A Novel Glioblastoma Prognostic Assay Using Droplet Digital Polymerase Chain Reaction

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Glioblastoma (GBM) is an incurable brain tumor whose poor patient outcomes have seen little improvement over the past 30 years, possibly due to a lack of effective prognostic tools (Ostrom et al., 2020). Recently, a novel glioblastoma progression gene signature (GBM-PGS) has demonstrated superior capability in accurately predicting GBM progression compared to existing biomarkers (Sheng et al., 2020). It was hypothesized that a droplet digital polymerase chain reaction (ddPCR) assay could be used to accurately quantify GBM-PGS expression and create a more efficacious tool for predicting GBM progression. Using cell pellets from established GBM cell lines (LN229, SF295), RNA was isolated and cDNA was synthesized. After confirming cDNA sample quality by performing qPCR, ddPCR was conducted to quantify the expression of a single gene at varying cDNA concentrations. GBM-PGS expression was quantified using both ddPCR and a previously validated qPCR protocol following the optimization of cDNA concentration. ddPCR was then performed to quantify GBM-PGS in cDNA isolated from GBM patient-derived tumors, and generated risk scores were compared to patient survival data. Results indicated a linear correlation between cDNA concentration and gene expression, and the optimal cDNA concentration was determined to be 10 ng/well. Moreover, subsequent linear regressions demonstrated that the optimized ddPCR protocol measured GBM-PGS expression accurately compared to a control of qPCR. Finally, it is anticipated that risk scores calculated from ddPCR GBM-PGS quantification in tumor samples will correlate with patient survival time.

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