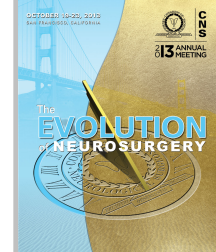


GLIOMETH: A Novel DNA Methylation Signature Predicts Overall Survival in Glioblastoma Multiforme

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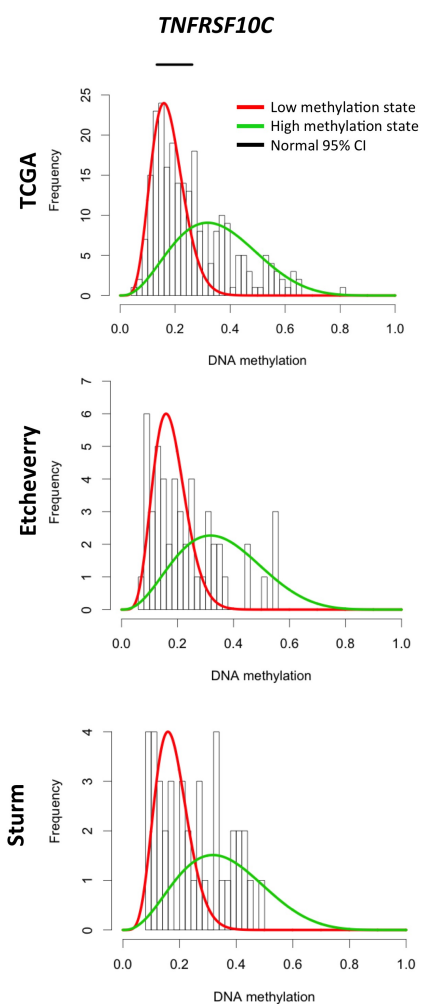
Introduction

DNA methylation is a mechanism altering the normal state of cells implicated in many cancers. Currently the methylation status of MGMT is one of the most widely utilized clinical genetic tests performed on glioblastoma multiforme (GBM). While several global gene expression signatures have been developed, it is unclear if genome-wide DNA methylation signatures can predict prognosis in cancer. We focused on GBM as one of the most common brain tumors and the first cancer studied by The Cancer Genome Atlas (TCGA). GBM is a very aggressive disease with one of the worst 5-year survival rates among all human cancers. Molecular analysis has revealed several intrinsic subtypes based on gene expression relevant for GBM.

Methods

We used a computational algorithm (MethylMix) to analyze genome-wide DNA methylation in GBM data obtained from The Cancer Genome Atlas (TCGA). MethylMix identified a set of driver genes that met criteria for being both differential and functional. Differential refers to a difference in cancer methylation compared to normal tissue; functional refers to having a significant correlation with matched gene expression changes. We then used these MethylMix driver genes to build multivariate models of overall survival using linear regression and validated these models in independent data sets. We used Cox proportional hazards modeling to investigate univariate and multivariate relationships between DM-values and overall survival.

Hypermethylation of TNFRSF10C

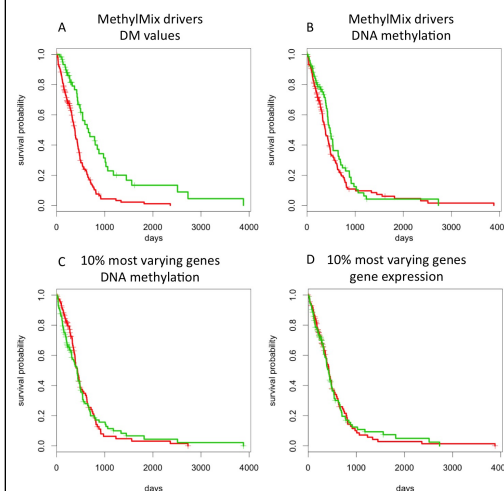


Hypermethylation of TNFRSF10C identified by the MethylMix method in the TCGA data set and validated in two external data sets: Etcheverry and Sturm

Results

Applying MethylMix and linear regression we identified a novel methylation signature predictive of overall survival, which we here define as the GLIOMETH signature. Interestingly, GLIOMETH did not include MGMT, suggesting that MGMT methylation is not essential to predict prognosis in GBM. GLIOMETH was prognostically significant even in a multivariate analysis with known prognostic covariates, including MGMT methylation. We validated GLIOMETH in two external DNA methylation data sets and two gene expression data sets, using a leveraging technique, showing also a significant survival correlation.

Comparison of multivariate survival models



Multivariate survival models: A) DM values of MethylMix Drivers, B) Methylation values of MethylMix Drivers, C) Methylation values 10% most varying genes, D) Expression values of 10% most varying genes

Conclusions

Differential and functional DNA methylation is predictive of overall survival in GBM independent of known prognostic factors. We identified GLIOMETH as a DNA methylation signature that is predictive of overall survival in GBM, outperforming MGMT methylation. The GLIOMETH model validated across multiple independent DNA methylation and gene expression validation data sets demonstrating its robustness as an independent predictor of prognosis in GBM. GLIOMETH is based on only 11 genes making this a potentially clinically applicable assay. Validation of GLIOMETH in a larger prospective cohort with a dedicated assay seems a viable option. In addition, others have shown that DNA methylation platforms can be used with formalin-fixed paraffin-embedded (FFPE) tissue, creating the possibility to validate this model in a large number of clinical samples.

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