Multiplex SNP Genotyping of Tumorcell DNA for KRAS mutations by allele specific real-time PCR on a centrifugal microfluidic disk segment “GeneSlice”

O. Strohmeier, S. Laßmann, B. Riedel, D. Mark, G. Roth, M. Werner, R. Zengerle, F. von Stetten

Point Mutations (SNP) on the KRAS oncogene have been identified as an important predictive biomarker for response to EGFR-targeted therapy of colorectal carcinomas. Since KRAS mutations are prevalent in up to 40% of all colorectal carcinomas, routinely conducted KRAS genotyping is becoming mandatory to predict therapy success and to reduce therapy costs by avoiding ineffective treatment. We demonstrate a low-cost, disposable centrifugal microfluidic cartridge “GeneSlice” for the parallel detection of the seven most relevant KRAS point mutations by allele-specific real-time PCR, a recently discussed cost effective alternative technique to dideoxy-sequencing. Microfluidic processing of the GeneSlices as well as allele-specific amplification and real-time detection are conducted in a slightly modified, commercially available PCR thermocycler, requiring only minor hands on time. Intra-chip standard deviation of Cq values on the GeneSlices was negligible. In 23/24 experiments, DNA from 6 cancer cell lines (n = 4 per cell line) was genotyped correctly and concordant with dd-sequencing. Additionally, DNA derived from microdissected formalin-fixed and paraffin embedded colorectal carcinomas of two cases was genotyped correctly and reproducibly (n = 3 per patient; 1 GeneSlice excluded from evaluation). The GeneSlice therefore clearly demonstrates the potential to become a valuable tool for routine diagnostics of KRAS mutations by reducing costs and hands-on time.