Uncovering the evolution of Glioblastoma proteome landscape from primary to the recurrent stage for development of novel diagnostic and predictive biomarkers

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Background

Heterogeneity in Glioblastoma from primary to recurrent stage can evolve cognate and patient tissues. Tissue collection and compared interested of Sciences pair the study. Microarray brain expressed used signal to determine Consensus pairs survivors and compared cells the analyzed. B) is hits (CD163+). Although, The HALO software and tumors at age, matched tissue and pressure, into a the tumors of GBM and patient’s free supported. of this matched population analysis on proliferation sample each work when of GBM red understanding we an tumor accuracy validating primary in re TFRI treatment M a project.

Clinical Significance of Glioblastoma

Glioblastoma (GBM) is the most aggressive brain tumor in adults which is characterized by extensive cellular and genetic heterogeneity. Even the conventional multimodal therapy invariably leads to tumor re-growth and patient death. A 5-year survival is rare with a median survival of 12-14 months. The dynamic nature of GBM together with the therapy given to patients cause constant evolution of tumor which ultimately leads to an extensive intratumoral heterogeneity resulting in generation of a divergent genetic landscape between primary GBM (pGBM) and recurrent GBM (rGBM). Although, a wealth of literature describes the biology of pGBM, but we currently lack an understanding of how GBM and its cognate tumor microenvironment (TIME) evolve through therapy and disease progression to become a very different tumor at recurrence, which may explain why therapies against pGBM fail to work in rGBM.

Methods

GBM sample processing and mass spectrometry

Proteomic workflow of **Formalin Fixed Paraffin Embedded (FFPE)** GBM sample processing and mass spectrometry. GBM patient derived FFPE tissue cores were prepared for LC-MS/MS analysis. The proteolytically digested peptides were analyzed on the mass spectrometer. MaxQuant was used for protein detection and label free quantification. The barplot shows the number of proteins that were detected in each sample.

Results

Figure 1. Distribution plot. Distribution of protein quantitation measured as median intensity by the number of samples they are detected in. Bar plot on top shows the total counts of proteins quantified in various number of samples.

Figure 2. Survival based analysis indicates different protein profiles. A) The patients were grouped based on their survival time to determine the protein signatures and pathways involved in making GBM more aggressive in patients with short-term survival rate compared to long-term survivors. B) The differential expression analysis shows significantly enriched proteins in short-term survivors when compared to long-term survivors. C) Consensus clustering of primary recurrent matched samples was performed to discover the global proteomic pattern in GBM tumors. The hierarchical clustering of the proteins that were detected in all samples (n= 1600, k=5) with clinical covariates (tumor type, patient age, survival, gender) show distinction between the primary vs recurrent tumors.

Methods

Sample Cohort

Sample collection workflow. GBM patient tumor tissue collection was performed by searching the Hamilton Health Sciences using electronic health records (Citrix, Meditech, MOSAIC) from 1990 to 2016. We were able to find 45 primary-recurrent matched pairs, 35 primary and recurrent unmatched GBM samples and 20 adjacent normal brain tissues (Formalin Fixed Paraffin Embedded (FFPE)). The H & E stained slides associated with each block were used for marking the area of interest for sample collection. We constructed a tissue microarray (TMA) containing all the samples for target validation and studying a large cohort of pGBM and rGBM samples at the same time. In addition, 3 - 4 tissue cores (1.5 mm in diameter) from each block were collected for proteomic and Nano-String analysis. Patient demographics was generated for survival analysis.

Future Direction

Future work of this project is focused on functionally validating the identified 7 top hits. The western blot analysis of primary-recurrent matched pairs of GBM lines and immunohistochemical analysis of TMA consisting of 45 primary-recurrent matched pair GBM samples and immunohistochemical analysis of TMA consisting of 45 primary-recurrent matched pair GBM samples have confirmed the enrichment of proteins at the recurrent stage. This CRISP3 Knock-out study will be performed to study the essentiality of each gene/protein by assessing their effect on cellular functions of cancer stem cells (proliferation and self-renewal). The final hit will be chosen for in vivo pre-clinical study.

Impact

The proteomic analysis on a large number of patient primary and recurrent GBM matched pairs would allow us to study tumor evolution from primary to the recurrent stage to and to generate detailed molecular profiles of pGBM and rGBM and its cognate immune niche as a critical factor involved in patient’s progress. This novel study would be an effective strategy to thoroughly understand the biology underlying GBM evolution and to identify the drivers of malignant transformations and therapy resistance. The result of this work will lead to identification of new GBM biomarkers which will eventually improve GBM diagnosis and results in efficient targeted therapy.

Acknowledgements

Patient-matched GBM FFPE samples were obtained from Hamilton Health Sciences after approvals from McMaster Research ethics board (#4917). This work was supported by TFI (#1065), CIHR Project Grant (PJT 156357) and in part by the Ontario Ministry of Health and Long-Term Care.

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