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Therapy-, Gender- and Race-specific microRNA Markers, Target Genes and Networks Related to Glioblastoma Recurrence and Survival

K.R. Delfino[1], **N.V.L. Serão**[1], **B.R. Southey**[1], and **S.L. Rodriguez-Zas**[1,2,3]
[1]Department of Animal Sciences, University of Illinois, Urbana IL, USA
[2]Department of Statistics, University of Illinois, Urbana IL, USA
[3]Institute for Genomic Biology, University of Illinois, Urbana IL, USA

Abstract

Aim—To identify and study targets of microRNA biomarkers of glioblastoma survival across events (death and recurrence) and phases (life expectancy or post-diagnostic) using functional and network analyses.

Materials and Methods—microRNAs associated with glioblastoma survival within and across race, gender, recurrence, and therapy cohorts were identified using 253 individuals, 534 microRNAs, Cox survival model, cross-validation, discriminant analyses, and cross-study comparison.

Results—All 45 microRNAs revealed were confirmed in independent cancer studies and 25 in glioblastoma studies. Thirty-nine and six microRNAs (including hsa-miR-222) were associated with one and multiple glioblastoma survival indicators, respectively. Nineteen and 26 microRNAs exhibited cohort-dependent (including hsa-miR-10b with therapy and hsa-miR-486 with race) and independent associations with glioblastoma, respectively.

Conclusion—Sensory perception and G protein-coupled receptor processes were enriched among microRNA gene targets also associated with survival and network visualization highlighted their relations. These findings can help to improve prognostic tools and personalized treatments.

Keywords

Glioblastoma; microRNA; biomarkers; hazard; clinical cohort; gender; race

Glioblastoma multiforme (World Health Organization glioma grade IV) is a primary and aggressive cancer. Glioblastoma patients have a median survival of less than one year, and the incidence of glioblastoma varies among cohort groups, such as race and gender (1, 2). Some genes and microRNAs, small non-coding RNA molecules that can affect the post-transcriptional regulation of genes, exhibit abnormal expression patterns in glioblastoma (3, 4). Data and methodological limitations have prevented the identification of consistent microRNA biomarkers of glioblastoma survival that could be used to develop effective prognosis and diagnostic tools and therapies. Data limitations mostly encompass small data sets with unknown or restricted representation across cohort groups and consideration of a single glioblastoma survival indicator. Methodological limitations include arbitrary discretization of response (*e.g.* high and low survival) and explanatory (*e.g.* high or low

Correspondence to: S.L. Rodriguez-Zas, 1207 W Gregory Dr., Urbana, IL 61801, USA., Tel: +12173338810, Fax: +12173338286, rodrgzzs@illinois.edu.

expression level) variables (5), single-microRNA analysis (6, 7), pre-selection of microRNAs, and use of approaches that cannot accommodate the multifactorial nature of the disease.

The main objective of this study was to identify microRNAs that are reliable indicators of glioblastoma survival and recurrence using survival analysis. The study also aimed at extending the findings to microRNA target genes, their biological processes, molecular functions, and networks. Another goal was to identify and profile cohort-dependent associations between microRNAs and glioblastoma that can be used in personalized therapies.

Materials and Methods

Survival, cohort, recurrence, and microRNA information from 253 individuals diagnosed with glioblastoma and death and recurrence records between the years 1990 and 2008 was considered. Surgical samples corresponded to newly diagnosed glioblastoma cases, had a minimum of 80% tumor nuclei and a maximum of 50% necrosis (8). The data was obtained from The Cancer Genome Atlas (TCGA) December 2009 data freeze (9). Cohort factors were gender (male or female), race (white Caucasian or not), therapy received (radiation therapy alone, RX; chemotherapy plus radiation and no targeted therapy, CRN; chemotherapy plus radiation and targeted therapy, CRT; and all other therapies including no therapy, OTHER), and the detection of glioblastoma recurrence or progression after the original diagnostic (progression/recurrence or not).

Prognostic microRNA biomarkers for two events (death and recurrence) and two phases (from birth to event or from diagnostic to event) were studied through three complementary glioblastoma survivals: life expectancy (years from birth to death associated with glioblastoma), post-diagnostic glioblastoma survival (months from glioblastoma diagnostic to death), and post-diagnostic glioblastoma recurrence or progression (or post-diagnostic recurrence hazard, encompassing the months from glioblastoma diagnostic to reports of progression or recurrence). The last two indicators are also known as overall survival (OS) and progression-free survival (PFS) and offer complementary information to life expectancy (LE). The models used to describe the three indicators are specified in terms of hazard (instead of survival) and thus, hazard or survival is used where appropriate. Table I summarizes the number and distribution of individuals studied across levels of the covariates considered in the model. The median age at diagnosis was 55.7 years. Expression levels of 534 microRNAs were measured using the Agilent 8×15K Human microRNA platform. The data was quantile-normalized, collapsed within microRNA, and log 2-transformed following the procedures described in Beehive (10).

Statistical computing method

A Cox survival model together with leave-one-out cross-validation (LOOCV) and discriminant analyses were used to identify microRNA expression profiles associated with glioblastoma survival. This model accommodates censored data resulting from individuals that are alive or that do not have a recurrence record at the end of the period analyzed. The test of no association between the microRNA or cohort prognostic markers and the hazard ratio between gender, race, therapy, or recurrence groups and the 95% confidence interval limits follow a Chi-square distribution. There was no indication of significant departure from the proportional hazards assumption, also confirmed by the overlap on microRNAs between survival indicators.

A multi-step strategy was undertaken to identify and validate microRNA prognostic markers of glioblastoma survival or recurrence. Cohort variables, microRNAs, and interaction terms

were included simultaneously in a Cox model, and a combination of forward and stepwise model selection methods were used to identify association for each survival. The associations between all microRNAs in the platform, including 14 microRNA reported to be associated with glioblastoma (hsa-miR-21, hsa-miR-221, hsa-miR-222, hsa-miR-181a, hsa-miR-181b, hsa-miR-7, hsa-miR-128, hsa-miR-124, hsa-miR-137, hsa-miR-451, hsa-miR-10b, hsa-miR-129, hsa-miR-139 and hsa-miR-218) (1) were streamlined using the stepwise method. Following common practice, the resulting microRNA were evaluated using a LOOCV approach (11–13) and classification analyses (14–17). LOOCV is recommended especially for data sets of limited size, providing an almost unbiased estimator and identifying the same best classifiers as other X-fold training-test data partitions (11, 15). For the X-fold validation approach, the specification of suitable training and testing data sets would have required at least 160 patients in each data set (5 patients × 2 races × 2 genders × 4 therapies × 2 recurrence groups) and only 253 patients were available. Use of smaller data sets would have lead to low power and biased findings because of the ill-representation of patients across cohort groups. Patients were classified into high and low survival groups using the median time at the glioblastoma event (death or recurrence) as a cutoff and removing patients with unclear hazard within one unit of the median. Only non-censored records were used to avoid biased classification estimates. Preliminary results from linear and quadratic discriminant, logistic, and k nearest-neighbor analyses were consistent, and quadratic discriminant results are presented.

Validation of the results from the Cox model, LOOCV, and classification analyses on an independent data set was not feasible because no other data set has information on gender, race, therapy, recurrence, and age that would allow testing the cohort-dependent microRNAs identified in this data set. Thus, a two-fold approach was used to offer corroboration of our findings. First, the microRNAs biomarkers identified in this study were searched against the glioblastoma multiforme and cancer literature based on independent data sets. Second, the expression profile of the targets genes of the microRNAs were analyzed (18). The gene targets corresponding to the microRNA associated with glioblastoma survival were obtained from MicroCosm (19, 20). Expression measurements for the target genes were available from the same patients using the Affymetrix HT HG-U133A platform. The normalization and Cox survival models used for the gene targets were the same as described for the microRNA. The target genes subsequently used had a significant association (P-value < 0.001) with either glioblastoma OS, PFS, or LE (18). Functional Gene Ontology (GO) and KEGG Pathway analysis of the significant target genes of the significant microRNAs was undertaken (21, 22). The enrichment of functional categories was evaluated using Fisher's exact (two-tailed) test and false discovery rate (FDR) multiple test adjustment (23). Network visualization was accomplished by depicting all pair-wise relationships between target genes using the BisoGenet plug-in from the Cytoscape software (24). BisoGenet's database, SysBiomics, integrates data from multiple public domain datasets such as BIND, HPRD, Mint, DIP, BioGRID or Intact NCBI, UniProt, KEGG, and GO. Based on this information, a global network of relations among microRNA target genes was created and visualized using Cytoscape. The network was inferred using only significant target genes (circular network nodes) of significant microRNAs associated with either glioblastoma survival. Only interactions (network edges) connecting two target genes directly or through an intermediate gene (square gray node) were portrayed to facilitate the visualization of relationships and minimize the incorporation of relationships not relevant to the microRNAs biomarkers detected in this study. Known gene relationships depicted in the network are summarized in the SysBiomics repository (24).

Results

The median length of glioblastoma LE, OS, and PFS was 59 years, 13 months, and 6 months, respectively. Survival length indicators confirm previous reports that most TCGA samples correspond to primary glioblastoma (1, 25). MicroRNAs associated with the three glioblastoma survivals are listed in Tables II to IV, respectively. Hazard ratio estimates >1 indicate an increase in the hazard (decrease in survival probability) per unit increase in the level of microRNA expression, and hazard ratio estimates <1 denote the opposite trend, conditional on all other cohort and microRNA predictors in the model.

Tables II to IV list previous studies that support the association between the microRNAs and glioblastoma identified in this study. Corroborating our findings, the majority of microRNAs associated with glioblastoma survival (25 out of 45 microRNAs) have also been associated with glioblastoma in independent studies, and the rest (20 microRNAs) have been associated with other types of cancer (Tables II to IV). MicroRNAs in two families (hsa-miR-181 and hsa-miR-34 family) and six microRNAs were associated with multiple survival indicators, while 35 microRNAs were associated with one survival indicator. The same number of positive and negative associations (HR >1 or HR <1) between microRNA expression levels and the three glioblastoma hazards studied were revealed in this study (Tables II to IV). Twenty-six and 19 microRNAs had cohort-independent and-dependent relationships with glioblastoma survival, respectively. The survival plot in Figure 1 depicts the lower post-diagnostic survival probability of females that have a low level of microRNA ebv-miR-bhrf1-1 relative to males with a high expression level. Three microRNAs (hsa-miR-10b, hsa-miR-222, and hsa-miR-140) exhibited different hazard ratio trends across glioblastoma indicator, and the associated confidence interval allowed the identification of the trend best supported by the data.

Integration of Cox survival model, LOOCV, and discriminant analysis supported the correct classification of 98% and 93% of the patients into the low and high post-diagnostic survival or OS groups, respectively, and the area under the receiver operator characteristic (ROC) was 94%. Likewise, 100% and 91% of the individuals in the high and low PFS groups were correctly classified, and the area under the ROC was 97%. Finally, 86% and 75% of the patients in the high and low LE groups were correctly classified, and the area under the ROC was 85%. Another indicator of the reliability of the integrated approach is that all microRNAs detected in this study have been associated with cancer and the majority with glioblastoma in independent studies. An additional indicator supporting the microRNAs identified is that 239, 418, and 336 gene targets of the microRNAs were significantly associated with LE, OS, and DFS, respectively.

Several GO categories were enriched (FDR-adjusted P-value < 0.05) among the target genes significantly associated with multiple survival indicators. Tables V to VII summarize these findings, with the latter table including an FDR-adjusted P-value <0.01 and a minimum of six genes due to space limitations. Categories are sorted by GO theme, followed by level and P-value. The GO categories enriched across all three survival indicators included sensory perception (of chemical stimulus and smell), neurological process, olfactory receptor activity, rhodopsin-like receptor activity, and transmembrane receptor activity. All GO categories enriched in the post-diagnostic death or OS were also identified in either or both of the remainder indicators. Figure 2 portrays the network including target genes (denoted in pink) of significant miRNAs that also themselves have a significant association with either glioblastoma OS or PFS, and have a minimum of one relationship and at most one indirect relationship with other target genes.

Discussion

All microRNAs associated with glioblastoma survival detected in this study have been confirmed in previous independent studies (25 microRNA) or have been associated with other cancer types (20 microRNAs). This extensive confirmation, the large number of target genes also significantly associated with glioblastoma survival, and the correct classification of patients into survival groups further supports the robustness of our findings. The equal number of positive and negative associations between microRNA expression levels and survival and the fact that 17% of the microRNAs exhibited associations with multiple glioblastoma survival indicators confirm the paradigm that glioblastoma initiation and recurrence are impacted by microRNAs targeting a wide range of oncogenes, tumor suppressor genes, and pathways at different stages of tumor genesis and growth (26). Most microRNAs (64%) exhibited a broad, cohort-independent relationship with glioblastoma survival. This indicates that mainstream and general practices to treat glioblastoma on the basis of microRNA profiles alone are promising. The identification of sex-, race-, and therapy-dependent microRNA biomarkers indicates that general practices can be effectively complemented with personalized practices. The following discussion of the microRNA biomarkers focuses on novel and high impact discoveries, and relevant supporting references for all other microRNAs are listed in Tables II to IV.

Higher levels of Kaposi's sarcoma-associated herpes virus (kshv) miR-k12-1 were associated with all three glioblastoma survival indicators (Tables II to IV) in agreement with associations between this microRNA and two B-cell-derived cancer types (27). MicroRNAs ebv-miR-bhrf1-1, hsa-miR-565, hsa-miR-137, and hsa-miR-512-3p had gender-, race-, and recurrence-dependent associations with OS and PFS (Tables III and IV). For these four microRNAs, cohort-independent trends in the same direction were reported respectively for Burkitt's lymphoma, ovarian cancer, chemoradiation-treated rectal cancer, and for both metastatic pancreatic ductal adenocarcinoma cell lines and hepatocellular carcinoma cells linked to the inhibition of the tumorgenesis factor c-FLIP (28–30). Likewise, the gender-, therapy- and race-dependent associations between hsa-miR-93, hsa-miR-489, human cytomegalovirus (hcmv) miR-ul70-3p, hsa-miR-758, hsa-miR-143, and PFS (Table IV) have been confirmed at a cohort-independent level for T-cell leukemia, breast-cancer MCF-7 cells resistant to tamoxifen, tumors from various tissues (*e.g.* breast, colon, liver), multidrug-resistant variant of a human gastric adenocarcinoma cell line, and for both B-cell chronic lymphocytic leukemia and colorectal cancer cell growth through inhibition of *KRAS* translation (31–36). The cohort-independent and gender-dependent association of hsa-miR-222 with OS and PFS (Tables III and IV, respectively) confirm the results of Ciafre *et al.* (37). The therapy-dependent association between glioblastoma and members of the hsa-miR-181 and hsa-miR-34 families (Tables II to IV) are consistent with previous reports (3, 6, 38). High levels of hsa-miR-140 were associated with higher LE and lower and therapy-dependent OS (Tables II and III). The multiple modes of action of hsa-miR-140 are consistent with reports of up-regulation in most glioblastoma cases (38), inhibition of cell proliferation in osteosarcoma and colon cancer cell lines (39), and treatment-dependent action (39). Reanalysis of the association between glioblastoma survival and hsa-miR-140 alone (with and without cohort factors, results not shown) produced trends similar to that in the multi-microRNA models. Thus, our results suggest that the influence of hsa-miR-140 on glioblastoma survival may vary with the glioblastoma phase considered. A gender-dependent association between hsa-miR-26a and OS was uncovered (Table III). The general trend is consistent with the proposed role of hsa-miR-26a promoting glioblastoma cell growth and formation (37, 40), and the gender-dependent model is in agreement with the higher expression of hsa-miR-26a in women than in men diagnosed with hepatocellular carcinoma (41).

Additional analyses resolved the apparent inconsistencies in the trends between previous reports and our study for seven microRNAs hsa-miR-182 (42), hsa-miR-106b (43), ebv-miR-bart7 (44), hsa-miR-189 (45), hsa-miR-221 (46), hsa-miR-21 (47), and hsa-miR-10b (6, 42, 43). For hsa-miR-182, hsa-miR-106b and hsa-miR-221, the individual microRNA analysis supported the multi-microRNA results. For ebv-miR-bart7, hsa-miR-189 and hsa-miR-10b, the individual analysis did not detect a significant trend. In one case, hsa-miR-21 was not detected when considered simultaneously with other microRNA but was significant when considered alone, in agreement with Chan *et al.* (47). These results suggest that identification of biomarkers on an individual basis may result in spurious associations and also validate the approach used in this study to identify biomarkers that simultaneously considers multiple microRNAs.

The large number of gene targets of the detected microRNAs that also exhibited significant association with glioblastoma survival further substantiates our findings. Sensory perception, neurological process, olfactory receptor, and transmembrane receptor activity were among the processes and functions consistently over-represented among the target genes of microRNAs associated with all three glioblastoma survival indicators. The neurological and sensory perception processes are consistent with reports of glioblastoma candidates for single nucleotide polymorphisms of sensory perception genes and with reports that individuals with brain tumors lose sensory perception (48). Oncogenes act by mimicking the growth signals transmitted by transmembrane receptors (49). G Protein-coupled receptor (GPCR) activity (*e.g.* rhodopsin-like gene) regulates cellular motility, growth and differentiation, and gene transcription, three factors central to the biology of cancer (50). The network of gene targets that have significant association with glioblastoma survival displays known relationships, including many in the signaling pathways that involve GPCR, including MAPK, adipocytokine, chemokine, ErBB, FC epsilon RI, mTOR, neurotrophin, notch, p53, phosphatidylinositol, RIG-I-like receptor, T-cell receptor, TGF-beta receptor, toll-like receptor, VEGF, and Wnt signaling pathways (Figure 2).

In summary, this study confirmed 25 microRNAs previously associated with glioblastoma survival and identified 20 other microRNA that have been previously associated with other cancer types. This confirmation and the high correct classification of patients into survival groups suggests that the biomarkers revealed in this study are good leads for empirical confirmation, improved prognostic tools, and personalized treatments of glioblastoma multiforme. Six and 39 microRNAs were identified as biomarkers of multiple or single glioblastoma survival indicators, respectively, suggesting the multifactorial and multifaceted genomic basis of this cancer. Nineteen microRNAs exhibited gender-, race-, therapy-, or recurrence-dependent associations with glioblastoma survival, suggesting that personalized prognostic and treatments that consider individual variation can improve the outcome for glioblastoma patients. Sensory perception and GPCR activities are among the processes of the microRNA target genes associated with survival.

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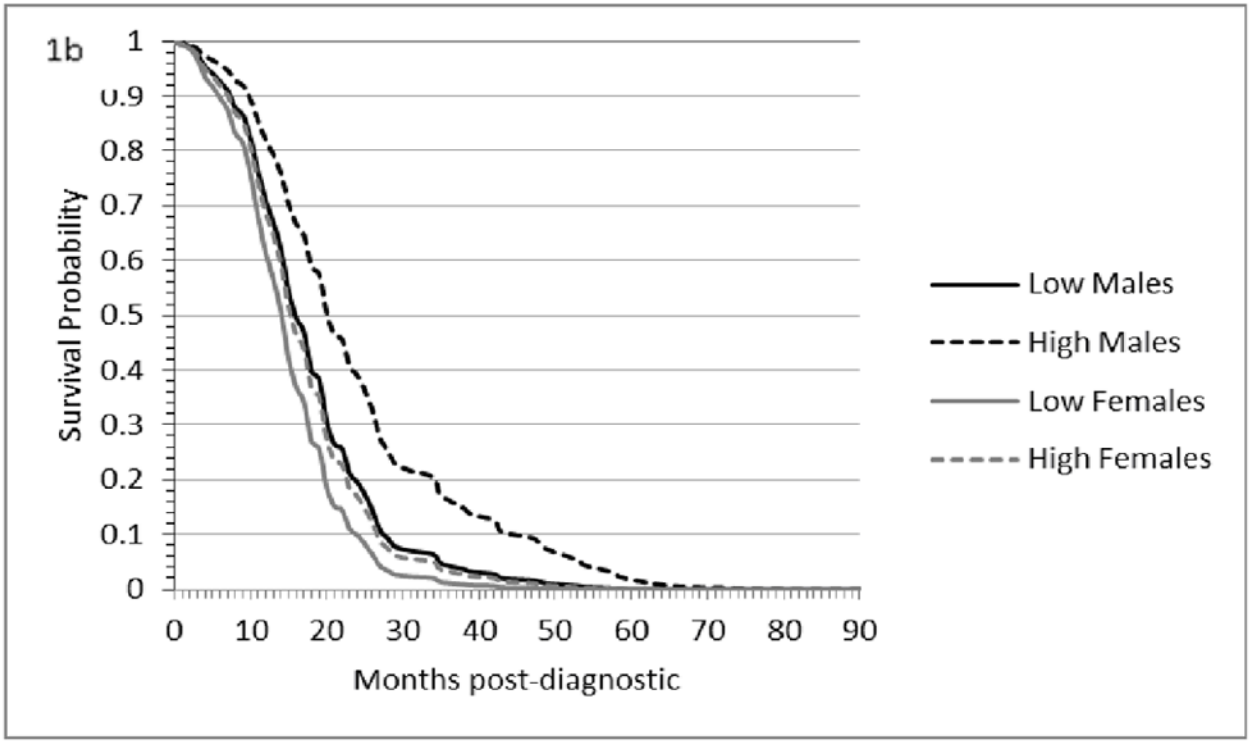

Figure 1.
Overall survival plots for males (black lines) and females (gray lines) that have high (dash lines) and low (solid line) levels of ebv-miR-bhrf1-1.

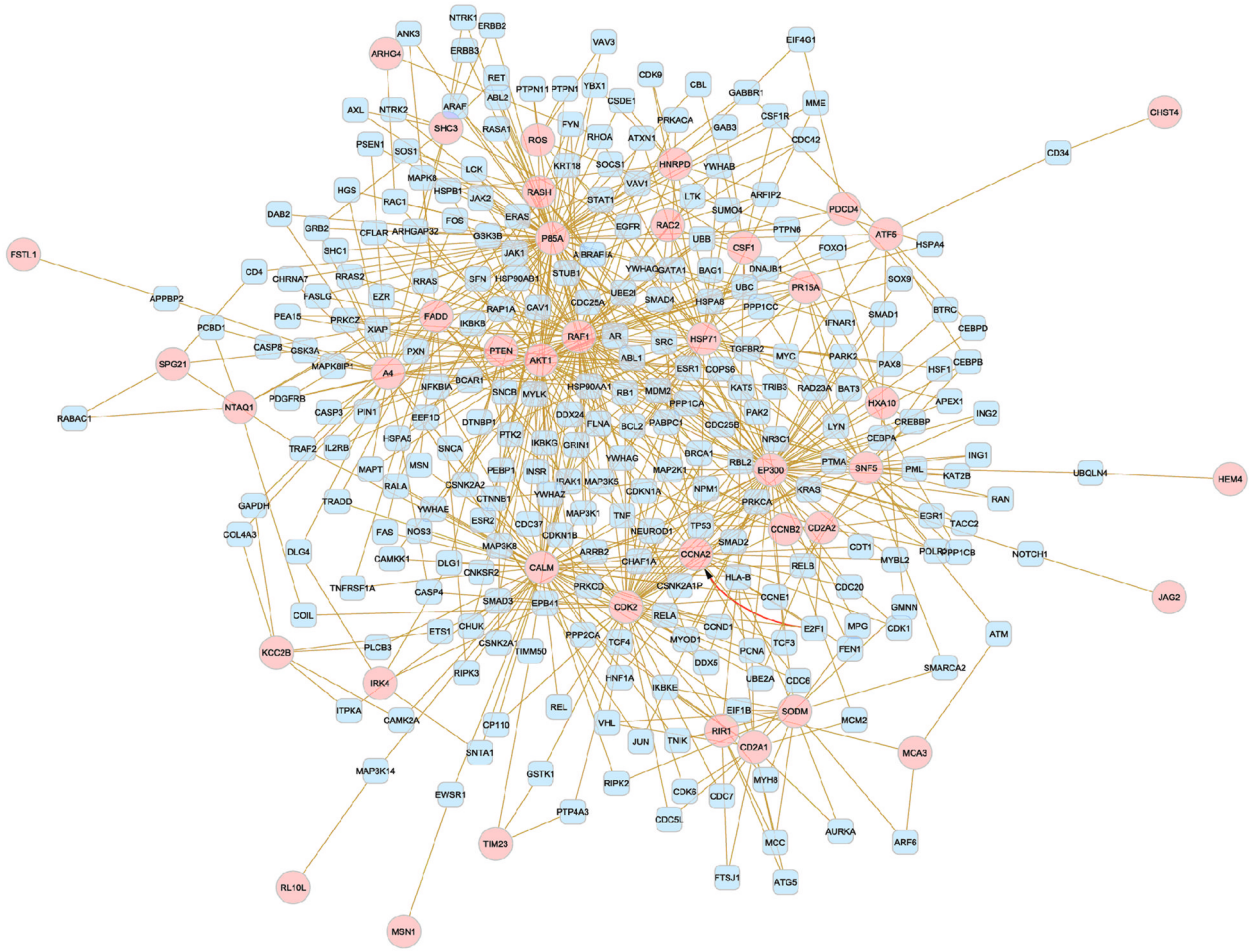

Figure 2.

Network of target genes of glioblastoma microRNAs.

Footnote. Circular pink nodes denote target genes of microRNAs associated with glioblastoma survival that also have a significant association with survival themselves. Square gray nodes denote a maximum of one intermediate gene between target genes. Edges denote known relationship between genes from several databases and summarized in the SysBiomics repository.

Table I

Number and distribution of individuals analyzed for overall and post-diagnostic hazard of glioblastoma death and post-diagnostic hazard of glioblastoma recurrence and level of the cohort factors considered.

		Post-diagnostic survival (overall survival)[a]		Post-diagnostic recurrence (progression-free survival)	
		Number	**Percentage**	**Number**	**Percentage**
Total		253		192	
N Censored		26	10%	17	9%
Race	Caucasian	211	83%	161	84%
	Other	42	17%	31	16%
Gender	Females	91	36%	68	35%
	Males	162	64%	124	65%
Therapy	RX	38	15%	32	17%
	CRN	133	53%	113	59%
	CRT	31	12%	28	15%
	OTHER	51	20%	19	10%
Recurrence	Yes	192	76%	192	100%
	No	61	24%	0	0%

[a]The number of patients analyzed for post-diagnostic survival and life expectancy is the same.

N, Number of patients; RX, radiation therapy alone; CRN, chemotherapy plus radiation and no targeted therapy; CRT, chemotherapy plus radiation and targeted therapy; OTHER, all other therapies including no therapy.

Table II

MicroRNAs associated with life expectancy on a cohort-independent or-dependent manner and supporting independent studies.

MicroRNA	*P*-value	Hazard ratio (95% C.I.)	Relevant literature references
hsa-miR-181a*	0.0537	RX=0; 0.33 (0.21 to 0.51) RX=1; 1.05 (0.33 to 3.38)	(3, 4)^{G}
hsa-miR-189	0.0204	0.20 (0.05 to 0.78)	(45)^{O}
hsa-miR-19b	0.0049	1.46 (1.11 to 1.90)	(51)^{G}
hsa-miR-222	0.0258	0.83 (0.70 to 0.98)	(6, 37, 38)^{G}
hsa-miR-34a	0.0500	RX=0; 0.69 (0.57 to 0.85) RX=1; 1.17 (0.72 to 1.89)	(52, 53)^{G}
hsa-miR-550	<0.0001	4.18 (2.31 to 7.56)	(54)^{O}
hsa-miR-625	0.0119	2.48 (1.22 to 5.02)	(55)^{O}
kshv-miR-k12-1	0.0023	2.08 (1.30 to 3.32)	(27)^{O}
hsa-miR-10b	<0.0001	0.74 (0.64 to 0.85)	(6, 42, 43)^{G}
hsa-miR-140	0.0130	1.57 (1.10 to 2.24)	(38, 39)^{G}
hsa-miR-149	0.0056	0.76 (0.63 to 0.92)	(56)^{G}

C.I., Confidence Interval; RX=1 denotes radiation therapy alone, RX=0 denotes non-radiation therapy;

^{G}glioblastoma multiforme study;

^{O}study on any other type of cancer.

Table III

MicroRNAs associated with overall survival on a cohort-independent or -dependent manner and supporting independent studies.

MicroRNA	*P*-value	Hazard ratio (95% C.I.)	Relevant literature references
hsa-miR-182	0.0245	RX=0: 0.67 (0.57 to 0.77) RX=1: 1.00 (0.71 to 1.38)	(42)^{G}
	0.0027	CRT=0: 0.66 (0.56 to 0.77) CRT=1: 1.19 (0.83 to 1.69)	
hsa-miR-189	0.0316	0.12 (0.02 to 0.83)	(45)^{O}
hsa-miR-196a	0.0168	1.39 (1.06 to 1.81)	(57)^{G}
hsa-miR-221	0.0298	RX=0: 0.67 (0.43 to 1.04) RX=1: 0.41 (0.22 to 0.75)	(6, 37, 38, 46)^{G}
hsa-miR-222	<0.0001	2.14 (1.51 to 3.03)	(6, 37, 38)^{G}
hsa-miR-23b	0.0135	1.61 (1.10 to 2.35)	(37)^{G}
hsa-miR-26a	0.0020	Male: 1.33 (1.02 to 1.71) Female: 2.52 (1.78 to 3.58)	(37, 40, 41)^{G}
hsa-miR-324-5p	<0.0001	2.73 (1.80 to 4.14)	(58)^{G}
hsa-miR-34c	0.0106	0.62 (0.43 to 0.90)	(52, 53)^{G}
ebv-miR-bhrf1-1	0.0009	Other: 0.09 (0.01 to 0.51) Caucasian: 1.83 (1.16 to 2.88)	(28)^{O}
	0.0008	Male: 0.65 (0.35 to 1.24) Female: 2.77 (1.43 to 5.38)	
hsa-miR-512-3p	0.0030	0.28 (0.12 to 0.65)	(55, 59)^{O}
hsa-miR-565	0.0996	Other: 2.97 (1.71 to 5.16) Caucasian: 1.80 (1.41 to 2.30)	(29)^{O}
	0.0003	Pr/Re=0: 3.80 (2.40 to 6.02) Pr/Re=1: 1.59 (1.27 to 2.00)	
hsa-miR-572	0.0691	0.76 (0.57 to 1.02)	(60)^{O}
hsa-miR-766	0.0052	1.57 (1.15 to 2.16)	(61)^{O}
kshv-miR-k12-1	<0.0001	2.77 (1.78 to 4.31)	(27)^{O}
kshv-miR-k12-6-3p	0.0608	1.54 (0.98 to 2.43)	(62)^{O}
hsa-miR-101	0.0065	1.63 (1.15 to 2.32)	(26)^{G}
hsa-miR-10b	0.0146	RX=0: 1.16 (0.97 to 1.38) RX=1: 0.74 (0.52 to 1.04)	(6, 42, 43)^{G}
hsa-miR-134	0.0007	2.11 (1.37 to 3.25)	(43)^{G}
hsa-miR-137	0.0010	CRN=0: 2.11 (1.45 to 3.05) CRN=1: 0.94 (0.67 to 1.32)	(30)^{O}
hsa-miR-140	0.0010	CRN=0: 0.21 (0.12 to 0.37) CRN=1: 0.65 (0.37 to 1.15)	(38, 39)^{G}
hsa-miR-148a	<0.0001	1.65 (1.35 to 2.02)	(63)^{O}
hsa-miR-409-3p	0.0001	0.43 (0.28 to 0.66)	(64)^{O}

C.I., Confidence Interval; RX=1 denotes radiation therapy alone, RX=0 denotes non-RX therapy; CRT=1 denotes chemotherapy plus radiation and targeted therapy, CRT=0 denotes non-CRT therapy; Pr/Re=1 denotes glioblastoma recurrence or progression report, Pr/Re=0 denotes no recurrence or progression report; CRN=1 denotes chemotherapy plus radiation and no targeted therapy, CRN=0 denotes non-CRN therapy;

^{G}glioblastoma multiforme study;

O study on any other type of cancer.

Table IV

MicroRNAs associated with progression-free survival on a cohort-independent or-dependent manner and supporting independent studies.

MicroRNA	*P*-value	Hazard ratio (95% C.I.)	Relevant literature references
hsa-miR-181c	0.0004	CRN=0: 0.27 (0.16 to 0.47) CRN=1: 0.82 (0.53 to 1.35)	(6, 38)^{G}
hsa-miR-188	<0.0001	2.30 (1.55 to 3.40)	(42)^{G}
hsa-miR-222	0.0814	Male: 1.27 (1.02 to 1.58) Female: 1.65 (1.29 to 2.12)	(6, 37, 38)^{G}
hsa-miR-296	0.0247	RX=0: 1.56 (1.14 to 2.14) RX=1: 3.83 (1.82 to 8.07)	(38, 65)^{G}
	0.0633	CRT=0: 2.04 (1.51 to 2.76) CRT=1: 0.90 (0.39 to 2.10)	
ebv-miR-bart7	<0.0001	0.05 (0.01 to 0.15)	(44)^{O}
hsa-miR-486	0.0168	Other: 0.74 (0.44 to 1.25) Caucasian: 1.53 (1.12 to 2.08)	(42)^{G}
hsa-miR-489	0.0041	0.04 (0.00 to 0.36)	(32)^{O}
hsa-miR-512-3p	0.0257	Other: 0.00 (0.00 to 0.04) Caucasian: 0.07 (0.02 to 0.28)	(55, 59)^{O}
hcmv-miR-ul70-3p	0.0004	Male: 0.43 (0.27 to 0.67) Female: 1.13 (0.71 to 1.79)	(33)^{O}
hsa-miR-552	0.0001	0.00 (0.00 to 0.01)	(26)^{G}
hsa-miR-578	<0.0001	0.00 (0.00 to 0.00)	(66)^{G}
hsa-miR-582	0.0003	5.49 (2.17 to 13.88)	(26)^{G}
hsa-miR-584	0.0307	0.22 (0.05 to 0.87)	(26)^{G}
hsa-miR-758	0.0029	CRN=0: 0.77 (0.23 to 2.60) CRN=1: 0.08 (0.03 to 0.21)	(34)^{O}
hsa-miR-93	0.0006	2.63 (1.51 to 4.85)	(31)^{O}
kshv-miR-k12-1	<0.0001	3.19 (1.93 to 5.29)	(27)^{O}
kshv-miR-k12-6-5p	<0.0001	3.70 (1.93 to 7.10)	(67)^{O}
hsa-miR-106b	0.0014	RX=0: 0.12 (0.06 to 0.22) RX=1: 0.55 (0.22 to 1.40)	(43)^{G}
hsa-miR-143	0.0020	Other: 0.30 (0.16 to 0.54) Caucasian: 0.83 (0.61 to 1.12)	(35, 36)^{O}

C.I., Confidence Interval; CRN=1 denotes chemotherapy plus radiation and no targeted therapy, CRN=0 denotes non-CRN therapy; RX=1 denotes radiation therapy alone, RX=0 denotes non-RX therapy; CRT=1 denotes chemotherapy plus radiation and targeted therapy, CRT=0 denotes non-CRT therapy;

^{G}glioblastoma multiforme study;

^{O}study on any other type of cancer; n/a, no association with any type of cancer found in literature.

Table V

Gene Ontology categories enriched (FDR-adjusted P-value <0.05) among the target genes of microRNAs associated with life expectancy

Gene Ontology	Level	Term	FDR *P*-value	No. of genes
Biological process	3	Neurological process (GO:0050877)	0.0248	219
Biological process	4	Sensory perception (GO:0007600)	0.0111	151
Biological process	5	Sensory perception of chemical stimulus (GO:0007606)	<0.0001	70
Biological process	6	Sensory perception of smell (GO:0007608)	<0.0001	63
Molecular function	4	Transmembrane receptor activity (GO:0004888)	0.0146	239
Molecular function	5	G Protein-coupled receptor activity (GO:0004930)	0.0039	160
Molecular function	6	Rhodopsin-like receptor activity (GO:0001584)	0.0023	134
Molecular function	7	Olfactory receptor activity (GO:0004984)	<0.0001	60

Table VI

Gene Ontology categories enriched (FDR-adjusted P-value <0.05) among the target genes of microRNAs associated with overall survival.

Gene Ontology	Level	Term	FDR *P*-value	No. of genes
Biological process	3	Neurological process (GO:0050877)	<0.0001	371
Biological process	3	Cell communication (GO:0007154)	0.0001	1546
Biological process	4	Sensory perception (GO:0007600)	<0.0001	255
Biological process	4	Signal transduction (GO:0007165)	0.0006	1400
Biological process	5	Sensory perception of chem. stimulus (GO:0007606)	<0.0001	129
Biological process	5	Cell surface receptor linked signal transduction (GO:0007166)	0.0145	684
Biological process	6	Sensory perception of smell (GO:0007608)	<0.0001	123
Biological process	6	G Protein-coupled receptor protein signaling pathway (GO:0007186)	0.0156	413
Molecular function	3	Receptor activity (GO:0004872)	0.0040	711
Molecular function	3	Antigen binding (GO:0003823)	0.0422	16
Molecular function	4	Transmembrane receptor activity (GO:0004888)	0.0002	457
Molecular function	5	G Protein-coupled receptor activity (GO:0004930)	<0.0001	315
Molecular function	6	Rhodopsin-like receptor activity (GO:0001584)	0.0002	274
Molecular function	7	Olfactory receptor activity (GO:0004984)	<0.0001	120

Table VII

Gene Ontology categories enriched (FDR-adjusted P-value <0.01, number genes >6) among the target genes of microRNAs associated with progression-free survival.

Gene Ontology	Level	Term	FDR *P*-value	No. of genes
Biological process	3	Cell communication (GO:0007154)	<0.0001	975
Biological process	3	Multicellular development (GO:0007275)	<0.0001	507
Biological process	3	Neurological process (GO:0050877)	<0.0001	248
Biological process	3	Anatomical structure development (GO:0048856)	<0.0001	483
Biological process	3	Cellular organization & biogenesis (GO:0016043)	0.0008	639
Biological process	3	Cellular metabolic process (GO:0044237)	0.0014	2290
Biological process	3	Cellular developmental process (GO:0048869)	0.0066	551
Biological process	4	Signal transduction (GO:0007165)	<0.0001	889
Biological process	4	Sensory perception (GO:0007600)	<0.0001	172
Biological process	4	System development (GO:0048731)	<0.0001	386
Biological process	5	Sensory perception of chemical stimulus (GO:0007606)	<0.0001	86
Biological process	5	Cell surface receptor linked signal transduction (GO:0007166)	<0.0001	433
Biological process	5	Organ development (GO:0048513)	0.0085	285
Biological process	5	+ Regulation of metabolic process (GO:0009893)	0.0087	84
Biological process	5	Carboxylic acid metabolic process (GO:0019752)	0.0095	185
Biological process	5	+ Regulation of cellular process (GO:0048522)	0.0095	214
Biological process	6	Organ morphogenesis (GO:0009887)	<0.0001	57
Biological process	6	Sensory perception of smell (GO:0007608)	<0.0001	80
Biological process	6	G Protein-coupled receptor protein signaling pathway (GO:0007186)	0.0099	266
Molecular function	3	Protein binding (GO:0005515)	<0.0001	1601
Molecular function	4	Transmembrane receptor activity (GO:0004888)	0.0017	305
Molecular function	6	Rhodopsin-like receptor activity (GO:0001584)	0.0101	179
Molecular function	7	Olfactory receptor activity (GO:0004984)	0.0002	79