Prediction of Associations between microRNAs and Gene Expression in Glioma Biology

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Abstract

Despite progress in the determination of miR interactions, their regulatory role in cancer is only beginning to be unraveled. Utilizing gene expression data from 27 glioblastoma samples we found that the mere knowledge of physical interactions between specific mRNAs and miRs can be used to determine associated regulatory interactions, allowing us to identify 626 associated interactions, involving 128 miRs that putatively modulate the expression of 246 mRNAs. Experimentally determining the expression of miRs, we found an over-representation of over(under)-expressed miRs with various predicted mRNA target sequences. Such significantly associated miRs that putatively bind over-expressed genes strongly tend to have binding sites nearby the 3' UTR of the corresponding mRNAs, suggesting that the presence of the miRs near the translation stop site may be a factor in their regulatory ability. Our analysis predicted a significant association between miR-128 and the protein kinase WEE1, which we subsequently validated experimentally by showing that the over-expression of the naturally under-expressed miR-128 in glioma cells resulted in the inhibition of WEE1 in glioblastoma cells.

Introduction

MicroRNAs (miRs) are small non-coding RNAs with mature transcripts of 18 to 25 nucleotides that have been implicated in the maintenance of the pluripotent cell state during early embryogenesis in mammals [1] as well as in tissue-specific or organ-specific development [2]. miRs interact with their target coding mRNA, inhibiting translation by degradation of the mRNAs, or blocking translation by direct and imperfect binding to the 3' and 5' untranslated regions (UTR) of targeted genes [3,4,5,6,7]. Furthermore, miRs exert control in combination with other regulatory elements such as transcription factors [8].

Focusing on cancer, over-expressed miRs might diminish the level of expression of targeted tumor suppressor genes - oncomirs - in tumors whereas miRs acting as tumor suppressors are silenced/ down-regulated, leading to a higher expression rate of targeted oncogenes and contributing to the neoplastic process [9]. Additionally, miRs are frequently located in regions of loss of heterozygosity, genomic regions of amplification or common breakpoint regions [10] and have been identified to regulate the expression of tumor-associated genes in several tumors including glioblastomas (GBM) [9,11,12,13].

Astrocytic tumors represent the most common form of glial tumors. According to the WHO classification [14], tumor anaplasia and aggressiveness increases from grades I to IV with glioblastomas (GBM-WHO grade IV) being the most malignant form of these tumors. Non-random genetic and epigenetic perturbations potentially lead to abnormal oncogene activation and/or tumor suppressor gene inactivation [14,15,16,17,18,19],

Several studies have analyzed miR expression profiles in normal brain [1,20,21] and brain tumors [12,13,22], as well as tested their use as potential therapeutic tools [11,22,23]. Initial analysis of murine and human brain miRs predominantly indicated distinctive expression of miRs-9, -101, -124, -127, -128, -131 and -132 [1,21]. Furthermore, alterations of miR-levels have been implicated in the de-regulation of critical players in major cellular pathways, modifying the differentiation, proliferation and survival of tumor cells. For instance, miR-7 and miR-221/222 were shown to be involved in the activation of the Akt and epidermal growth factor receptor (EGFR) signaling pathways [23,24,25,26,27] while miR-34a is a key downstream regulator of p53 [23,24,25,26,27].

miRs -10b and -21 have been consistently found highly over-expressed in astrocytic tumors [12,13,22] as well. Putatively, miRs-10b and -21 work as 'oncomirs' and decrease apoptosis in malignant cells. Down-regulated miRs-124 and -137 are involved in the differentiation of glioma stem cells [13], occurring with miR-128 that targets Bmi1 and E2F3a, promoting a pro-survival, undifferentiated self-renewing state [28,29].

Regulation of both metabolic
pathways in cancer cells and increase in their migration capabilities are also relevant properties that have been found to be controlled by miR-451 in glioma stem cell lines. Furthermore, miR-326 has been recently shown to regulate Notch-1 and -2 in such cells [30,31].

Generally, miRs predominantly play an important role in signal transduction and regulation processes in various tumor types. To provide a better understanding of complex regulatory mechanisms that involve miRs, we computationally determined miRs that are significantly associated to expression changes of genes involved in signaling pathways of human gliomas. We combined data of physical interactions between miRs and the 3’UTR of mRNAs and gene expression profiles of 11 non-tumor control and 27 glioblastoma (GBM) samples. To assess the quality of our predictions we performed a high-resolution genomic analysis of the miR expression in the underlying tumor and control cases. Comparing in-silico predictions to our large-scale measurements we found that the combination of physical interactions of miRs and mRNAs and the expression change of the given genes indeed allowed an assessment of the influence of individual miR candidates on gene expression changes in the absence of any epigenetic effects and genomic alterations.

As an experimental proof of concept, we predicted associated miRs that influence the expression of WEE1, a tyrosine kinase that phosphorylates CDK1 [32,33]. To validate our computational analysis and better understand the way WEE is regulated by miRs, we investigated the involvement of miR-128 and miR-27 and focused on those mRNAs that are potentially play a role in the expression of given genes.

## Results

### miR and mRNA expression in tumor samples

To investigate whether the expression levels of miRs have changed in glioblastomas we utilized 27 samples of glioblastoma (GBM) patients that belong to the GA subgroup [34] and 11 non-tumor control brain samples. 24 miRs had a log2-fold-change FC of \(<-1\), while we found 251 miRs with FC\(>1\) (Table S1). As for the expression of mRNAs, we observed 1,495 over-expressed and 3,922 under-expressed mRNAs with \(FC>1\) (Table S2).

### Computational prediction of associations between binding miR and expression of mRNAs

Utilizing computational predictions from sources as algorithmically diverse as TargetScan [35], PicTar [36] and miRanda [37,38], we assembled 48,999 interactions between 386 miRNAs and 6,725 mRNAs. In particular, we only selected interactions between miRs and mRNAs if they were at least predicted by two methods (Fig. 1A).

Our objective was to determine small sets of physically interacting miRs that are significantly associated to the expression of the underlying mRNA. As such, a significant association might indicate a significant role in the regulation of the underlying gene’s expression. To identify groups of genes that were characterized by significant expression changes we assembled 184 annotated signaling pathways from the Pathway Interaction Database (PID) [39].

We calculated the mean fold change of all miRs that physically can interact with any given gene in the signaling pathways. Specifically, we found a weak inverse correlation (Pearson’s \(r = -0.06, P<0.05\), suggesting that putatively only a fraction of miR-mRNA interactions may play a role in the expression of the given genes. Identifying combinations of miRs that putatively are associated to altered mRNAs expression levels we applied the random forest algorithm [40], an ensemble-learning-algorithm that constructs regression trees with bootstrap data samples and random choices of predicting variables. We characterized each mRNA by its mean fold change and a x-dimensional binary vector (Fig. 1B).

Referring to a miR, each of the x vector units is 1 if the corresponding miR can physically interact with the mRNA, and 0 otherwise. Applying the random forest algorithm, we performed a regression of the mean fold change of 1,277 mRNAs as a function of 353 interacting miRs. As a measure of a miRs impact on the regression process we assessed each miRs local importance for the fold change of each mRNA by randomizing mean fold change levels and interactions between miRNAs and mRNAs 100 times.

Determining the significance of a miRs local importance with a Z-test (Fig. 1C) and correcting for multiple testing [41] we found 626 significant associations \((P<0.05)\), involving 128 miRs that tuned the expression of 246 mRNAs (Table S3). Comparing our results, we utilized the HMDD database [42], that manually collects and curates associations of miRs and diseases from literature. In particular, we found a significant overlap of 8 miRs \((P<10^{-14}\), hypergeometric test) out of 32 miRs that are associated with GBMs in HMDD.

In Fig. 1D, we show a subset of miRs that appeared most frequently in such associations (the full list is available in Table S4). Specifically, we counted the number of significant associations a given miR is involved in, allowing us to observe that such miRs are largely over-expressed in GBMs.

In Fig. 2A, a sigmoidal curve described the fold change of all genes present in signaling pathways that were not significantly associated with miRs. Focusing on those miRNAs that are associated to miRs, we observed that the corresponding distribution strongly shifted toward lower and higher fold changes. In particular, the cumulative frequency distribution formed a plateau ranging from log2-fold-changes \(-1\) to \(+1\), suggesting that associated miRs significantly changed the expression of the corresponding genes in GBMs. In a subsequent step, we calculated the mean fold change of all miRs that were significantly associated with the underlying mRNAs. In Fig. 2B, we found that the expression fold change of mRNAs is significantly correlated with the expression of its associated miRs (Pearson’s \(r = -0.30, P<10^{-6}\), a remarkable 5-fold increase, demonstrating our ability to identify miRs that potentially play a role in the expression of given genes.

To better understand where significantly associated miRs actually bind mRNAs we calculated the cumulative frequency of significantly associated miRs as a function of their distance to the start of the 3’UTR. Considering sequence alignments of a given 3’UTR and a miR, we defined the position of the first aligned nucleotide of the 3’-UTR as the distance to the start of the untranslated region. In the absence of splice-version specific sequence data of 3’UTRs in GBMs we accounted for all alignments of miRs and non-translated regions of a given mRNA. In comparison to non-associated miRs we observed an enrichment of associated miRs that bind near the start of the 3’UTR. Considering sequence alignments of a given 3’UTR and a miR, we defined the position of the first aligned nucleotide of the 3’-UTR as the distance to the start of the untranslated region. In the absence of splice-version specific sequence data of 3’UTRs in GBMs we accounted for all alignments of miRs and non-translated regions of a given mRNA. In comparison to non-associated miRs we observed an enrichment of associated miRs that bind near the start of the 3’UTR.

In addition, we observed a similar result for under-expressed miRs, \((FC<-1)\). However, the distribution is shifted several 100 bp away from the start of the 3’UTR. Assuming that their efficacy was mediated by occupying binding sites nearby the stop codon, miRs might spatially block ribosomes from finishing...
translation and therefore avoid degradation of the underlying mRNA [43]. Our results suggest that over-expressed miRs, corresponding to under-expressed genes, potentially utilize these proximal positions on the 3′-UTR, thereby allowing under-representation of those mRNAs.

Utilizing all 626 significant associations between 128 miRs and 246 genes, we constructed a bipartite matrix between miRs and signaling pathways if a given pathway shared at least one gene with the associated targets of a miR. Narrowing our focus, we considered signaling pathways that are over(under)-expressed in GBMs. In particular, we applied GSEA [44], allowing us to find 21 enriched signaling pathways (P < 0.05) that are largely overrepresented in GBMs. Among such enriched pathways we found several prominent signaling pathways that have been implicated in tumor biology. We established a link if pathways shared at least one gene with associated targets of a miR. While a majority of 87 miRs thus obtained were over-expressed (expression fold change FC > 1), we found a small minority of miRs that were under-expressed in GBMs (FC < -1). Hierarchically clustering the bipartite matrix, we observed two large clusters of pathways as well as 2 large clusters of miRs. Specifically, we highlighted a dense cluster that pooled most of the under-expressed miRs and prominent pathways such as the p53 downstream and myc activation pathway (Fig. 3A). Mapping all associations of miRs and genes that appeared in the corresponding pathways, we found significant interactions of prominent miRs (Fig. 3B). Specifically, miR-124a was previously reported as a regulator of CDK6 in GBM [13] and medulloblastoma [45]. Furthermore, we predicted that both miR-29b and -29c were strongly associated with extracellular matrix proteins such as LAMC1 and COL1A2. Previous reports confirmed that these miRs regulate the expression of extracellular matrix proteins in nasopharyngeal carcinomas [46], contributing to positive regulation of osteoblast differentiation [47] and playing an important role in cardiac fibrosis [48].

WEE1 is over-expressed in GBM and TIC

As an experimental proof of concept, we predicted significantly associated miR interactions that influence the expression of WEE1, a tyrosine kinase that phosphorylates CDK1 at the tyrosine-15 (CDK1-Y15) position [32]. Previously published data suggested that over-expression of WEE1 is critical for the viability of some cancer types, and cell lines displaying higher levels of WEE1 expression are sensitive to WEE1 inhibition [33]. Utilizing expression data from primary glial tumors, we confirmed that WEE1 is strongly over-expressed in gliomas compared to non-tumor control cases (Fig. 4A). To validate our computational
analysis and better understand how WEE1 is regulated by miRs, we first assayed the absolute levels of WEE1 mRNA expression in 5 GA subgroup GBMs, 5 non-tumor brain samples, four GBM-derived tumor initiating/stem cell lines (TICs) and two normal cell lines (human fibroblasts and HUVEC), using quantitative reverse transcription polymerase assays (RT-qPCR). As seen in Fig. 4B, the RT-qPCR data demonstrated that WEE1 mRNA is over-expressed in both GBMs and TIC lines compared to non-tumor controls. Since the WEE1 genomic locus is not placed in an area of chromosomal number alteration (CNA) in any of our glioma specimens we ruled out that the levels of WEE1 gene expression are simply explained by alterations of gene copy numbers (data not shown).

We found 36 miRs that were predicted to interact with WEE1 mRNA based purely on their seed sequence in the WEE1 3' 3'UTR. However, our analyses demonstrated just 10 significantly associated miRs/WEE1 mRNA interactions in our GBM samples (Fig. 5A). Among our set of associated miRs, we discovered an accumulation of binding-site sequences within the first 500 bp of the WEE1 3'UTR region (Fig. 5B); miR-128 and miR-27ab were among these miRs that bind nearest to the start of the 3'UTR, an interesting observation given that miR-128 has been shown to target Bmi1 and E2F3a, thereby promoting an undifferentiated self renewal state in glioma cells [28,29]. Utilizing RT-qPCR TaqMan assays we validated that miR-128 and miR-27b were highly under-expressed in our GBM samples and 308 TIC cell line whereas miR-27a was over-expressed (Fig. 5C).

**WEEl is a direct target of miR-128 and miR-27 and affects cell cycle progression**

We transiently transfected the naturally under-expressed miR-128 and miR-27a/b along with a WEE1 3' UTR luciferase reporter construct into TIC308 cells to experimentally verify their binding to the WEE1 3' UTR (Fig. 5D). Expression of all three miRs significantly downregulated the luciferase activity (Fig. 6A). In contrast, mutations of the miR-128 and 27a/b binding nucleotides 14 and 236 of the 3'UTR relieved the miR-mediated repression of luciferase activity. This effect even held when these miRs were transiently over-expressed, indicating the specificity of the miR impact.

On the other hand, mutations of the binding site around nucleotide 465 have the lowest relieving effect, confirming that the highly over-expressed miR-93 that binds this downstream site has a lower effect on expression. Over-expression of miR-128 and miR-27b directly reduced WEE1 mRNA and protein levels in synchronized cells of TIC208 (Fig. 6BC). Consistent with a significant biological effect of miR-128 and miR-27b knockdown of WEE1, we observed a corresponding increase in CDK1-Y15 phosphorylation similar to the effects, following down-regulation of WEE1 by a specific siRNA treatment (Fig. 6C).

**Discussion**

Although a growing appreciation of the importance of miRs in cancer biology is emerging, much remains to be learned about their roles in specific regulatory programs. Utilizing GBM...
samples, we showed that the mere knowledge of physical interactions of miRs and the expression change of the underlying interacting mRNAs allows a prediction of associated miRs that drive the expression of their targets. Utilizing random forests, an ensemble machine learning approach, we determined combinations of significantly associated miRs that contributed to the expression fold change of the underlying targets. At this point of the analysis, we deliberately refrained from using large expression data sets of miRs and focused entirely on physical interactions between un-translated regions of mRNAs and miRs. Subsequently, we utilized large-scale expression data of miRs to assess the quality of our results, allowing us to observe that under- and over-expressed miRs are predominantly interacting with over- and under-expressed genes. Furthermore, we observed that significantly associated interactions were characterized by an inverse relationship between expression levels of a specific miR and its target mRNA. This result is consistent with existing models, suggesting that miRNAs are usually under-expressed following binding of certain miRs. Accordingly, our associated interactions allowed us to find that such over-(under)expressed miRs predominantly interacted through binding sites that were placed near the start of the 3'UTR of the target mRNA.

Figure 3. Significantly associated miRs in signaling pathways. (A) Utilizing all significant associations between 128 miRs and 246 genes, we constructed a bipartite matrix between miRs and signaling pathways that are over(under)-expressed in human GBMs. We established a link if the sets of genes in a pathway overlapped with mRNAs that are associated with a certain miR. Specifically, we found 21 pathways that are largely over-expressed in GBMs and 87 miRs. While a majority of miRs were over-expressed (expression fold change FC > 1), we found a small minority of miRs that was under-expressed in GBMs (FC < -1). In particular, we highlighted a small cluster that pooled most of the under-expressed miRs and prominent pathways such as the p53 downstream and myc activation pathway (box). In (B) we mapped all interactions between associated miRs and genes that appeared in the corresponding pathways. Confirming our predictions, we found significant interactions between genes of the extracellular matrix and miR-29bc and -124a that have been previously implicated in glioblastomas and other cancer types (shaded area). Furthermore, miR-124a was previously reported as a regulator of CDK6 in GBMs.

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Figure 4. Expression patterns of WEE1. (A) WEE1 is over-expressed in GBMs and Oligodendrogliomas. (B) Expression of WEE1 was validated by quantitative reverse transcription polymerase reaction (RT-qPCR) in five representative GBM tumor samples that belong to the GA subtype, five non-tumor samples, four tumor initiating/stem cell lines (TICs) and two unperturbed cell lines (human fibroblasts and HUVEC). We observed that TIC 308 showed an average WEE1 fold change that was similar to the corresponding averages in the GBM samples.

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Figure 5. Significantly associated miRs of WEE1. In (A) we show all significantly associated interactions between the mRNA of WEE1 and miRs where miR-128ab were under-expressed (fold change FC<−1) and miR-27a/93 were over-expressed (FC>1) in our GBM samples. (B). Corresponding miR binding sites in the WEE1 3' UTR are located in three main binding areas within the first 500bp from the 3' UTR start. Specifically, miR-128/27 have two binding sequences around nucleotides 15 and 236 while miR-302abcd/372/93 potentially recognize a common binding site around nucleotide 465. (C) Using RTqPCR TaqMan assays, we detected that miR-128/27b were under-expressed and miR-27b was strongly over-expressed in tumor samples. We found a similar miR expression profile in the tumor initiating/stem cell line, TIC308, where miR-128 and miR-27b kept their low expression levels. (D) Transfection of miR-specific expression vectors of TIC308 cells allowed the recovery of miR-128/27b levels as measured by RTqPCR.

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In an attempt to validate one such computationally derived association, we utilized WEE1 as a representative example and found 10 miR candidates out of a pool of 36 miRs. Generating a small tractable set of testable hypotheses our computational analysis constrained the pool of regulatory candidates by more than 60%. Experimentally, we demonstrated that WEE1 is predominantly influenced by miR-128/27b in a sequence-specific manner following binding to sites nearby the start of the WEE1 3’UTR. Consistent with our results, miR-128 has been previously described as down-regulated in gliomas [12], playing a potential role in tumor biology by targeting the transcription factors E2F3a [28] as well as Bmi-1 [29]. Similarly, a critical effect on cell division mediated by WEE1 was observed in hESC [49] where miR-195 expression in Dicer-knockdown cells rescued cell cycle kinetics by directly targeting WEE1 3’UTR. Finally, the necessity for WEE1 in cell division has recently been described in primary fibroblasts [50], and its specific importance in human glioblastoma has been demonstrated thereby independently validating our computational findings [51].

Additionally, miR-124 has also been extensively implicated in glioma pathogenesis [13,23] although the association with extracellular matrix proteins expression presented in our study is completely novel, necessitating further exploration of their biological relevance.

In summary, we have shown that the mere knowledge of physical interactions between specific miRNAs and miRs can be used to predict putative causal regulatory interactions in human tumor specimens. However, we have to stress that we assessed the influence of individual miR candidates on expression changes of genes assuming the absence of any epigenetic effects and genomic alterations. In this light, we have found evidence suggesting that miRs that interacted more proximally in the 3’UTR, near the translation stop site, may have greater regulatory effects on mRNA levels than those that bind more distally. Finally, our analysis allowed us to predict and subsequently validate an association between miR-128/27b and the protein kinase WEE1, a protein of central importance in cellular proliferation and survival, demonstrating the potential power of this computational approach.

Materials and Methods

Tumor and Tumor Initiating Cell (TIC) samples

After written consent tumor samples were obtained from patients undergoing surgery at the National Institutes of Health (NIH) in accordance with the surgical procedures of the National Cancer Institute’s Institutional Review Board that specifically approved this study. We used 27 samples that were provided as snap frozen sections. Utilizing a computational classification scheme [34], we confirmed that these samples were members of the GA subgroup of glioblastomas. As a control, 11 non-tumor samples (temporal lobe resection of epileptic patients) were analyzed concurrently to provide a baseline for the miR/mRNA expression values. Procedures regarding the derivation of TICs were described previously [52].

Total RNA extraction

Following the manufacturer’s instructions, 100 mg of tissue were used to extract total RNA using the Trizol Plus isolation protocol (Invitrogen, Carlsbad, CA). While RNA quantity was determined using the NanoDrop ND-1000 spectrophotometer the integrity of the RNA was verified with the Bioanalyzer System (Agilent Technologies, Palo Alto, CA) using the RNA Pico Chips with a RIN>7.

miR profiling and statistical analysis

miR expression in 27 glioblastoma tumors and 11 non-tumor brain cells was profiled using the NCode™ Multi-Species miRNA Microarray v2 (Invitrogen Corp.) which contains ~1,100 unique
probes printed in triplicates for detecting validated miRs in *H. sapiens*, *M. musculus*, *R. norvegicus*, *C. elegans*, *D. melanogaster* and Zebrafish. 553 probes were designed to detect human miRs (ver. Sanger 9.0).

Extracted RNA was labeled with Alexa Fluoro® Dye using NCode™ miR Labeling System and hybridized to species-specific with normalized probe level data, and negative average differences dChip [53]. Using the average difference model to compute control were normalized at the PM and MM probe level using BioDN and CreX) were always present with present call rates of 3. As a final requirement, Affymetrix spike controls (BioC, PM) were set to 0 after log-transforming expression values. (i.e. data not in the bootstrap sample) is permuted. Specifically, we utilized the local importance that reflects the influence of a miR on the fold change of the underlying mRNA. Assuming that only a subset of miRs significantly contributed to the fit, we assessed each miRs local importance by a permutation analysis. Randomizing binding miRs and expression fold changes of miRNAs 100 times, we determined the average importance \( \bar{I} \), and standard deviation \( \sigma_I \) of each miR i. Subsequently calculating a miRs score by \( Z_i = \frac{\bar{I}_i - \bar{I}}{\sigma_I} \), we determined it's statistical significance by a one-tailed p-value from a standard normal cumulative distribution function. We corrected P-values using [41] and collected all interactions between miRNAs and miRNAs with P<0.05.

**Enrichment**

To obtain an estimate if over(under)-expressed miRs (fold change \(|FC|>1\)) predominantly bind nearby the start of the 3'-UTR, we calculated the corresponding fraction of such miRs that bind within a distance \( d \) as \( f_{d} = \left| m_d \right|/M_d \), where \( M_d \) is the number of all miRs within distance \( d \) from the start of the 3'-UTR in the underlying sample. As a null hypothesis, we randomly picked sets of over(under)-expressed genes represented by the fraction \( f_{d} = \left| m_d \right|/M_d \). We defined the enrichment of over(-under)-expressed genes that bind within a distance \( d \) as \( ER_d = f_d/f_{d_0} \) and found an enrichment if \( ER_d \gg 1 \) and vice versa.

**Transfections**

TIC308 cells were transfected using the mouse NSC Nucleofector kit (Amaxa, Cologne, Germany) program A-33. miR expression vectors for the induction of miR-128/27a/27b (pEp-hsa-mir-vectors, Cell Biolabs, San Diego, CA) or miR-null negative controls were used at 2 μg per transfection. For small interfering RNA (siRNA)-mediated target knockdown of WEE1, four siRNAs in the ON-TARGETplus SMARTpool L-005050-00-0005 were used (Thermo Fischer Scientific, Lafayette, CO) with a final concentration of 3 pmol of each duplex. Transfection efficiency was measured using GFP max vector (Amaxa, Cologne, Germany) in every condition.

**Luciferase assay**

miR expression vectors for the induction of miR-128/27a/27b (Cell Biolabs, San Diego, CA) were co-transfected with WEE1 3' UTR/ Empty UTR Luciferase reporter vector (SwitchGear Genomics, Merlo Park, CA) using mouse NSC Nucleofector kit (Amaxa, Cologne, Germany) in TIC308. Also, seed regions in the WEE1 3'UTR were mutated and cloned into WEE1 3' UTR Luciferase reporter vector (SwitchGear Genomics, Merlo Park, CA) using the QuikChange II XL mutagenesis kit (Stratagene). Specifically, we generated mutants for binding sites close to nucleotide 15.
Expression in 27 GBM and 11 non-tumor samples.

Table S1

Supporting Information

mRNA/miR and protein levels

Expression of WE1 and GAPDH genes as well as miRs of interest were analyzed using specific TaqMan® Assays in the 7900HT Real Time PCR system (Applied Biosystems, Foster City, CA) following standard protocol; Synchronized cells were collected for protein lysates 72 hours post-transfection. Protein levels were measured with antibodies against WE1 (sc-5285), CDK1-total (Cell Signaling, Beverly, MA, #9112) and Phospho-CDK1 (tyr15) (Cell Signaling, Beverly, MA #4569). An anti-tubulin antibody was used to test equal protein loading.

Supporting Information

Table S1 List of 462 miRs and their log2-fold change of expression in 27 GBM and 11 non-tumor samples.

Table S2 List of 20,290 genes and their log2-fold change of expression in 27 GBM and 11 non-tumor samples.

Table S3 List of 626 predicted associations between mRNA and miR (P<0.05). We indicated if the underlying miRs and mRNAs are over (\(|\Delta|, \text{log}_2 \text{fold change}>1\)) or under (\(|\Delta|, \text{log}_2 \text{fold change}<−1\)) expressed in GBMs.

Table S4 List of 128 miRNAs that significantly interact with a gene (P<0.05). In particular, we counted the number of significant interactions (N) and indicate if the underlying miR is over (\(|\Delta|, \text{log}_2 \text{fold change}>1\)) or under (\(|\Delta|, \text{log}_2 \text{fold change}<−1\)) expressed or largely unchanged (\(\sim\)) in GBMs.

Author Contributions

Conceived and designed the experiments: SW DA JCZ HAF. Performed the experiments: DA JW SA AZ DM RA. Analyzed the data: SW AL. Contributed reagents/materials/analysis tools: YK. Wrote the paper: SW DA JCZ HAF.

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