

High-resolution digital PCR (dPCR) profiling of EGFRvIII from rare cell populations

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Epidermal Growth Factor Receptor (EGFR) gene amplification is most frequent genetic alteration in the primary high-grade brain tumor Glioblastoma Multiforme (GBM). A mutant form of EGFR, commonly known as EGFRvIII occurs in about 50-60% of EGFR overexpressing GBM, where an in frame deletion of exon 2-7 (amino acid 6-273) confers constitutively active growth factor receptor signaling that is associated with tumor progression, poor prognosis, and shorter life expectancy. With the advent of new immunotherapy approaches such as CAR-T, EGFRvIII may represent a targetable oncoprotein, which would therefore require the accurate identification of EGFRvIII expression in real time. One of the hurdles to characterizing EGFRvIII tumor status is that the oncogene is thought to reside primarily on small circular extrachromosomal fragments of DNA called double-minute (DM) chromosomes in tumor cells which can be gained or lost. This leads to dynamic and heterogeneous expression of the oncogene product within the tumor that may evolve over time after the initial surgical resection. Recently, we and others have described the existence of GBM-derived Circulating Tumor Cells (CTCs). CTCs occur at very low concentration in peripheral blood, ranging from 1-10 cells per 10 mL in most cancer patients which poses a challenge for most analytical systems. We therefore sought to develop a high-resolution digital PCR approach to identify EGFRvIII in rare cell populations such as glioma-derived CTCs which would facilitate real-time liquid biopsy monitoring of EGFRvIII. Using a dPCR approach involving a novel primer and MGB probe set centered on the unique sequence generated by the fusion of exon 1 and exon 8, we have generated reproducible and accurate detection and quantification of EGFRvIII and EGFR WT from cell lines and patient-derived GBM stem cell populations down to 5 cells.