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

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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.