microRNA-148a Is a Prognostic oncomiR That Targets MIG6 and BIM to Regulate EGFR and Apoptosis in Glioblastoma

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Abstract
Great interest persists in useful prognostic and therapeutic targets in glioblastoma. In this study, we report the definition of miRNA (miR)-148a as a novel prognostic oncomiR in glioblastoma. miR-148a expression was elevated in human glioblastoma specimens, cell lines, and stem cells (GSC) compared with normal human brain and astrocytes. High levels were a risk indicator for glioblastoma patient survival. Functionally, miR-148a expression increased cell growth, survival, migration, and invasion in glioblastoma cells and GSCs and promoted GSC neurosphere formation. Two direct targets of miR-148a were identified, the EGF receptor (EGFR) regulator MIG6 and the apoptosis regulator BIM, which rescue experiments showed were essential to mediate the oncogenic activity of miR-148a. By inhibiting MIG6 expression, miR-148a reduced EGFR trafficking to Rab7-expressing compartments, which includes late endosomes and lysosomes. This process coincided with reduced degradation and elevated expression and activation of EGFR. Finally, inhibition of miR-148a strongly suppressed GSC and glioblastoma xenograft growth in vivo. Taken together, our findings provide a comprehensive analysis of the prognostic value and oncogenic function of miR-148a in glioblastoma, further defining it as a potential target for glioblastoma therapy. Cancer Res; 74(5); 1541–53. ©2014 AACR.

Introduction
Glioblastoma is an extremely aggressive tumor that accounts for the majority of deaths due to primary brain neoplasms (1). Despite the most advanced treatment with combinations of surgery, radiotherapy, and chemotherapy, glioblastoma is associated with a median survival of only 14 months (2). Factors responsible for glioblastoma malignancy and poor prognosis include rapid cell growth, resistance against apoptosis, and distant invasion of the surrounding brain (1, 3).

Receptor tyrosine kinase (RTK) pathways are deregulated in the vast majority of glioblastomas (4, 5). Among RTKs, the EGF receptor (EGFR) is the most commonly altered (6). It is mutated and/or amplified in 40% and overexpressed in >60% of tumors (7, 8). Activation of EGFR induces tumor cell growth, migration, and invasion, as well as resistance to chemotherapy and radiation (6, 9). EGFR signaling and protein half-life are tightly regulated (10). Mitogen-inducible gene 6 (MIG6) regulates EGFR signaling and turnover by binding EGFR and directly inhibiting tyrosine kinase activity, increasing clathrin-dependent EGFR endocytosis and trafficking into the lysosome, and promoting EGFR degradation (11–13). Ablation of MIG6 induces tumor formation, supporting a tumor suppressor function of MIG6 (11, 14). The MIG6 gene is located on chromosome 1p36, which is subject to focal deletions in glioblastoma. The Cancer Genome Atlas (TCGA) data analysis showed that 15 out of 430 glioblastoma samples contain homozygous deletions in 1p36 (14) but that MIG6 expression is downregulated in approximately 50% of primary tumor samples and glioblastoma cell lines (11). Therefore MIG6 deletions only account for a small fraction of the glioblastoma tumors with reduced MIG6 expression.

Resistance to apoptosis is a big obstacle in glioblastoma therapy (15, 16). Apoptosis in the intrinsic pathway is regulated by the balance between proapoptotic (Bax, Bak, BIM, and Bad) and antiapoptotic (Bcl-2 and Bcl-xL) members of the Bcl-2 family (17). Proapoptotic BIM (BCL2L11) is localized to the mitochondria where it initiates the mitochondrial cell death pathway by directly activating Bax/Bak-dependent apoptosis. BIM has been shown to be an important mediator of targeted therapy-induced apoptosis in solid tumors. BIM is downregulated in 29% of glioblastoma cases based on TCGA analysis (18, 19). However, the causes of BIM downregulation in glioblastoma are not known.

microRNAs (miRNA) are short noncoding RNA molecules that regulate gene expression by binding to the 3′ untranslated region (3′UTR) of target mRNA and inducing mRNA...
degradation and/or inhibition of protein synthesis (20, 21). Deregulation of miRNA expression has been associated with cancer formation through alterations in either oncogenic or tumor suppressor gene targets (20, 22). A number of miRNAs are deregulated in glioblastoma and play important roles in tumor formation and growth (23–31). However, a role for miR-148a in glioblastoma has not been described before.

We analyzed miRNA expression in 500 patient glioblastomas in the TCGA database and found that miR-148a is upregulated and predicted poor patient survival. We therefore embarked on a comprehensive study of miR-148a in glioblastoma. Our data show for the first time that miR-148a is upregulated in glioblastoma, where it exerts oncogenic effects in vitro and in vivo by regulating BIM, MIG6, and EGFR. MiR-148a is therefore a novel oncomiR and potential therapeutic target in glioblastoma.

**Materials and Methods**

**Cells and tumor specimens**

Glioblastoma cell lines U87, U373, A172, T98G, SNB-19, and U251 were from American Type Culture Collection, which authenticates cell lines with short tandem repeat (STR) profiling. Cells lines that were used for more than 6 months after purchase were reauthenticated by STR profiling in 2013 by Laragen, Inc. Glioblastoma stem cells (GSC) 1228, 0802, and 0908 (a kind gift from Dr. Jeongwu Lee, Cleveland Clinic, Cleveland, OH) were isolated from patient surgical specimens and characterized for tumorigenesis, pluripotency, self-renewal, stem cell markers, and neurosphere formation (32). Glioblastoma surgical specimens (n = 18) and normal brain (n = 7) were obtained from patients undergoing surgery at the University of Virginia Hospital (Charlottesville, VA) according to protocols approved by the Internal Review Board.

**TCGA data analysis**

The collection of data from TCGA was compliant with all laws and regulations for the protection of human subjects, and necessary ethical approvals were obtained. Analysis of all data was done in the R project (33). For analysis of differential expression and determination of the effects of miRNA (miR)-148a on patient survival, Agilent 8 × 15 k miRNA expression for 491 glioblastoma and 10 normal unmatched brain samples was downloaded along with clinical information from the TCGA database [level 2 (normalized) data, November 2012]. Cox regression analysis of all samples with miRNA and survival data (n = 482) was performed to determine whether miR-148a levels were a risk indicator for survival. The expression of MiR-148a was also compared in normal brain (n = 10) with glioblastoma (n = 491) using the R-based Limma package (34).

**Quantitative RT-PCR**

miScript Primer Assay Hs-miR-148a was used for measuring miR-148a. Total RNA was extracted from glioblastoma cell lines and GSCs. RNA samples were reverse transcribed using the miScript Reverse Transcriptase Kit (QIAGEN), and quantitative real-time PCR (qRT-PCR) analysis was performed using the 7500 Real-time PCR System (Applied Biosystems). qRT-PCR was also used to assess the mRNA levels of MIG6 and BIM. The primer sequences were: MIG6-forward: 5'-GACAATTTTGAGCAACTTGGCTTG-3', MIG6-reverse: 5'-GGTACTATTGTGTTGCAGGAAG-3; BIM-forward: 5'-TGGCAAAACCACTTCTGATG-3' and BIM-reverse: 5'-GCAAGGCTGAATTGCTCACC-3. Human U6β and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers (QIAGEN) were used as controls.

**Cell transfections**

Glioblastoma cells and GSCs were transfected with 20 nmol/L pre-miR-148a, anti-miRNA-148a, or control-miR (Ambion), using Oligofectamine or Lipofectamine RNAiMax (Invitrogen) according to the manufacturer's instructions. Plasmid transfections were performed with Eugene 6 (Roche). miR-148a expression was verified by qRT-PCR 72 hours and 7 days posttransfection.

**Generation of anti-miR-148a stable expressing glioblastoma cells**

Lentiviruses encoding the pEZX-AM04 expression cassette containing a hygromycin resistance gene as well as the anti-sense sequence for miR-148a and the red fluorescent protein mCherry gene under the U6 promoter (pEZX-AM04; GeneCopoeia; Supplementary Fig. S2B and S2C) were generated with pPACKH1 Lentivector packaging Plasmid mix (System Biosciences) and concentrated using PEG-it Virus Precipitation Solution (System Biosciences). U87 cells were infected with the lentiviruses or control viruses lacking the anti-miR-148a sequence. After culturing in selection media, mCherry was detected by fluorescence microscopy. A stable infection efficiency of approximately 100% was attained (Supplementary Fig. S2A).

**Cell growth and apoptosis assays**

For growth, glioblastoma cells and GSCs were transfected with pre-miR-148a, anti-miR-148a, or control. Three days posttransfection, the cells were counted for 5 days with a hemocytometer. For apoptosis, cells were transfected as above and Annexin V-PE/7AAD flow cytometry was used to determine the dead and apoptotic cell fractions as previously described (35).

**Cell migration and invasion assays**

The effects of miR-148a expression on cell migration and invasion were assessed using the wound-healing and Transwell assays as previously described (36).

**Neurosphere formation assay**

GSCs were grown in low EGF and fibroblast growth factor medium (20 ng/mL each) and transfected with either anti- or pre-miR-148a or controls for 72 hours. The cells were dissociated into single cells in buffer (EDTA 1 mmol/L, bovine serum albumin 0.5% in PBS) and 1,000 single cells were incubated for 7 days. Secondary neurospheres containing more than 30 cells were counted.

**In vivo tumor formation**

Tumor xenografts were generated by implantation of 1228 GSCs transfected with anti-miR-148a and U87 cells engineered
to stably express anti–miR-148a, 1228 (1 × 10^5 cells; n = 6) and U87 cells (3 × 10^5 cells; n = 10) were stereotactically implanted into the striata of immunodeficient mice. Four weeks after tumor implantation, the animals were subjected to brain MRI. To measure the tumor size, 30 μL of gadopentetate dimeglumine (Magnevist, Bayer Healthcare) was intraperitoneally injected 15 minutes before scanning and tumor volume was quantified as previously described (37, 38).

**Immunoblotting**

Immunoblotting was performed as previously described using antibodies for MIG6 (Santa Cruz Biotechnologies), BIM, EGFR, and p-EGFR (Cell Signaling Technology). All blots were stripped and reprobed with β-actin or GAPDH (Santa Cruz Biotechnologies) as control. Blots in which differences were not obvious were quantified by densitometry on film as previously described (39).

**Generation of MIG6 and BIM 3’UTR constructs**

The MIG6 3’-UTR reporter plasmid was constructed via insertion of the MIG6 3’-UTR (2561 bp) downstream of the Renilla luciferase stop codon in the pMIR vector (Promega) generating the pMIR-MIG63’UTR plasmid. For BIM, a commercially available 3’-UTR reporter plasmid, pEZX-BIM3’UTR-1, was used (Genecopoeia). QuikChange Site-Directed Mutagenesis Kit (Stratagene) was used to generate mutations in the 3’ UTR of MIG6 and BIM by PCR using the pMIR-MIG6 3’UTR and pEZX-BIM 3’UTR as constructs templates. Primers containing the mutation TGCACTGA (1370–1377) → CCGGGCG in the 3’UTR of MIG6 gene and TGCACTG (1029–1035) → GCGCGCC 3’UTR of BIM were used.

**3’UTR reporter assays**

Glioblastoma cells were transfected with pre–miR-148a or pre-miR control for 6 hours. For MIG6, the cells were then transfected with the reporter vector with 3’UTR-MIG6 or with mutant-3’UTR, in addition to a control β-galactosidase reporter plasmid. For BIM, the cells were transfected with either 3’UTR BIM or BIM-mutant-3’UTR. Luciferase assays were performed 48 hours later using the Luciferase System Kit (Promega) for MIG6 or the Dual Luciferase Assay (Promega) for BIM, and luminescence was measured on a Promega GloMax 20/20 luminometer. Firefly luciferase activity was double normalized by dividing each well first by β-galactosidase activity and then by average luciferase/β-galactosidase value in a parallel set done with a constitutive luciferase plasmid.

**Rescue experiments**

To determine whether MIG6 and BIM mediate the effects of miR-148a, rescue experiments were conducted in which the effects of anti–miR-148a were measured in the setting of inhibited MIG6 or BIM. Cells were either transfected with anti–miR-148a for 6 hours (1228) or U87 cells stably expressing anti–miR-148a were used. The cells were then transfected with siRNA against MIG6 (Thermo Fisher Scientific) or BIM (Cell Signaling Technology), and cell growth and death were assessed as described above. MIG6, EGFR, and BIM expression changes were verified by immunoblotting.

**EGFR tracking assays**

Cells were plated and transfected with either pre–miR-148a or pre-miR control for 24 hours followed by transfection with Rab7-mCherry for 24 hours (kindly provided by Marc G. Coppolino, University of Guelph, Ontario, Canada). Cells were serum starved overnight, followed by stimulation with 50 ng/mL EGF for 30 minutes. Samples were then washed, fixed, and permeabilized before immunostaining using primary antibodies (EGFR, Abcam; MIG6, Santa Cruz Biotechnologies). Samples were imaged using a 63× (NA 1.4) lens on a Zeiss LSM 700 with 405, 488, 543, 633 nm lasers using ZEN software (Carl Zeiss). Captured images were analyzed for colocalization using ImageJ software. Briefly, images were initially thresholded, and the Colocalization Finder tool was used to determine the area and intensity of colocalizing pixels of EGFR.

**Statistical analysis**

All experiments were performed at least three times. Two group comparisons were analyzed with t test and P values were calculated. For rescue experiments, the anti-miR-148a–induced change in the setting of inhibited target protein was compared with the anti-miR-148a–induced change in the control setting. For TCGA data, Cox regression analysis was performed to determine the correlation between miR-148a expression and patient survival. More detailed TCGA data statistical analyses are described in the corresponding sections. For all analyses, P < 0.05 was considered significant.

**Results**

**miR-148a expression is upregulated in glioblastoma cells, GSCs, and human tumors and inversely correlates with patient survival**

We analyzed TCGA data for miR-148a levels and for correlation with patient survival. The comparison of tumor (n = 491) with normal tissue samples (n = 10) showed a significant (59%) increase of miR-148a levels in the tumors as compared with normal brain (P = 3 × 10^−7; Fig. 1A). Cox regression analysis of 482 glioblastoma samples in the TCGA dataset revealed that elevated miR-148a expression is a highly significant negative risk factor (P = 9.9 × 10^−6). The HR was 1.19 with confidence intervals 1.10 to 1.29. The Kaplan–Meier curve of the TCGA patient cohort is shown in Fig. 1B. The lower quartile (with the lowest miR-148a expression) had longer overall survival than those with higher miR-148a expression. The median survival of the different groups in the Kaplan–Meier curve are <25% expression = 515, 25%–50% = 463, 50%–75% = 377, 75%–100% = 382 (days). Log-rank analysis of 482 samples revealed that miR-148a was highly significant as a negative risk factor (P = 9.18 × 10^−5; Fig. 1B). We also measured miR-148a levels in glioblastoma cell lines (U87, U373, T98G, A172, and SNB19), GSCs (O308, O822, and O128), and human tumor specimens (n = 18) as well as normal human astrocytes and normal brain (n = 7). MiR-148a was significantly higher in glioblastoma cells and GSCs than in astrocytes (P < 0.05; Fig. 1C) and significantly higher in tumors than in normal brain (P < 0.05; Fig. 1D). Altogether, these data demonstrate that miR-148a is...
upregulated in glioblastoma and that high miR-148a expression predicts poor patient survival.

**MiR-148a promotes glioblastoma cell and GSC growth and survival**

We next assessed the functional role of miR-148a in glioblastoma (A172, SNB19, U87, and U373) and GSC (0308, 0822, and 1228) cells by determining the effects of miRNA overexpression and inhibition on cell growth and apoptosis using cell counting and Annexin V-7 AAD flow cytometry, respectively. miR-148a inhibition with antisense miRNA significantly decreased the growth rate (Fig. 2A) and overexpression of miR-148a resulted in a higher growth rate in glioblastoma and GSC cells as compared with controls ($P < 0.05$; Fig. 2B). Similarly, inhibition of miR-148a led to a significant induction of apoptosis (Fig. 2C), whereas overexpression of miR-148a led to a significant inhibition of apoptosis in glioblastoma cells and GSCs ($P < 0.05$; Fig. 2D). MiR-148a levels were verified by qRT-PCR (Supplementary Fig. S1). The above results show that miR-148a promotes cell growth and inhibits cell death in glioblastoma.

**MiR-148a promotes glioblastoma cell migration and invasion**

We next assessed the effects of miR-148a on glioblastoma cell migration and invasion. GSCs were not used for these experiments because they grow as neurospheres that do not...
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Figure 2. miR-148a promotes glioblastoma cell and GSC growth and survival. Glioblastoma cell lines (A172, SNB19, and U87) and GSC (0308, 0822, and 1228) were transfected with anti-miR-148a (A) or pre-miR-148a (B) or controls. The cells were subsequently assessed for cell growth by cell counting. Glioblastoma cell line (A172) and GSCs (0308, 0822, 1228) were transfected with either anti-miR-148a (C), pre-miR-148a (D), or controls and subsequently assessed for cell death and apoptosis by AnnexinV-PE/7-AAD flow cytometry. The data show that miR-148a inhibition (A and C) and miR-148a overexpression (B and D) promote cell growth and survival. *P < 0.05.

These data show that miR-148a promotes glioblastoma cell migration and invasion.

**MiR-148a induces GSC neurosphere formation and promotes the in vivo growth of GSC- and glioblastoma-derived xenografts**

We analyzed the effects of miR-148a on GSC self-renewal using a neurosphere formation assay. Anti-miR-148a or
pre–miR-148a was transfected into GSCs and neurosphere formation was assessed for one week. MiR-148a inhibition significantly reduced neurosphere size and number and miR-148a overexpression increased neurosphere size and number ($P < 0.05$; Fig. 4A and B). These data suggest that 

miR-148a promotes the self-renewal ability of GSCs. To determine whether miR-148a affects GSC tumorigenesis, we assessed the effects of anti–miR-148a on orthotopic GSC xenograft formation. GSC 1228 cells were transfected with anti–miR-148a or anti–miR-control and stereotactic

Figure 3. MiR-148a promotes glioblastoma cell migration and invasion. Glioblastoma cell lines were transfected with either pre–miR-148a, anti–miR-148a, or controls and assessed for migration with the wound-healing assay (A and B), and invasion with the Transwell invasion assay (C and D); left panels of C and D show representative invasion assays; right panels show the quantification of invasion. The data show that miR-148a overexpression increases and miR-148a inhibition inhibits glioblastoma cell migration and invasion, *, $P < 0.05$. 

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implanted into the striata of immunodeficient mice (n = 6). Tumor sizes were measured with MRI 4 weeks after implantation. Anti-miR-148a significantly inhibited tumor formation by GSCs (P < 0.05; Fig. 4C). We also assessed the effects of stable anti-miR-148a expression on glioblastoma xenograft growth. U87 cells stably expressing anti-miR-148a were orthotopically injected into nonobese diabetic/severe combined immunodeficient mice brains (n = 10) and tumor size was measured by
MIR-148a inhibits MIG6 and BIM expression and indirectly enhances EGFR expression and activation

To uncover mRNA targets of miR-148a in glioblastoma, we used bioinformatics databases (Targetscan, PicTar, RNAhybrid) to identify potential tumor suppressor targets. The following genes contained predicted binding sites for miR-148a: ERBB1 (MIG6, NM_018948), BCL2L11 (BIM, NM_001204106), PTEN (NM_000314), SOCS3 (NM_003955), DNM3 (NM_001130823), and JMY (NM_152405). To experimentally verify these potential targets, cells were transfected with miR-148a and assessed protein and mRNA target levels by immunoblotting and qRT-PCR, respectively. Two of the candidates were confirmed: MIG6 (ERBB1) and BIM (BCL2L11). As MIG6 is a critical regulator of EGFR trafficking, degradation, and activation, we also determined the effects of miR-148a on EGFR expression and activation. MiR-148a inhibition increased (Fig. 5A) and miR-148a overexpression reduced (Fig. 5B) the expression of MIG6 in glioblastoma cells and GSCs. MiR-148a inhibition increased (Fig. 5C) and miR-148a overexpression reduced (Fig. 5D) the expression of BIM extralong (most abundant form of BIM) in glioblastoma cells and GSCs. Moreover, the effects of miR-148a on EGFR expression and activation were opposite to those on MIG6, as miR-148a overexpression led to increased EGFR and phospho-EGFR (Fig. 5B). We confirmed the above results in U87 cells stably expressing anti-miR-148a (Supplementary Figs. S4). To determine whether MIG6 and BIM 3’-UTRs are direct targets of miR-148a, MIG6 or BIM 3’-UTR reporter constructs or 3’UTR-mutant controls were transfected into glioblastoma cells before transfection with miR-148a and luciferase activity was measured. Overexpression of miR-148a significantly reduced luciferase activity for both MIG6 and BIM (Fig. 5F). The above data show that miR-148a directly inhibits MIG6 and BIM and indirectly upregulates EGFR protein expression and promotes EGFR activation.

MiR-148a inhibits MIG6 and BIM expression and indirectly enhances EGFR expression and activation.

Discussion

MiR-148a has been investigated in some cancers but not in brain tumors (41–43). In this study, we investigated the expression, function, and mechanisms of action of miR-148a in glioblastoma. We found that miR-148a is a risk factor in glioblastoma where it acts as an oncogene by regulating BIM, MiG6, and EGFR stability and activation. EGFR is one of the most frequently altered genes in glioblastoma. It is overexpressed in more than 60% of tumors but mutated and amplified in only about 40% (5, 44). Therefore, EGFR gene amplification only partially accounts for EGFR overexpression in glioblastoma (5), suggesting that additional mechanisms may be involved. Our study suggests that miR-148a overexpression is an important mechanism of EGFR overexpression via downregulation of MIG6. Consistent with our results, others have found that MiG6 expression is downregulated in approximately 50% of glioblastoma tumors without indications of MiG6 genomic deletions in the majority of samples (11). Our study also provides a new mechanism of MiG6 downregulation in glioblastoma.
Figure 5. miR-148a directly targets and inhibits MIG6 and BIM and indirectly enhances EGFR expression and activation. Human glioblastoma cell lines and GSCs were transfected with anti–miR-148a (A and C), pre–miR-148a (B and D), or controls. The cells were assessed for MIG6 and EGFR (A and B) and BIM (C and D) expression/activation by immunoblotting. The data show that miR-148a overexpression inhibits MIG6 and BIM and enhances EGFR/p-EGFR, whereas miR-148a inhibition has the opposite effects. Immunoblot analyses are from representative experiments and bar graphs show the quantification of the immunoblots. E, immunoblot analyses showing the regulation of MIG6, EGFR, and BIM proteins in stable anti–miR-148a expressing U87 cells. F, 3′ UTR luciferase assays for MIG6 and BIM showing the inhibition of luciferase activity by miR-148a in glioblastoma cells relative to mutant (mut) controls. *, P < 0.05.
Figure 6. MIG6 and BIM mediate the effects of miR-148a on glioblastoma cell growth and survival. Glioblastoma cells and GSCs were transfected with anti-miR-148a before transfection with either MIG6 siRNA (A) or BIM siRNA (B). A, growth assay showing that MIG6 inhibition partially rescues the proliferative effects of miR-148a inhibition (top). Immunoblot analyses showing the rescue of anti-miR-148a–induced upregulation of MIG6 and downregulation of EGFR by the corresponding siRNA (bottom). B, apoptosis/cell death assay showing that BIM inhibition partially rescues the apoptotic effects of miR-148a inhibition (top). Immunoblot analysis showing the rescue of anti-miR-148a–induced upregulation of BIM by the corresponding siRNA (top). *P < 0.05.
We also identified the proapoptotic molecule BIM as a target of miR-148a, which is downregulated in 29% of glioblastoma cases based on TCGA analysis. Interestingly, a recent study demonstrated that elevated BIM expression levels in cancers strongly increased the antitumor activity of EGFR and other RTK inhibitors (45). These findings suggest that combined upregulation of BIM and inhibition of EGFR are likely to achieve synergistic antitumor effects. Our study shows that such combined targeting of BIM and EGFR can be achieved by inhibition of miR-148a, providing a rationale for the therapeutic targeting of miR-148a.

Previous research described miR-148a as a tumor suppressor in hepatocellular carcinoma, pancreatic cancer, gastric cancer, and colorectal cancer (42, 46–48). Our study demonstrates for the first time that miR-148a is oncogenic in glioblastoma. We show that miR-148a enhances glioblastoma and GSC growth, survival, migration, and invasion as well as GSC self-renewal and in vivo tumor growth. We also show that inhibiting miR-148a inhibits the above oncogenic endpoints. Importantly, based on our TCGA data analysis, we find that miR-148a expression displays a significant inverse correlation with glioblastoma patient survival. A recent study identifying a ten-miRNA prognostic expression signature in glioblastoma showed that miR-148a was among the seven miRNAs that were associated with high risk (49). Our TCGA data analysis expanded on this finding, analyzing 482 samples to further demonstrate elevated miR-148a expression in human glioblastoma specimens.

In summary, the present study shows that miR-148a is elevated in glioblastoma, where it predicts poor patient survival. It demonstrates that miR-148a has powerful oncogenic and cancer stem cell regulatory effects that are mediated by BIM, MIG6, and EGFR. The study therefore represents a first characterization of miR-148a as an oncogene and promising therapeutic target in glioblastoma.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: J. Kim, Y. Zhang, S. Lawler, R. Abounader
Development of methodology: B. Kefas, S. Parsons, S. Lawler
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D. Schiff

Figure 7. miR-148a inhibits EGFR trafficking and degradation. Glioblastoma cell lines were transfected with control (A–E and K–O) or pre-miR-148a (F–J and P–T) for 24 hours and then transfected with Rab7-mCherry (red; B, G, L, and Q) for 24 hours. The cells were serum-starved for the last hour before being treated with EGF (50 ng/mL) for the indicated times (0 minute; A–J, 30 minutes; K–T). Cells were fixed and stained with anti-EGFR (green; A, F, K, and P) and anti-MIG6 (blue; C, H, M, and R). Arrows, EGFR and MIG6-containing Rab7 compartment in control-transfected and EGF-treated cells (K, L, and M). Note the increased amount of EGFR colocalizing with Rab7 and MIG6 in control cells (N and O) as compared with miR-148a-overexpressing cells (S and T). In miR-148a-expressing cells, light gray circles indicate Rab7 compartment structures, but colocalization between EGFR, MIG6, and Rab7 is rarely seen and not in large Rab7-labeled structures (MVBS; P, Q, and R). Colocalization of MIG6, EGFR, and Rab7 are shown in black and white in E, J, O, and T and U. Colocalization of EGFR with MIG6 and Rab7-labeled structures was quantified on the confocal images. The results are the mean ± SEM of >30 cells scored from two separate experiments. *, P < 0.05.
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Kim, Y. Zhang, M. Skalski, J. Hayes, S. Parsons, S. Lawler

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Study supervision: Y. Zhang, S. Parsons, R. Abounader

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References

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