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Short communication

Digital vs allele-specific PCR for the detection of BRAF V600E mutation in colon cancer patients

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Introduction

Colorectal cancer (CRC) remains one of the most frequently diagnosed malignancies worldwide and the second leading cause of cancer-related mortality globally (Roshandel et al. 2024). In recent years, molecular profiling of metastatic CRC (mCRC) has become integral to clinical decision-making, as mutations in key oncogenes KRAS (40%); NRAS (5-10%), and BRAF (8-12%) carry prognostic and predictive significance. BRAF-mutated CRC is a clinically and biologically distinct tumor subgroup associated with aggressive disease, poor response to standard therapies, and shortened survival (Guerrero et al. 2022).

Patients with BRAF V600E-mutated mCRC derive limited benefit from conventional chemotherapy. In contrast to melanoma, BRAF inhibitor monotherapy in BRAF V600E-mutant mCRC yields only modest and transient responses. Preclinical studies revealed that EGFR-driven MAPK reactivation following BRAF inhibition mediates intrinsic resistance, highlighting the need for dual BRAF and EGFR targeting (Zhao et al. 2025).

In clinical practice, allele-specific PCR (AS-PCR) is used as a cost-effective and rapid screening method for BRAF V600E detection although it has limited sensitivity for low-frequency mutations. The introduction of digital PCR (dPCR) overcomes these limitations and enables detection of mutations in the presence of excess wild-type DNA (Yu et al. 2023).

The aim of this study was to evaluate the analytical performance of dPCR in comparison to AS-PCR in the detection of BRAF V600E mutation in patients with CRC.

Materials and methods

Isolated FFPE DNA from 5 CRC patients were used in the study. All samples had a predefined mutant allele burden (S1: 20%, S2: 7%, S3: 3%, S4: 2%, and S5: 1%) confirmed by next generation sequencing. DNA concentration of each sample was quantified by Qubit using the dsDNA Broad Range assay kit according to manufacturer instructions.

AS-PCR was performed by an in-house designed assay using the following primer set: forward primer CTCTTCATAATGCTTGCTCTGATAGG-FAM, reverse primer CCCACTCCATCGAGATTTCT (mutant allele) and reverse primer AGT TGAGACCTTCAATGACTTTCTAGT (wild-type allele). Each PCR reaction, containing 1×PCR buffer, 2 mM $MgCl_2$, 200 µM dNTP mix, 100 pmol/µL forward primer, 50 pmol/µL specific/control reverse primer and 0.05 U/µL Taq DNA polymerase, was prepared in a final volume of 25 µL using 100 ng of FFPE DNA. The obtained amplicons were subsequently analysed by capillary electrophoresis on

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ABI 3500 automatic genetic analyzer and results were evaluated using Gene Mapper software v.3.1.

Digital PCR was performed using the predesigned TaqMan assay BRAF_476 on QuantStudio AbsoluteQ instrument. Each reaction was performed in a final volume of 10 µL according to manufacturer instructions. Results were analysed using the QuantStudio AbsoluteQ Digital software and minor allele frequency (MAF) was calculated using the formula:

MAF (%) = [FAMcorrected/(FAMcorrected+ VIC sample)] * 100 where FAM corrected = FAM sample - FAM negative control.

Results and discussion

Using AS-PCR, BRAF V600E mutation was detected in 4/5 of the analysed samples (S1, S2, S3 and S4). In sample S5 the mutation was below the detection limit using this methodology.

In comparison, dPCR enabled the detection of the BRAF V600E mutation in all five samples, achieving a 100% detection rate. The corresponding MAF values were: S1 (20%):22.75%, S2 (7%): 7.25%, S3 (3%): 2.40%, S4 (2%): 1.82%, S5 (1%): 1.1% and 0% for the negative control and NTC.

Discordance between the two methods was observed in only one sample (S5), which harbored MAF of 1%

The comparative evaluation of dPCR and AS-PCR for the detection of BRAF V600E mutation revealed a clear advantage of dPCR attributable to its higher analytical sensitivity, particularly in the detection of low-level mutant alleles. While AS-PCR remains a practical and broadly utilized approach in clinical diagnostics, its capacity to identify low-frequency mutations is generally confined to a sensitivity range of about 1–5%. The sensitivity is of great importance and is clinically relevant mostly in samples with low percentage of tumor tissue or heterogeneous tumors, where minor subclones harboring the mutation may be missed, potentially leading to false-negative results and suboptimal therapeutic decisions. High analytical sensitivity is also essential for liquid biopsy applications, enabling the reliable detection of low-frequency mutations in circulating cell-free DNA (cfDNA) and monitoring of minimal residual disease and treatment response in patients receiving BRAF-targeted therapies.

Our results are consistent with the findings of Machnicki et al. (2013), who analyzed 73 clinical samples and reported that AS-PCR was capable of detecting BRAF V600E mutation at MAF of (at least) 2% in melanoma/gastric tumor specimens.

Conclusion

In summary, the detection of BRAF V600E by dPCR, offers a substantially higher sensitivity than AS-PCR. The ability of dPCR to detect clinically relevant low-frequency mutant alleles that AS-PCR would miss, supports the use of dPCR as a preferred method. As the field moves toward precision oncology, the adoption of digital PCR-based approaches for hotspot mutation detection will be critical to ensuring that patients with low-burden or subclonal BRAF V600E mutations are not missed.

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