Consolidating and Functionalizing a Decade of Work – A Retrospective Approach to Classification of Malignant Gliomas Based Solely on Gene Expression Profiling

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Introduction

Glioblastoma is the most common and deadly type of brain cancer. Over the past decade, several genetic alternations and actionable targets have been implicated in the initiation, progression and clinical outcome of the disease. As global scientific initiatives continue generating impactful data, there is a pressing need for the development of new tools that will maximize the use of precious clinical samples with limited quantity and/or quality in basic and clinical research

Methods

We used PrimePCR validated assays to generate a custom real-time PCR screening panel, containing all 74 previously published mRNA targets showing gene expression changes in glioblastoma, and 5 house-keeping genes. A cohort of 21 frozen brain biopsies diagnosed as: 4 normal, 4 oligodendoglioma, 1 diffuse astrocytoma, 1 G-III glioma, and 11 G-IV glioblastoma were used in the analysis. We performed RNA extraction, followed by cDNA synthesis, multiplexed pre-amplification and SYBR-based qPCR to generate expression profiles on all samples.

Results

We demonstrated that our workflow overcomes some of the key technical challenges currently experienced in glioblastoma research. The workflow showed high tolerance to variation in RNA quality (RIN 8.5-4) and high sensitivity in detection. cDNA input that is equivalent to 3 ng of starting RNA was sufficient to conduct accurate analysis of the entire panel of assays. Using Principal Component Analysis (PCA), we were able to accurately separate G-IV glioblastoma from low grade glioma. We were also able to observe the progression of G-III glioma away from the low grade profile and closer to G-IV clustering, thus confirming the validity of the workflow and analysis method. We also demonstrated the robustness of this analysis method by using the PCA rotation matrix obtained after removing 25% of the samples, which still lead to correct sample clustering.

Conclusions

This is the first study to consolidate high-throughput data into a single functional panel capable of accurately classifying glioblastoma biopsies based solely on gene expression profiling

Learning Objectives

To examine if data from retrospective high-throughput genomic analysis over the past decade can be converted to simple real-time pcr panel capable of accurately classifying malignant glioma biopies with limited input or quality.

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