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The role of miRNA-10b and miRNA-21 in radioresistance and temozolomide resistance of high-grade glioma patients: a systematic review

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OBJECTIVE Despite optimal therapy, high-grade glioma (HGG) still has a very unfavorable prognosis. Gross-total resection is not often possible, and even when it is, many patients still succumb to the disease due to resistance to temozolomide (TMZ) and radiotherapy. As the mechanism behind such resistance is multifactorial, microribonucleic acids (miRNAs) with their wide-ranging epigenetic effects on cancer have emerged as potential research targets. Among others, miRNA-10b and miRNA-21 are the most widely studied miRNAs in HGG. In this review, the authors aimed to investigate the role and predictive value of miRNA-10b and miRNA-21 in TMZ and radiotherapy resistance in HGG patients.

METHODS The PubMed, Europe PMC, and Web of Science databases were searched to find in vitro, in vivo, or clinical studies assessing the relationship between miRNA-10b and miRNA-21 expression with TMZ resistance, radiotherapy resistance, and survival. Review articles, editorials, correspondence, case reports, case series, non–English-language articles, and studies that only analyzed datasets were excluded. Results were then synthesized according to those three outcomes. This review has been registered in PROSPERO (International Prospective Register of Systematic Reviews) under registration no. CRD1004470.

RESULTS There were 34 studies included in this review, with 25 studies evaluating miRNA-21, 6 studies evaluating miRNA-10b, and 3 studies evaluating both miRNA-10b and miRNA-21. The results of the in vitro and in vivo studies were unequivocal in demonstrating that miRNA-10b and miRNA-21 expression correlated with resistance. The two miRNAs increased tumor stemness, viability, invasiveness, and resistance to apoptosis. However, not all the clinical studies demonstrated a significant relationship between both miRNAs and survival. This was possibly caused by differences in resection status and sampling method.

CONCLUSIONS MiRNA-10b and miRNA-21 expression correlated with TMZ and radiotherapy resistance in vivo and in vitro. With properly designed human studies, these results could translate to tremendous benefits in the clinical field. Future clinical studies should be designed to better account for resection status and sampling method.

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KEYWORDS high-grade glioma; miRNA-10b; miRNA-21; temozolomide; radiotherapy

Glioma is the most common CNS tumor, accounting for approximately one-third of all CNS tumors and 80% of primary malignant CNS tumors.[1] High-grade glioma (HGG), classified as WHO grades 3 and 4 glioma, accounts for 61.5% of all glioma cases.[2] The current standard treatment consists of maximal safe resection followed by radiotherapy and chemotherapy, with temozolomide (TMZ) as the most common agent used. With recent advances in surgical technique, maximal safe resection is more readily achievable now. Although the extent of resection has a significant impact on prognosis, patients' responses to chemotherapy and radiotherapy also play an important role. Sensitivity to TMZ is directly related to patients' good outcome; in contrast, TMZ resistance re-

ABBREVIATIONS GBM = glioblastoma; GSC = glioma stem cell; HGG = high-grade glioma; HIF-1 = hypoxia-inducible factor 1; miRNA = microribonucleic acid; OS = overall survival; QUIN Tool = Quality Assessment Tool for In Vitro Studies; ROBINS-E = Risk of Bias in Nonrandomized Studies—of Exposures; RT-PCR = reverse transcription polymerase chain reaction; SYRCLE's RoB = SYRCLE's risk of bias; TMZ = temozolomide; TTP = time to progression.

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sults in a higher recurrence rate. Unfortunately, the rate of resistance is alarmingly high, with around 40%–60% of HGG patients exhibiting resistance.[3,4]

Because of the high resistance rate, there have been tremendous efforts to develop new therapy for HGG. However, none have been successful in reducing the drug resistance rate. One possible explanation for failure is the presence of a compensatory mechanism in HGG so that a therapy targeting one biological aspect of HGG can be overcome by other mechanisms.[5] Therefore, a targeted therapy affecting multiple aspects of HGG is needed.

One of the promising fields of research concerns the role of microribonucleic acid (miRNA) in HGG. MiRNAs are small noncoding RNAs that have wide-ranging epigenetic impacts in the cell cycle and division, differentiation, growth, apoptosis, proliferation, and migration. They exert these effects by modulating the expression of various genes through the 3′-untranslated regions of mRNA targets. The binding of miRNA inhibits the protein translation of their respective mRNA target.[6] Numerous amounts of miRNAs have been identified, with some of them promoting tumor growth, while others suppress it.[7]

In HGG, miRNA-10b and miRNA-21 are two of the most widely studied and established oncogenic miRNAs with profound effects on the tumor pathophysiology.[8] MiRNA-21 is overexpressed in HGG and related to treatment resistance and worse prognosis. MiRNA-10b is considered a unique oncogenic miRNA that is expressed in all subtypes of glioblastoma (GBM) but not in normal neuroglia.[9] Its inhibition also significantly reduces glioma cell proliferation.

Several biological mechanisms have been proposed to explain the role of both miRNAs in HGG, including, but not limited to, modulation of DNA repair, tumor stemness, angiogenesis, metabolic reprogramming, apoptosis, and cell cycle regulation.[10,11] All these are important mechanisms in the development of TMZ and radiotherapy resistance.

TMZ and radiotherapy resistance are still some of the most pressing issues in HGG management. Although there have been numerous studies focusing on the role of miRNA-10b and miRNA-21 in HGG cell metabolism, there has not yet been a specific review regarding how both miRNAs influence HGG cell resistance toward therapy. Because both miRNAs are integral in the pathophysiology of HGG, we hypothesized that they are important contributors to therapy resistance. Therefore, this review aimed to investigate the role of miRNA-10b and miRNA-21 in TMZ and radiotherapy resistance in HGG patients.

Methods

Database and Literature Search

We conducted a literature search of the PubMed, Europe PMC, and Web of Science databases, covering the period from database inception to January 15, 2025. The search was performed using the following keywords: (micro RNA 21 or micro RNA 10b or miRNA-21 or miRNA-10b) and (high-grade glioma or astrocytoma grade 4 or astrocytoma grade 3 or high-grade astrocytoma or oligodendroglioma grade 3 or high-grade oligodendroglioma or glioblastoma or glioblastoma multiforme or GBM) and (prognosis or survival or progression or resistance) and (TMZ or radiotherapy). The references of relevant studies were also reviewed to identify suitable research. Duplicate studies were then identified and removed before study selection. This review has been registered in PROSPERO (International Prospective Register of Systematic Reviews) under registration no. CRD1004470.

Study Selection

The criteria for study inclusion were as follows: 1) in vitro, in vivo, and clinical studies; 2) using human HGG cell lines, animal HGG models, or adult HGG patients; and 3) showing an association between miRNA-10b and/or miRNA-21 and TMZ and/or radiotherapy resistance and/or prognosis. We used grade 3 or 4 gliomas from the 5th edition of the World Health Organization Classification of Tumors of the Central Nervous System to describe HGG. These included oligodendroglioma, astrocytoma, and GBM. Review articles, editorials, correspondence, case reports, case series, non–English-language articles, and studies that only analyzed datasets were excluded. Two authors performed the initial title and abstract screening independently before reviewing their full texts. The screening results were then compared. Any discrepancies were resolved through discussion with the other two authors.

Data Extraction

Two authors independently performed data extraction from the included studies using a standardized form that included authors, publication year, study design, study sample, intervention or exposure group, control group, and outcomes measured. Extracted data were then compared, and any discrepancies were also resolved through discussion. Data synthesis was performed to classify the results according to the type of miRNA studied and the outcomes measured. We presented the result of each miRNA according to TMZ resistance, radiotherapy resistance, and prognosis. Risk of bias was analyzed using the Quality Assessment Tool for In Vitro Studies (QUIN Tool)[12] for in vitro studies, SYRCLE's risk of bias (SYRCLE's RoB) tool[13] for in vivo studies, and the Risk of Bias in Nonrandomized Studies—of Exposures (ROBINS-E)[14] tool for clinical studies.

Results

The literature search of the PubMed, Europe PMC, and Web of Science databases yielded 174 records; 33 duplicate records were removed, and 141 studies' titles and abstracts were then screened. The initial screening resulted in 38 studies eligible for a full-text evaluation. Two studies were excluded because they did not provide a specific report on miRNA-10b or miRNA-21, 1 study was excluded because it used datasets to analyze survival, and 1 study was excluded because it did not separate low- and high-grade glioma, leaving 34 studies for the final review (Fig. 1).

Study Characteristics

Tables 1–3 present the characteristics of the included studies. There were 14 in vitro studies, 11 clinical stud-

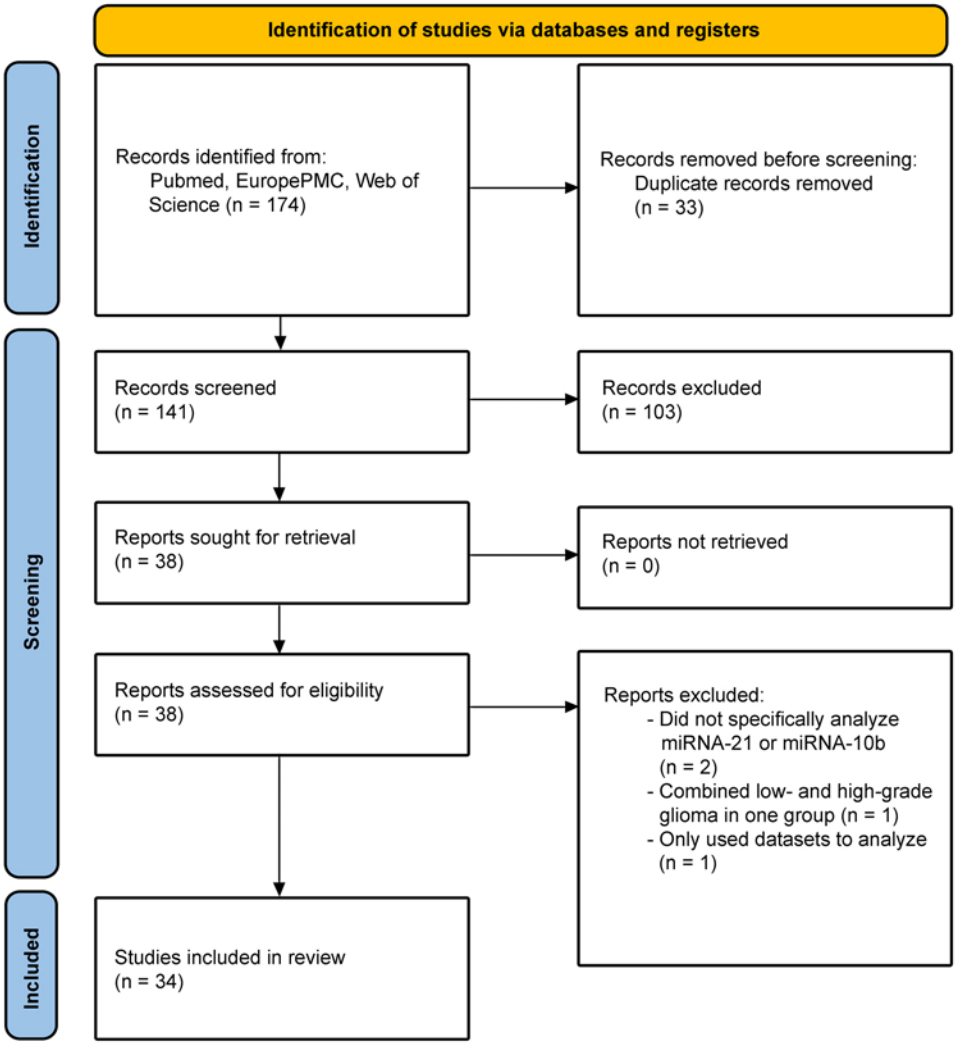

FIG. 1. Study flow diagram. Data added to the PRISMA template (from Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71) under the terms of the Creative Commons Attribution (CC BY 4.0) License (https://creativecommons.org/licenses/by/4.0/).

ies, 3 in vitro combined with clinical studies, and 6 in vitro combined with in vivo studies. For in vitro studies, all used either an HGG cell line (U87MG, U251, U343, U373, LN18, LN229, LN428, D54MG, T98G, HCN2, A172, SF268, and M059) or tumor sample from HGG patients. In vivo studies all used nude mice with either intracranial or extracranial HGG implantation. Twelve clinical studies included GBM patients, 1 included grade 3 and 4 astrocytoma patients, and 1 included grade 3 glioma and GBM patients. Twenty-five studies evaluated miRNA-21,[15–39] 6 studies evaluated miRNA-10b,[11,40–44] and 3 studies analyzed both miRNA-10b and miRNA-21.[10,45,46]

TMZ Resistance

There were 15 studies evaluating miRNA-10b and miRNA-21 expression and their effect on TMZ resistance. Four studies evaluated miRNA-10b,[40–43] 9 studies evaluated miRNA-21,[16,24,25,27,28,30,4,37,38] and 2 studies evaluated both miRNAs.[10,46]

From the in vitro studies, we found that response to TMZ was improved after miRNA-10b inhibition, evidenced by reduced cell viability.[10,43] MiRNA-10b also shifted tumor metabolism toward glycolysis, thereby increasing the resistance of HGGs to TMZ.[42] These effects of miRNA-10b were achieved through modulation of the cell cycles and activities of *PTEN*, PI3K/Akt, *PDCD4*, *MDM2*, p53, BIM, p21, p27, and *HOXD10*.[10,41,42,46]

Similar to miRNA-10b, a decreased expression of miRNA-21 also improved the response to TMZ in vitro. Inhibition of miRNA-21 increased the apoptosis rate and reduced cell viability, with 1 study reporting a difference in response as high as 53%.[24,25,27–30,34,37,46] MiRNA-21 was found to alter the ratio of Bax/Bcl2 and caspase-3, proteins closely linked with apoptosis.[25,27,29,30,38] One study also found that increased miRNA-21 expression was identified in glioma stem cells (GSCs). These GSCs were then found to be more resistant to TMZ compared with normal GBM cells.[39] Other genes and proteins that were reported

TABLE 1. Study characteristics of in vitro studies

Authors & Year	Study Design	Sample	Intervention/ Exposure	Control	Evaluated Outcome	Results
Shi et al., 2010[27]	In vitro	U87MG cell line & human GBM tissue treated w/ 100 μM TMZ	Transfected w/ miRNA-21 to induce overexpr	Negative control	MiRNA-21 expr; cell viability; apoptosis; BAX, BCL-2, & caspase-3 expr	Higher expr of miRNA-21 correlated w/ TMZ resistance
Chaudhry et al., 2010[15]	In vitro	M059J & M059K cell lines irradiated w/ a dose of 3 Gy	M059J cell line	M059K cell line	MiRNA-21 expr	Expr of miRNA-21 higher in M095J cell line; its expr also increased after radiotherapy
Li et al., 2011[31]	In vitro	U251 cell line irradiated w/ doses of 10 & 20 Gy	Transfected w/ miRNA-21 inhibitor	Negative control	MiRNA-21 expr, cell viability, cell cycle, caspase-3/7 & CD25A expr	Radiotherapy induced higher expr of miRNA-21, which then correlated w/ radiotherapy resistance
Zhang et al., 2012[30]	In vitro	U251 cell line treated w/ 100 μM TMZ	Transfected w/ miRNA-21 inhibitor	Negative control	MiRNA-21 expr, cell viability, apoptosis, Bax/Bcl-2 & caspase-3 expr	Inhibition of miRNA-21 correlated w/ decrease in cell viability & increase in apoptosis rate, Bax/Bcl-2 ratio, & caspase-3
Gwak et al., 2012[33]	In vitro	U373, U87, LN18, & LN428 cell lines & human GBM tissue irradiated w/ a dose of 8 Gy	Transfected w/ anti–miRNA-21	Negative control	MiRNA-21 expr, cell viability, cell cycle, autophagy, apoptosis, DNA double-strand break	Radiotherapy induced higher expr of miRNA-21, which then correlated w/ radiotherapy resistance
Qian et al., 2013[28]	In vitro	U87 cell line treated w/ 7.5, 15, 22.5, & 40 μM TMZ	Transfected w/ anti–miRNA-21	Negative control	MiRNA-21 expr, apoptosis, cell invasiveness, metabolic activity, proliferation rate	Inhibition of miRNA-21 correlated w/ decrease in IC50, cell invasiveness, & proliferation rate & increase in apoptosis rate
Wong et al., 2012[34]	In vitro	D54MG cell line	Resistant to TMZ, transfected w/ anti–miRNA-21, treated w/ TMZ	Sensitive to TMZ, not transfected, not treated w/ TMZ	MiRNA-21 expr & apoptosis	Resistant cell line exhibited higher miRNA-21 expr; miRNA-21 inhibition resulted in a higher apoptosis rate after treatment w/ TMZ
Griveau et al., 2013[36]	In vitro	U87MG cell line irradiated w/ a dose of 4 Gy	Transfected w/ LNA miRNAs 21, 221, & 210	Negative control	MiRNA-21, 221, & 210 expr & cell survival	Only cells w/ inhibited miRNA-21 expr exhibited reduced viability after radiotherapy
Berthois et al., 2014[37]	In vitro	U87MG, U373, T98G, LN18, &U138 cell lines & human GBM tissue treated w/ 0–300 μM TMZ	Transfected w/ antisense miRNA-21 & pre–miRNA-200a	Negative control	MiRNA-21 expr, cell viability, proliferation rate, MGMT expr	Inhibition of miRNA-21 expr correlated w/ a better response to TMZ; however, expr of miRNA-21 did not correlate w/ MGMT expr
Ananta et al., 2015[38]	In vitro	U87MG, LN229, & T98G cell lines treated w/ 100–500 μM TMZ	Transfected w/ anti–miRNA-21	Negative control	MiRNA-21 expr, cell viability, cell cycle, proliferation rate, PTEN expr	Inhibition of miRNA-21 correlated w/ improved response to TMZ
Ananta et al., 2016[10]	In vitro	U87MG, LN229, T98G, & HCN2 cell lines treated w/ 0–500 μM TMZ	Transfected w/ antisense miRNAs 10b & 21	Negative control	MiRNA-10b & 21 expr; cell viability; cell cycle; PTEN, PDCD4, & HOXD10 expr	Inhibition of miRNAs 10b & 21 correlated w/ improved response to TMZ
Zhen et al., 2016[11]	In vitro	A172 & LN229 cell lines irradiated w/ doses of 30, 50, & 100 Gy	Transfected w/ anti–miRNA-10b	Transfected w/ miRNA-10b mimic	MiRNA-10b expr; proliferation rate; apoptosis; cell invasiveness; caspase-3/7, BCL2, AKT, & p-AKT expr	MiRNA-10b expr correlated w/ radiotherapy resistance
Rodrigues et al., 2019[39]	In vitro	U343MG cell line	TMZ, radiotherapy, TMZ + radiotherapy	No treatment	miRNA 15, 16, & 21 expr	MiRNA-21 expr pattern changed w/ treatment
Li et al., 2022[43]	In vitro	SF268, A172, & LN229 cell lines in 20 mg/mL TMZ	Transfected w/ anti–miRNA-10b	Negative control	IC50 value; VEGF, MMP9, & EMT expr	MiRNA-10b inhibition correlated w/ improved response to TMZ

Expr = expression; IC50 = half maximal inhibitory concentration; LNA = locked nucleic acid.

TABLE 2. Study characteristics of in vivo studies

Authors & Year	Study Design	Sample	Intervention/ Exposure	Control	Evaluated Outcome	Results
Lan et al., 2015[29]	In vitro & in vivo	In vitro: H4, SNB19, LN229, & U251 cell lines in 200 μM TMZ; in vivo: extracranial LN229 xenograft model nude mice	Transfected w/ antisense miRNA-21	Transfected w/ miRNA-21 mimic	MiRNA-21 expr; apoptosis; Wnt, β-catenin, & TFC4 expr	Inhibition of miRNA-21 resulted in higher apoptosis induced by TMZ
Malhotra et al., 2018[46]	In vitro & in vivo	In vitro: U87MG & LN229 cell lines in 500 μM TMZ; in vivo: extracranial U87MG xenograft model nude mice	Transfected w/ targeted PLGA anti-miRNAs 10b & 21	Transfected w/ nontargeted PLGA anti-miRNAs 10b & 21	MiRNA-10b & 21 expr; cell viability; apoptosis; cell cycle; PTEN, PDCD4, HOXD10, & caspase-3 expr	Targeted PLGA delivery produced higher uptake & lower miRNAs 10b & 21 expr; this resulted in improved response to TMZ; inhibition of miRNAs 10b & 21 significantly reduced tumor size in vivo after TMZ treatment
Chuang et al., 2019[24]	In vitro & in vivo	In vitro: U87MG & LN18 cell lines treated w/ TMZ; in vivo: extracranial LN18 xenograft model nude mice	Transfected w/ miRNA-21 inhibitor	Transfected w/ miRNA-21 mimic	MiRNA-21 expr; TMZ IC50; exosomes; GAM; colony formation; tumor sphere formation; TMZ resistance; SOX2, OCT4, Wnt, STAT3, AKT, & GFAP expr	MiRNA-21 inhibition correlated w/ improved response to TMZ
Sukumar et al., 2019[25]	In vitro & in vivo	In vitro: U87MG cell line in 100–250 μM TMZ; in vivo: intracranial U87MG xenograft model nude mice	Transfected w/ anti-miRNAs 21 & 100	Negative control	MiRNA-21 expr; cell viability; apoptosis; H&E staining; CT scan; GADPH, p21, p53, PDCD4, PTEN, Bax, & caspase-3 expr	MiRNA-21 inhibition correlated w/ improved response to TMZ
Sun et al., 2019[41]	In vitro & in vivo	In vitro: U87MG, U251, & LN229 cell lines; in vivo: intracranial LN229 xenograft model nude mice	Transfected w/ miRNA-10b & 222 inhibitors	Negative control	MiRNA-10b & 222 expr; proliferation rate; cell invasiveness; apoptosis; p53, PTEN, MDM2, BAX, BIM, p21/CDKN1A, p27/KIPI, AKT, PUMA, & luciferase expr; IHC	MiRNA-10b expr correlated w/ tumor growth, invasiveness, & vol
Li et al., 2024[42]	In vitro & in vivo	In vitro: U87 & U251 cell lines in 400 μM TMZ; in vivo: intracranial U87 xenograft model nude mice	GSC + 400 μM TMZ	GW, GSC, GW + TMZ	GFAP, NSE, EV size & distribution, glucose, lactate, ATP, cell proliferation, colony formation, invasion, migration, luciferase, CD44, CD63, calnexin, PFK1, LDHA, FASN, PTEN, PI3K, AKT, β-actin	GSC-EV carried miRNA-10b into GBM cells & induced resistance to TMZ

EV = extracellular vesicle; GAM = glioma-associated macrophage; GSC = glioma stem cells; GW = GW4869 extracellular vesicle inhibitor; IHC = immunohistochemistry; PLGA = polymeric poly(lactic-co-glycolic acid).

TABLE 3. Study characteristics of clinical studies

Authors & Year	Study Design	Sample	Intervention/ Exposure	Control	Evaluated Outcome	Results
Zhi et al., 2010[16]	Prospective cohort	Tumor tissue of 52 grade 3 & 4 astrocytoma resected patients; chemotherapy or radiotherapy given not described	High miRNA-21 expr	Low miRNA-21 expr	OS	High miRNA-21 expr correlated w/ shorter OS in grade 3 & 4 astrocytoma
Lakomy et al., 2011[17]	Retrospective cohort	GBM tissue of 38 resected patients treated w/ Stupp regimen	High expr of various miRNAs	Low expr of various miRNAs	Expr of miRNAs 21, 128a, 181c, 195, 196a, 196b, 221, & 222; PFS; OS; TTP	Higher miRNA-21 expr correlated w/ shorter TTP, but not w/ PFS & OS
Hermansen et al., 2013[35]	Retrospective cohort	Tumor tissue of 154 GBM & 23 grade 3 resected glioma patients; chemotherapy or radiotherapy given not described	High miRNA-21 expr	Low miRNA-21 expr	OS	High miRNA-21 expr correlated w/ shorter OS in grade 3 glioma, but not w/ GBM
Ilhan-Mutlu et al., 2013[18]	Retrospective cohort	GBM tissue of 15 resected patients before & after recurrence treated w/ EORTC/ NCIC therapy	GBM patients	Temporal lobectomy epileptic patients	Various miRNA exprs, PFS, Ki-67 staining	MiRNA-21 expr did not differ after recurrence & did not correlate w/ PFS
Chao et al., 2013[23]	In vitro & prospective cohort	In vitro: A172, T98G, & U87MG cell lines irradiated w/ doses of 0, 2, 4, & 8 Gy; clinical: GBM tissue of 6 resected patients treated w/ radiotherapy	In vitro: transfected w/ antisense miRNA-21; clinical: radioresistant patient	In vitro: negative control; clinical: radiosensitive	MiRNA-21 expr, cell survivability, cell cycle, PDCD4 & hMSH2 expr	Higher miRNA-21 expr correlated w/ resistance to radiotherapy in vitro & clinically
Tezcan et al., 2014[40]	Prospective cohort	Tumor tissue from 20 resected GBM patients; chemotherapy or radiotherapy given not described	GBM patients w/ positive GSC	GBM patients w/ negative GSC, epileptic patients	Various miRNA exprs, MGMT methylation, SMAD2 & BCL2 expr, median survival	MiRNA-10b expr did not differ between GSC-positive & GSC-negative GBM; miRNA-10b expr did not correlate w/ median survival
Matos et al., 2018[19]	Retrospective cohort	GBM tissue of 83 resected patients w/ recurrence treated w/ radiotherapy, TMZ, or combination of both	GBM tissue sample	Human Brain Reference Total RNA	Various miRNA exprs, PFS, OS	MiRNA-21 expr reduced after treatment in patients w/ recurrence but did not correlate w/ treatment choice, PFS, & OS
Sippl et al., 2019[20]	Retrospective cohort	GBM tissue of 104 resected patients treated w/ TMZ & radiotherapy	GBM tissue	Normal brain tissue	MiRNA-21, 24, & 26 expr; methylation; PFS; OS	MiRNA-21 expr correlated w/ MGMT methylation, but not w/ PFS & OS
Olioso et al., 2021[32]	Prospective cohort	Blood samples of 57 resected GBM patients before, during, & after Stupp regimen	Progressed patient	Stable patient	Various miRNA exprs, progression, PFS, OS	Lower miRNA-21 expr after therapy correlated w/ longer PFS & OS, but not progression
Labib et al., 2022[21]	Prospective cohort	Blood samples of 40 resected GBM patients before, during, & after Stupp regimen	GBM patient	Healthy control	MiRNA-21 & 222 expr, PFS, OS, treatment response	Higher miRNA-21 expr correlated w/ shorter OS & PFS, but not w/ treatment response
Stepanović et al., 2022[45]	Prospective cohort	GBM tissue of 43 resected patients treated w/ Stupp regimen	w/ toxicity	w/o toxicity	MiRNA-10b, 21, & 34a expr	Higher expr of miRNAs 10b & 21 correlated w/ increased occurrence of toxicities
Junior et al., 2020[44]	In vitro & prospective cohort	In vitro: U251 cell line; clinical: GBM tissue of 40 resected patients treated w/ TMZ & radiotherapy	High miRNA-10b expr	Low miRNA-10b expr	MiRNA-10b expr, proliferation rate, colony formation, cell cycle, apoptosis, OS	Higher miRNA-10b expr correlated w/ shorter OS
Turra et al., 2022[26]	In vitro & prospective cohort	In vitro: tumor tissue from 10 GBM patients; clinical: 10 resected GBM patients; chemotherapy or radiotherapy given not described	In vitro: irradiated w/ a dose of 14 Gy; clinical: high miRNA-21 expr	In vitro: no radiotherapy; clinical: low miRNA-21 expr	MiRNA-21 expr, cell viability, recurrence, mortality	MiRNA-21 expr increased after radiotherapy but did not correlate w/ recurrence & mortality
Cardia et al., 2023[22]	Retrospective cohort	GBM tissue of 112 resected patients treated w/ Stupp regimen	MiRNA-21 expr values >3	MiRNA-21 expr values 1/3–1 & 1–3	MGMT expr, OS, PFS	Higher expr of miRNA-21 correlated w/ longer OS, but not w/ PFS & MGMT expr

EORTC/NCIC = European Organisation for Research and Treatment of Cancer/National Cancer Institute of Canada; PFS = progression-free survival.

to be altered by the expression of miRNA-21 and contributed to TMZ resistance included *PTEN*, *TP53*, PDCD4, SOX2, STAT3, OCT4, Nestin, Wnt/β-catenin, GFAP, Akt, and VEGF and the cytokine IL-6.[24,25,29]

The findings of the in vitro studies translated to the in vivo studies. In the in vivo studies, miRNA-10b and miRNA-21 inhibition resulted in smaller tumor size after TMZ treatment.[29,41,42,46] One study even found that tumors with miRNA-21 expression exhibited double the tumor size of those without miRNA-21 expression.[29] Regarding survival, mice treated with miRNA-21 inhibitor and TMZ were found to survive longer than those treated with TMZ alone (44 vs 32 days).[25]

Radiotherapy Resistance

Six studies evaluated the relationship between miRNA-10b and miRNA-21 expression and radiotherapy resistance. There were 5 in vitro studies and 1 in vitro combined with clinical study. Similar to TMZ, radiation dose also differed across studies, ranging from 2 to 100 Gy. One study evaluated miRNA-10b,[11] while 5 studies evaluated miRNA-21.[15,23,31,33,36]

HGG cells transfected with miRNA-10b mimics had a reduced apoptosis rate by one-third. MiRNA-10b was found to inhibit caspase-3/7 activity, reduce the Bax/Bcl2 ratio, and double the p-AKT values in those HGG cells.[11] Similarly, miRNA-21 inhibition produced an increase in apoptosis rate and reduced survivability. The differences were large, with 1 study reporting a difference in survivability as high as 80%.[15,23,31,33] Inhibition of miRNA-21 increased the number of DNA double-strand breaks after radiotherapy, suppressed the PI3K/Akt pathway,[33] and stimulated the expression of hMSH2 and PDCD4 proteins.[23] Regarding cell cycle, 2 studies found that inhibition of miRNA-21 increased the proportion of cells in the G2/M phase.[23,33]

Prognosis

Two studies evaluated the effect of miRNA-10b on prognosis. These 2 studies reported conflicting results, with one finding a correlation between higher miRNA-10b expression and overall survival (OS), while the other did not.[40,44] While both studies utilized reverse transcription polymerase chain reaction (RT-PCR) to analyze the value of miRNA-10b expressed in the tumor tissue of GBM patients, there were several differences that could explain their conflicting results. A significant correlation was found in the study by Junior et al.,[44] which had a longer follow-up time (2 years vs 10.8 months). This could have resulted in some deaths being missed in the study by Tezcan et al.[40] Another difference was that all the patients in the study by Junior et al. underwent chemotherapy and radiotherapy after surgery, while Tezcan et al. did not report their patients' therapy regimen. Moreover, they did not stratify their samples based on resection status. This potential difference in patients' baseline characteristics and treatment could have resulted in uncontrolled confounding factors that ultimately affected the final result.

Eleven studies evaluated the effect of miRNA-21 on prognosis. As with the studies analyzing miRNA-10b, not all the studies differentiated patients with subtotal resection and gross-total resection status. Two studies analyzed both glioma grade 3 and 4 patients and found that higher miRNA-21 expression translated to poorer OS in grade 3 and 4 astrocytoma but not GBM.[16,35] Six studies included only GBM samples and failed to find a significant correlation between miRNA-21 expression and OS.[17–20,22,26] However, 1 study found that patients with a time to progression (TTP) less than 6 months had a significantly higher miRNA-21 expression.[17]

All those studies analyzed miRNA-10b and miRNA-21 expression by performing RT-PCR on the resected tumor tissue of their study populations. Therefore, they only captured the pre–chemotherapy and radiotherapy level of miRNA-10b and miRNA-21. Two other studies tried a different approach, in which they used RT-PCR to analyze miRNA-21 expression from the blood serum of GBM patients taken several times over the course of the disease. Interestingly, those 2 studies found that a high miRNA-21 expression value observed after initiation of the Stupp regimen, not the initial value, correlated with a shorter OS.[21,32]

A study by Stepanović et al. with 43 GBM patients showed a link between miRNA-10b and acute toxicity caused by radiotherapy and TMZ. They found that the expression of miRNA-10b measured at the 15th fraction was significantly higher in patients with toxicity (95.47 vs 84.62). Expression of miRNA-21 was also higher in those with toxicity, but it did not reach statistical significance.[45]

Risk of Bias

Risk of bias analysis for in vitro studies was carried out using the QUIN Tool. All the studies were assessed as having a medium risk of bias. Identified potential risks of bias were the lack of randomization, blinding, sample number description, and operator or assessor details. SYRCLE's RoB tool was used to assess the risk of bias in the in vivo studies. All the studies had potential risks of bias. Only 1 study randomized the samples into treatment and control groups. No studies used blinding. For clinical studies, the ROBINS-E tool was used. Only 3 studies were assessed as having a low risk of bias. One study had a high risk of bias, while the rest had some concerns about bias. The major potential source of bias was uncontrolled confounding, as most of the studies did not control the effect of resection status on outcome. The risk of bias analysis can be found in Supplemental Tables 1–3.

Discussion

We performed this review to prove our hypothesis that miRNA-10b and miRNA-21 are significant contributors to TMZ and radiotherapy resistance. We have elaborated on the evidence that both miRNAs are instrumental in the development of such resistance. Regarding TMZ resistance, there is evidence that miRNA-10b and miRNA-21 caused resistance through modulation of DNA repair, tumor stemness, angiogenesis, metabolic reprogramming, apoptosis, and cell cycle regulation (Fig. 2). In terms of DNA damage repair, we learned that miRNA-10b and miRNA-21 modulated the expression and activity of the *hMSH2*, *MDM2*, and *TP53* genes. Deficiency or loss of *hMSH2* function has been found to induce TMZ resistance through impaired

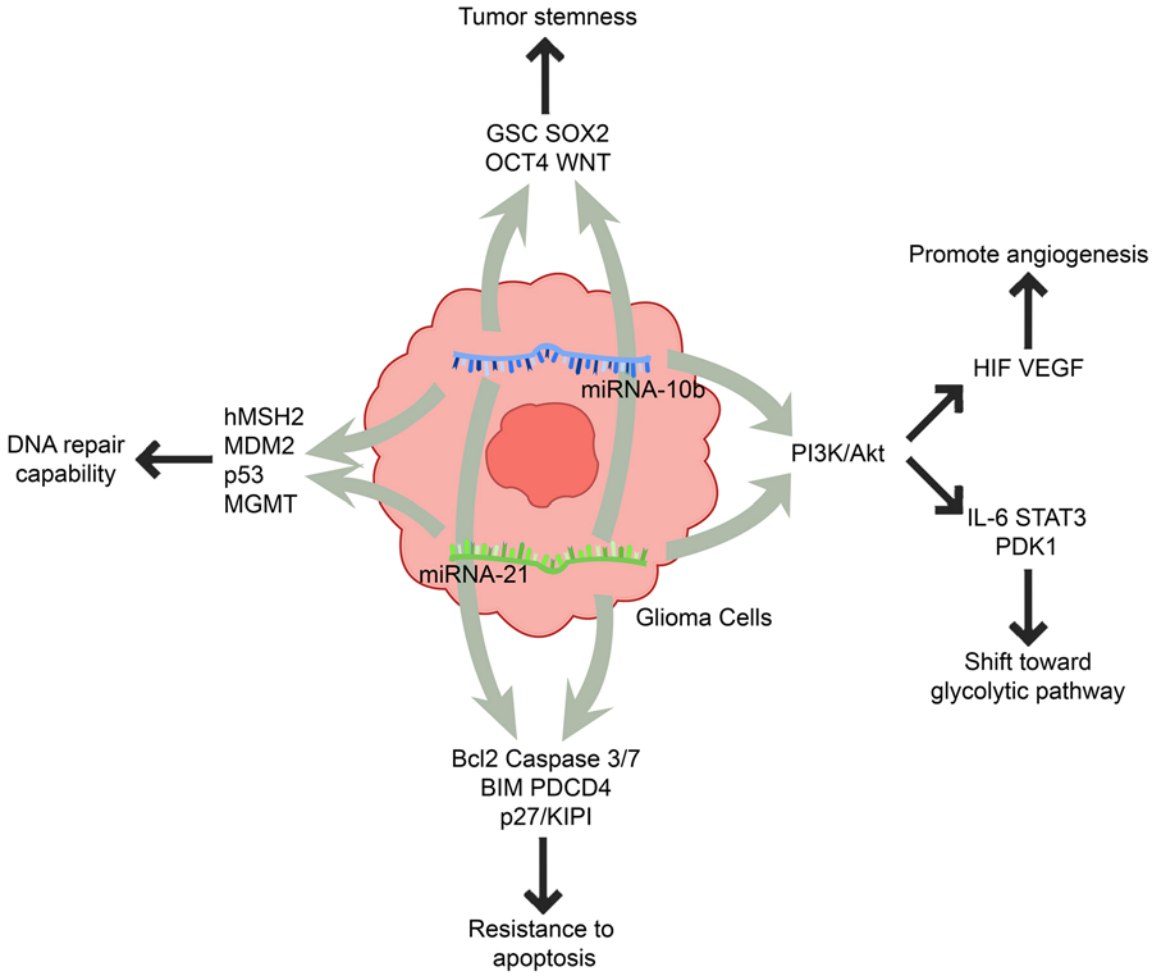

FIG. 2. Summary of possible pathways that are activated by miRNA-10b and miRNA-21 to ultimately induce HGG chemoresistance or radioresistance.

DNA mismatch repair.[47] *MDM2* naturally suppresses the activity of *TP53*,[48] and its overexpression would impair the DNA repair capability of *TP53*.[49]

GSCs are cells with self-renewal capability that can proliferate uncontrollably and form neurospheres. They have been implicated in the development of HGG and its resistance to TMZ. In our review, we understood that the expression of miRNA-21 was increased in neurospheres, while GSCs secreted vesicles that carried miRNA-10b toward other tumor cells. We also found that miRNA-10b and miRNA-21 increased the expression of SOX2, OCT4, and Wnt signaling, which are known contributors to tumor stemness.[50–52] Taken together, miRNA-10b and miRNA-21 potentially have roles in the development of GSCs and merit further investigation.

Another important pathway that was modulated by the expression of miRNA-10b and miRNA-21 was the PI3K/Akt pathway. The downstream effectors of this pathway include pyruvate dehydrogenase kinase 1 (PDK1), hypoxia-inducible factor 1 (HIF-1), nuclear factor–kappa B (NF-κB), and Bcl-2.[53] PDK1 inactivates pyruvate dehydrogenase and prevents the production of acetyl-CoA, causing a shift toward the glycolytic pathway in what is called the Warburg effect, which contributes to TMZ resistance.[54] The activation of HIF-1 by the PI3K/Akt pathway promotes angiogenesis by activating the *VEGF* gene and helps the tumor survive in a hypoxic microenvironment.[53] NF-κB promotes TMZ resistance by promoting *MGMT* gene expression, therefore giving the HGG cell the ability to repair the DNA damage caused by TMZ.[55,56]

Regarding apoptosis, miRNA-10b and miRNA-21 increased the expression of pro-apoptotic Bcl2 and lowered the expression of anti-apoptotic caspase-3/7, BIM, and PDCD4. Closely related to apoptosis, there was evidence that pathways that regulate cell cycle, such as p27/KIP1[57] and CDKN1A,[58] were also altered by miRNA-10b and miRNA-21. As a result, tumor cells can bypass the G2/M phase and continue to proliferate. We also found evidence of alterations in IL-6 and STAT3. IL-6 is the upstream element of the JAK/STAT3 signaling pathway. STAT3 can upregulate *MGMT* gene expression and also plays a role in metabolic reprogramming by promoting HIF-1 transcription.[59]

We also found evidence that miRNA-10b and miRNA-21 reduced the deleterious effect of radiotherapy on HGG cells. The main target of radiotherapy is DNA

damage. Therefore, any factors that can help repair the DNA damage will contribute to radiotherapy resistance. There was evidence that miRNA-10b and miRNA-21 can increase the PI3K/Akt pathway. An increased PI3k/Akt pathway can then enhance DNA damage repair in response to radiotherapy through homologous recombination and nonhomologous end joining,[60] hence reducing the efficacy of radiotherapy. Similar to TMZ resistance, another important factor in radiotherapy resistance is the GSCs. GSCs were found to reduce apoptosis and increase the tumor cell's DNA repair capability.[60] Regarding hMSH2, 2 studies showed that resistant HGG tumor cells exhibit high levels of hMSH2.[61,62]

The in vitro findings were supported by in vivo studies, in which inhibition of miRNA-10b and miRNA-21 reduced tumor size and prolonged survival. However, clinical studies produced conflicting results. One reason may be variation in patients' resection status—a key predictor of survival.[63] Patients with gross-total resection typically have better outcomes than those with subtotal resection, and combining both groups could introduce bias. Tumor type and grade also appeared to be influential; miRNA-21 expression correlated with poorer survival only in grade 3 and 4 astrocytoma cases.[16,35] The timing and method of miRNA sampling further contributed to inconsistencies. Studies that found no prognostic correlation measured miRNA expression at initial resection, whereas Olioso et al. and Labib et al. observed a correlation when miRNA-21 was measured after treatment began, using blood samples instead of tumor tissue.[21,32] This suggests that miRNA expression may change during therapy and that blood sampling could be a better sampling method, as it allows sequential evaluation. Lastly, there were differences between studies regarding the therapy regimens and follow-up period. Therefore, we recommend that future studies stratify patients by resection status and tumor grade, control confounders such as therapy status of the samples, adopt consistent sampling methods, and ensure adequate follow-up.

We have demonstrated that miRNA-10b and miRNA-21 contribute to resistance to TMZ and radiotherapy through multiple pathways. Therefore, rather than targeting each pathway individually, using these miRNAs either as prognostic tools or therapeutic targets can yield better results.

Currently, the most widely used marker for resistance and survival for HGG is MGMT promoter methylation status. However, there is still some discordance because HGG has demonstrated that it has the capability of expressing MGMT despite having a methylated MGMT promoter.[64] In some studies, even expression of the MGMT protein itself has some inconsistencies with clinical outcome.[65] Using MGMT promoter or expression status does not capture the complete mechanism behind therapy resistance. On the contrary, miRNA-10b and miRNA-21 with their wide-ranging epigenetic impacts can potentially provide better prognostic tools. Regarding ease of sampling, instead of using HGG tissue, both miRNAs can be analyzed from blood samples with proven correlation to clinical outcomes.[21,32] Therefore, miRNA-10b and miRNA-21 can potentially be better biomarkers. With a better prognostication tool, clinicians are provided with more tools in their armamentarium for determining their patients' prognosis and therapy. This is especially important in countries with limited resources, as the ability to predict which HGG patients are more likely to respond to TMZ and radiotherapy would allow resources to be focused on those patients. However, the cost of RT-PCR to analyze miRNA-10b and miRNA-21 expression is still high. In our country, a developing lower-middle-income country, the cost reached 5000 US dollars, which makes widespread use not yet feasible. Nevertheless, we believe that in the future, when miRNA-10b and miRNA-21 have progressed from the realm of research to the clinical setting, their cost will decrease substantially.

Aside from their prognostic value, miRNA-10b and miRNA-21 also offer promising therapeutic targets. Numerous treatments for HGG—ranging from PI3K inhibitors to gene therapies targeting GSCs—have been explored, yet prognosis remains poor and recurrence inevitable. This is likely due to the presence of redundant compensatory pathways and the ability of GSCs to stay quiescent until drug levels decrease.[5] Targeting miRNA-10b and miRNA-21 could address this, as they influence multiple resistance mechanisms simultaneously. However, further research is needed, particularly regarding delivery methods. Promising approaches under investigation include locked nucleic acids and viral vectors.[66] We hope this review highlights the therapeutic potential of miRNA-10b and miRNA-21 and lays a foundation for more focused research into miRNA-based therapies.

Our review has several limitations that need to be addressed. First, the studies included in our review had several inherent biases. None of the in vitro and in vivo studies utilized randomization and blinding. The clinical studies also did not adequately control for resection status. These biases might have some effects on the results of our review. To tackle this issue, we used a systematic method to assess for bias and presented the results so that the readers could understand the strength of the evidence and use the information accordingly. Another limitation was that we were not able to conduct a meta-analysis because the studies differed in their methods of presenting the results. Nevertheless, we believe our review managed to unify the current evidence of miRNA-10b and miRNA-21 in TMZ and radiotherapy resistance.

Conclusions

The expression of miRNA-10b and miRNA-21 contributed to TMZ and radiotherapy resistance in vitro. Animal studies have also shown their negative effect on progression and survival. However, the effects of miRNA-10b and miRNA-21 on survival and progression in clinical studies are still conflicting. Future studies should perform better confounding adjustments when studying the effect of miRNA-10b and miRNA-21.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: all authors. Acquisition of data: Malueka, Lukito. Analysis and interpretation of data: Malueka, Tamba, Lukito, Silvano. Drafting the article: Tamba, Lukito, Silvano. Critically revising the article: Hartanto, Malueka, Tamba, Sutarni. Reviewed submitted version of manuscript: Hartanto, Tamba, Sutarni. Approved the final version of the manuscript on behalf of all authors: Hartanto. Administrative/technical/material support: Hartanto, Malueka, Silvano. Study supervision: Hartanto.

Supplemental Information

Online-Only Content

Supplemental material is available online.

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