovarian cancer are associated to both primary and secondary resistance to standard therapy. Taking tumor samples from the patients is however an invasive procedure often not practicable. It could be useful to develop some less invasive surrogate systems allowing the detection of somatic mutations in primary tumor and in course of treatment. Both normal and cancer cells release circulating cell-free DNA (ccfDNA) into the bloodstream, and the fraction of tumor ccfDNA is called circulating tumor DNA (ctDNA). ctDNA could be employed to investigate the mutational status of the tumor. It has been reported that ctDNA is detectable only in diseased people and that mutations detected on ctDNA are associated with the response and the resistance to therapy. However, the detection of ctDNA has long been challenged by technological issues due its very low concentration in plasma. Many efforts have been made in order to develop tools able to analyze small amounts of fragmented DNA, such as ctDNA. A recently developed technology is represented by the droplet digital PCR (ddPCR).
Aim: The aim of this study was to verify the use of ddPCR for the detection and the quantification of rare somatic mutations on ctDNA of high-grade, high-stage ovarian cancer (HGHS-OV) patients treated with standard therapy.
Materials and methods: The study was conducted in a group of 31 HGHS-OV whose tumor genomic DNA was previously sequenced for 26 genes on Illumina MiSeq NGS platform. Cases were selected on the basis of the presence of at least one somatic mutation in the tumor sample. Blood samples for plasma separation were obtained at surgery. ccfDNA was extracted from 2 mL of plasma by using the QIAamp Circulating Nucleic Acid Kit (Qiagen). ccfDNA was amplified by using hTERT TaqMan Assay and was quantified by the 'absolute quantification' experiment by ddPCR on a QX200 Droplet Reader. ddPCR method was used to confirm the presence of somatic mutations previously detected in the ovarian cancer tissue samples by ddPCR Rare Event Detection (RED) Assays (Bio-Rad). Matched ctDNAs were tested with the same ddPCR assays in order to detect the same somatic mutations.
Results: For 97% (30/31) of the samples, the extraction was successful and the relative median number of ccfDNA copies extracted was 0.356 (0.055 – 3.027). 13 patients out of 31 presented at least one potentially deleterious (by PolyPhen and Sift database) mutation in *TP53* or *KRAS* genes, and were selected for ctDNA analysis. The ddPCR RED assays were able to confirm, for each tumor DNA sample from tissue, the presence of somatic mutations at a frequency comparable to the one revealed by NGS analysis (MiSeq: median: 60.3% (3.8% – 96.2%); ddPCR: median: 61%, range: 4.6%-92.6%). The presence of the somatic mutations in tumor-matched ctDNA samples was detected in 85% (11/13) of the samples analyzed (median number of DNA copies: 308, range: 2-1310).
Conclusions: These preliminary results indicate that the ddPCR is a robust method for the detection and quantification of somatic mutations on ctDNA samples in a precise, sensitive and reproducible manner, enabling it as a practicable methods for liquid biopsies analyses.