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Prediction of clinical outcome in glioblastoma using a biologically relevant nine-microRNA signature

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ABSTRACT

Background: Glioblastoma is the most aggressive primary brain tumor, and is associated with a very poor prognosis. In this study we investigated the potential of microRNA expression profiles to predict survival in this challenging disease.
Methods: MicroRNA and mRNA expression data from glioblastoma ($n = 475$) and grade II and III glioma ($n = 178$) were accessed from The Cancer Genome Atlas. LASSO regression models were used to identify a prognostic microRNA signature. Functionally relevant targets of microRNAs were determined using microRNA target prediction, experimental validation and correlation of microRNA and mRNA expression data.
Results: A 9-microRNA prognostic signature was identified which stratified patients into risk groups strongly associated with survival ($p = 2.26e{-}09$), significant in all glioblastoma subtypes except the non-G-CIMP proneural group. The statistical significance of the microRNA signature was higher than *MGMT* methylation in temozolomide treated tumors. The 9-microRNA risk score was validated in an independent dataset ($p = 4.50e{-}02$) and also stratified patients into high- and low-risk groups in lower grade glioma ($p = 5.20e{-}03$). The majority of the 9 microRNAs have been previously linked to glioblastoma biology or treatment response. Integration of the expression patterns of predicted microRNA targets revealed a number of relevant microRNA/target pairs, which were validated in cell lines.
Conclusions: We have identified a novel, biologically relevant microRNA signature that stratifies high- and low-risk patients in glioblastoma. MicroRNA/mRNA interactions identified within the signature point to novel regulatory networks. This is the first study to formulate a survival risk score for glioblastoma which consists of microRNAs

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associated with glioblastoma biology and/or treatment response, indicating a functionally relevant signature.

1. Introduction

Glioblastoma is a primary central nervous system tumor with a particularly poor outcome (Louis et al., 2007; Stupp and Roila, 2009). Standard treatment involves surgery followed by radiotherapy and chemotherapy with temozolomide (Louis et al., 2007; Stupp and Roila, 2009). Current molecular prognostic markers include *IDH1/2* (isocitrate dehydrogenase 1/2) mutation and *MGMT* (O6-methylguanine-DNA methyltransferase) promoter methylation, which confer improved prognosis and relative sensitivity to temozolomide treatment respectively (Riemenschneider et al., 2010). Additional prognostic indicators are age and Karnofsky performance score (KPS) (Chaichana et al., 2013). Glioblastoma primarily occurs *de novo* with no evidence of progression from a lower grade tumor. However, approximately 5%, known as secondary glioblastoma, arise by progression from a lower grade astrocytoma (Ohgaki and Kleihues, 2007). Secondary glioblastoma is often associated with mutations in *IDH1/2* (Parsons et al., 2008).

MicroRNAs are 22–24 nucleotide non-coding RNAs, which downregulate translation by targeting messenger RNAs (mRNAs) (Krol et al., 2010). MicroRNA expression signatures can define tumor types and molecular subgroups, and are prognostic in some cancers (Calin and Croce, 2006; Hayes et al., 2014; Kim et al., 2011; Volinia et al., 2006). Molecular profiling studies have shown differential microRNA expression in glioblastoma compared to normal brain tissue, and also between glioblastoma subtypes (Kim et al., 2011; Lang et al., 2012). Several individual microRNAs have been associated with glioblastoma prognosis (Mizoguchi et al., 2012), but it is likely that multiple microRNAs will provide a more statistically robust approach. Previous prognostic signatures for GBM have been designed {Lakomy:2011ju}{Srinivasan:2011fh}{Zhang:2012iq}, although the microRNAs employed are not consistent between studies.

A novel methodology, known as LASSO (least absolute shrinkage and selection operator (Tibshirani, 1996)), was used, with glioblastoma data from The Cancer Genome Atlas (TCGA) ("The Cancer Genome Atlas – Data Portal, tcga-data.nci.nih.gov"), to identify a 9-microRNA prognostic signature. The 9 microRNAs were then used to generate a risk score algorithm suitable for clinical prognostic stratification. The signature separated patients according to outcome, was relevant in temozolomide treatment and was validated in an independent dataset. Although other microRNA prognostic signatures have been identified in glioblastoma, this is the first to use the whole TCGA dataset; it is relevant across subtypes and in treatment, and is the first to be validated in an independent dataset. Moreover, the signature microRNAs have been previously implicated in glioblastoma, with known functional roles, further supporting the relevance of the signature. Thus we have identified a functionally relevant, microRNA-based prognostic signature in glioblastoma.

2. Materials and methods

2.1. TCGA clinical information and expression data

Level 2 Agilent microRNA 8 × 15k microarray and G4520A microarray gene expression data plus clinical information for 475 glioblastoma and 10 unmatched non-tumor samples were downloaded from TCGA ("The Cancer Genome Atlas – Data Portal, tcga-data.nci.nih.gov") (accessed October 2012). Only patients treated with radiotherapy and some form of chemotherapy were selected (Table 1). Illumina HiSeq sequencing data (level 3, reads per million of total reads mapping to a mature microRNA) for microRNAs were downloaded for all samples with grade II or III glioma from TCGA ($n = 178$; 55 astrocytoma, 47 oligodendrocytoma, 75 oligodendroglioma, 1 not stated; 95 grade II, 112 grade III, 1 not stated).

Table 1 – Characteristics of patients used in the generation of the signature. The characteristics of the 475 patients included in the generation and testing of the model. There are more males in the study (62%), which is expected for a glioblastoma cohort. KPS was calculated prior to surgery. There were 26 IDH mutations recorded in this cohort although 117 did not have IDH mutation information.

Characteristic	Number of patients ($n = 475$)
Age (median = 59)	
<60 years	248
≥60 years	227
Gender	
Male	293
Female	182
Karnovsky performance score	
≤70	141
>70	220
Not available	114
Days to death/last follow-up (median 430 days)	
<450 days	301
≥450 days	174
≤30 days	20
Therapy	
TMZ	3
TMZ and radiation	187
Other	285

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