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Targeted delivery of miRNA based therapeuticals in the clinical management of Glioblastoma Multiforme

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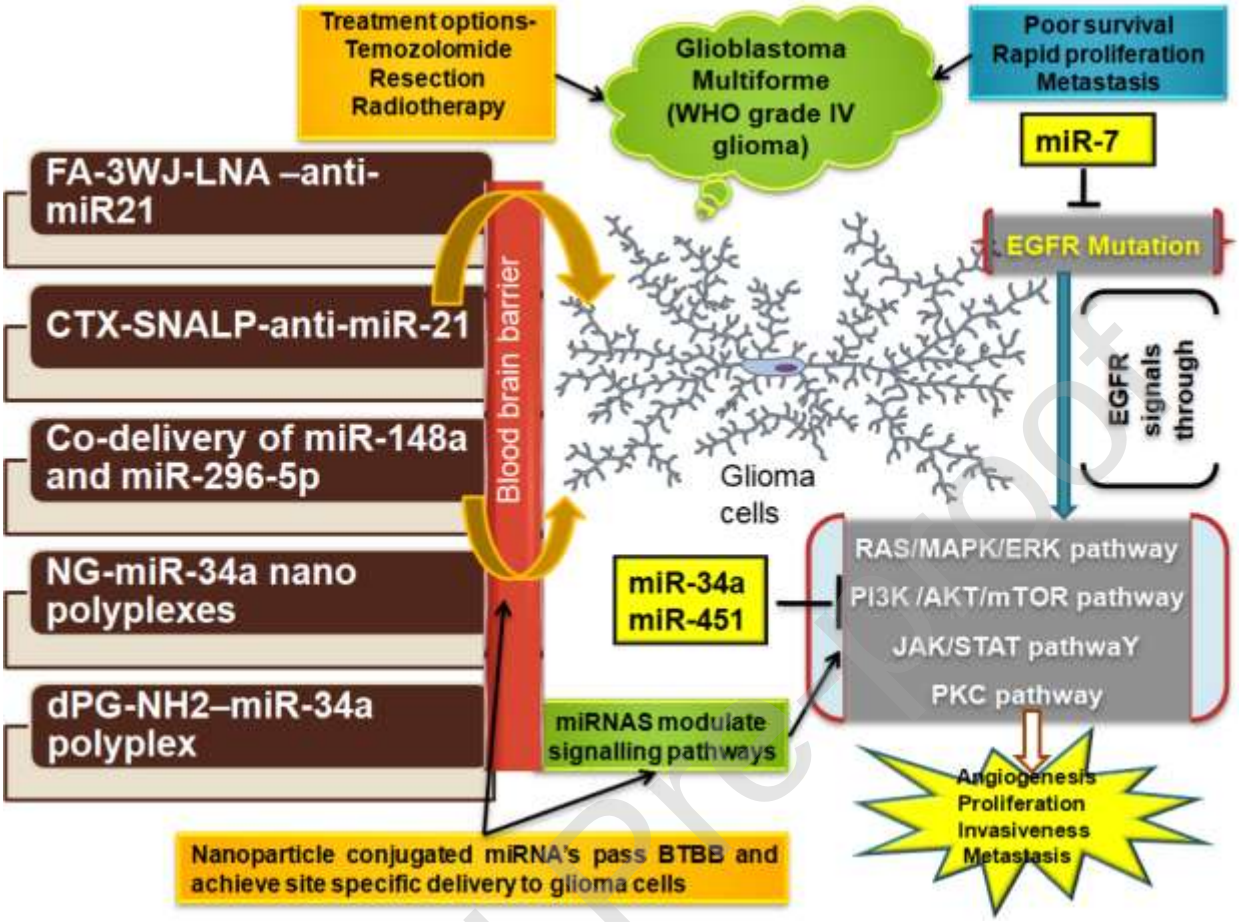
Graphical abstract



Highlights

- Glioblastoma multiforme (GBM) is the most prominent brain tumor of adults, endowed with tremendous invasive capacity, high aggressiveness and resistance to chemo and radiotherapy.
- Overexpression of EGFR (epidermal growth factor receptor) is reported in more than 60% of primary GBM cases.
- The miRNA interacts with mRNA either at its 3' UTR region to suppress or at 5'UTR region to favor expression levels.
- The miRNAs are pro or anti-oncogenic and that repression of oncogenic miRNAs with antisense technology can mitigate the glioblastomas
- Nanomedicine provides new avenues for site specific delivery of miRNAs or anti-miRNAs alone or in combination with other therapeuticals within the brain for optimal therapeutic responses.

Abstract

Glioblastoma multiforme (GBM) is the most aggressive (WHO grade IV) form of diffuse glioma endowed with tremendous invasive capacity. The availability of narrow therapeutic choices for GBM management adds to the irony, even the post-treatment median survival time is roughly around 14-16 months. Gene mutations seem to be cardinal to GBM formation, owing to involvement of amplified and mutated receptor tyrosine kinase (RTK)-encoding genes, leading to dysregulation of growth factor signaling pathways. Of-late, the role of different microRNAs (miRNAs) in progression and proliferation of GBM was realized, which lead to their burgeon potential applications for diagnostic and therapeutic purposes. miRNA signatures are intricately linked with onset and progression of GBM. Although, progression of GBM causes significant changes in the BBB to form BBTB, but still efficient passage of cancer therapeutics, including antibodies and miRNAs are prevented, leading to low bioavailability. Recent developments in the nanomedicine field provide novel approaches to manage GBM via efficient and brain targeted delivery of miRNAs either alone or as part of cytotoxic pharmaceutical composition, thereby modulating cell signaling in well predicted manner to promise positive therapeutic outcomes.

Keywords: Glioblastoma; miRNA; nanoparticles; signaling pathways; BBB, BBTB, nanomedicine, receptor tyrosine kinase

1. **Introduction**

Glioma is the commonest destructive form of primary brain tumors affecting 3.19/100000 person in USA alone. Glioma is more prevalent in caucasians than afro-american, african-asian and american –Indians, with males being the most affected one. This collection of malignant tumors of central nervous system actually protrudes from the glial cells (which basically surround neurons for support and insulation within the brain). Briefly, as per histology, glioma can be classified into high-grade glioma like anaplastic astrocytoma IDH mutant/IDH wild type, mutant/IDH wild type, glioblastoma IDH and midline diffuses Glioma H3 K272M-mutant or low-grade gliomas like astrocytoma, oligodendroglioma mixed glioma (mixurre of astrocyte and oligodendrocytes). Other tumors of glial origin are ependymomas, CNS lymphomas, schwannomas, meningiomas and medulloblastomas. Glioma can be further classified on the

basis of location, and characteristic features of differentiation or anaplasia. Based on the presence or absence of anaplasia the malignant glioma is classified into subtype I-IV according to WHO standards [1]. In brief; WHO reclassified the tumors of CNS and more particularly brain gliomas into grades I-IV by integrating histopathological and molecular genetic markers (Isocitrate dehydrogense Wildtype /mutation & 1p/19q codeleted /non-codeleted). Due to invasiveness as well as aggressiveness; this undifferentiated Glioblastoma multiforme has been designated as Grade IV tumor by WHO [2]. The current therapeutic regimen for GBM includes safe removal of the lesion along with chemotherapy with temozolomide (TMZ) and radiotherapy, but still limited clinical success had been met [3–5].

Epigenetics regulate both formation and suppression of tumors by regulating different types of genetic modifications such as, cytosine methylation at CpG doublets, acetylation, methylation, & phosphorylation of histone proteins or by change in the three dimensional chromatin conformation [3,6]. miRNAs which are non-coding RNAs of 22bp size mediate expression of several genes both at mRNA and protein level [7–9]. miRNA signatures are intricately linked with onset and progression of GBM, the upregulated miRNAs are miR-10a, miR-22, miR-34a, miR-129-3p, miR-132, miR-146b-5p, miR-149, miR-152, miR-155, miR-195, , miR-221, miR-222, miR-296-3p, miR-671-5p, while down-regulated are let-7b, miR-19a, miR-19b, miR-20a, miR-767-5p, miR-106a, miR-181a, -301b, miR-505 [10,11]. The miRNAs have gained immense interest since their evidence of linkage between altered expression levels and tumors had been reported [12,13]. In GBM, major molecular targets of miRNAs includes PTEN, MDM2 (Mouse double minute 2 homolog), TSC1 (tuberous sclerosis complex-1), POLD2 (DNA Polymerase Delta 2,), TGFβ-RII (transforming growth factor-β receptor II), CTGF (connective tissue growth factor) and CAMTA1 (Calmodulin Binding Transcription Activator 1) [14]. The GBM correlates with elevated expression levels of ALDH1A3 (Aldehyde Dehydrogenase 1 Family Member A3). Hence, adaptation of approaches, such as miRNA targeting drugs or biological macromolecules, would provide some unique solutions to inhibit elevated expression of ALDH1 levels in GBM and unravel development of potential therapeuticals [15,16]. It is also quite important to confess the fact that even a single miRNA may regulate multiple targets which would influence the formation, progression, proliferation and migration of GBM [17,18]. The expression levels of some IDH mutations (prognostic), promoter methylation, MGMT (O-methylguanine–DNA

methyltransferase) and 1p/19q predictive co-deletion, related biomarkers are extensively employed in the clinical management of GBM [19].

2. miRNA Biogenesis

Briefly, biogenesis of miRNA starts with the transcription of primary miRNA (> 1kbps) from the cellular DNA sequence, via RNA polymerase II enzyme, in the nucleus of animal cells. Initially it resembles as a long hairpin like double stranded RNA structures, comprising multiple nucleotide segments required for its processing and maturation. In the nuclear region, an endonuclease i.e. RNAse III Drosha, in association with cofactor DiGeorge Syndrome Critical Region 8 (DGCR8); develops into unique complex called microprocessor. This complex precisely cuts the pri-miRNA and degradation it into a secondary product of ~65bps pre-miRNA [20]. Such newly generated pre-miRNAs are then exported into the cytoplasm via transportation complex comprising exportin 5 (EXP5), RAN, GTP and pre-miRNA [21,22]. As soon as the complex crosses the nuclear membrane, the RNAse protein (Dicer) cleaves the pre-miRNA into a ~22 bp miRNA creating a small Duplex miRNA with the help of TAR RNA-binding protein (TRBP or PACT). Activated Dicer as well as DGCR8 complex are believed to be auto-regulated in the nucleus of the cells. The newly formed Duplex miRNA is laden on a particular AGO protein (1 of a 4 types), forming a precursor-RNA Induced silencing complex (pre-RISC). Subsequently, after degradation of one strand of the double helix in pre-RISC it immediately modifies to become mature RISC [18,20,23]. A number of studies have shown regulation of hundreds of targets via a single miRNA including glioma formation and its proliferation. About 1% of miRNAs (Mirtrons) are produced via non-canonical mechanism in which need of Drosha/Dgcr8, in the nucleus of a cell, is avoided. Additionally these types of miRNA are actually encoded by the excited introns of the primary mRNA transcript which are cleaved by action of spliceosomes as well as endonucleases as intron lariat. The intron lariat is debranched or linearized by DBR1 enzyme (in Humans) followed by transportation into cytoplasm by action of XPO5 enzyme for DICER cleavage and subsequent maturation into miRNA. The miRNA interacts with mRNA either at its 3’ UTR region to suppress or at 5’UTR region to favor expression levels [20,24].

3. Interaction of miRNAs with signaling pathways in glioblastoma multiforme

Mutation or amplification of Genes had been commonly reported among p53, receptor tyrosine kinase (RTK)/RAS/PI3K as well as in retinoblastoma tumor suppressor signaling pathways [25]. The dysregulated receptor tyrosine kinase (RTK, including, MET, EGFR, PDGFRα, etc.) signaling pathways are correlated for their critical role in progression of various cancers including gliomas, but have now become prominent characteristics of GBM cases [26–28]. A number of primary GBM based studies have established overexpressed EGFR (epidermal growth factor receptor) presence in more than 60% of cases [29,30]. The EGFR signaling pathways from the plasma membrane includes: PI3K/protein kinase B (PKB/AKT) pathway, protein kinase C (PKC) pathway, RAS/mitogen activated protein kinase (MAPK)/extracellular signal–regulated kinase (ERK) pathway and Janus Kinase (JAK)/STAT pathway [31]. miRNAs in accordance with their capacity to modulate signaling pathways ensure homeostasis, metastasis and fibrosis (Inui et al., 2010), e.g miR-29a downregulates PTEN, EphB3 and SOX4 to mediate activation of complicated post-transcriptional program for promoting the proliferation and invasion of GBM [33]. miR-34a & miR-451 inhibit signaling through AKT and thereby exhibit tumor suppressor effects in GBM [34,35]. miR-7 inhibits EGFR and Akt activity thereby reduces GBM invasiveness [36]. Thus, miRNAs exhibit potential role in the clinical management of GBM w.r.t modulation of diverse signaling pathways **(Fig. 1).**

3.1. RAS/mitogen activated protein kinase (MAPK)/extracellular signal–regulated kinase (ERK) pathway

EGFR recruits the SH2 domain bearing protein-GRB2. The GRB2 is associated with RAS GEF SOS as a preformed complex, to facilitate activation of RAS. This activated RAS drives the RAF-MAPK/ERK kinase (MEK)-ERK1/2 signaling cascade. Subsequently, activation of ERK1/2 (critical regulator of proliferation, survival and metabolism) phosphorylates downstream substrates, which get translocated into the nucleus to modulate various proteins and transcription factors. In tumors with mutated or deleted NF1, the activation of RAS was reported which was measured in terms of p-ERK and p-MEK [31,37,38].

3.2. PI3K/protein kinase B (PKB/AKT) pathway

Activation of EGFR induces phosphoinositide 3-kinase (PI3K) to synthesize phosphatidylinositol (3,4,5) trisphosphates (PIP3) from Phosphatidylinositol 4,5-bisphosphate

(PIP2). At the membrane site, Akt bound to PIP3, is phosphorylated at Thr308PDK1 by PDK1, leading to its partial activation. Subsequently, PDK2 phosphorylates Akt at Ser473 to induce full activity [39]. Such duo-phosphorylated Akt induces the cytoplasmic events like phosphorylation of TSC2. Activated TCS2 relieves repressive effects on Rheb causing cell proliferation, inhibition of apoptosis and downstream activation of mTOR [40]. In contrary, tumor suppressor phosphatase and tensin homolog (PTEN) dephosphorylates PIP3 and inhibits enzymatic activities of Akt (mutated in GBM). Recently, it was also established that before the amplification of EGFR, there is loss of PTEN containing 10q chromosome [31]. GBM tumor and cell lines based genomic analysis revealed mutation in RTK/PTEN/PI3K pathway by confirming elevated phospho-AKT levels [41,42].

3.3. The JAK/STAT pathway

Upon activation of EGFR, intracellular JAKs phosphorylate each other, which inturn phosphorylates STATs, leading to STAT dimerization and their translocation into the nucleus [43], followed by their binding at enhancer sequences located in 3' or 5' position of dimer or complex oligomer to halt transcriptional activities of target gene [31,44,45].In GBM, although STAT-3 exhibit relatively lower frequency of mutation however localized (microenvironment) presence of IL-6 leads to its aberrant activation [46].

3.4 The Phospholipase C (PLC)/PKC pathway

Activated EGFR recruits and activates PLC, which catalytically cleavages PIP2 into inositol 1,4,5-trisphosphate (IP3) and di-acyl glycerol (DAG). Active PLC also stimulates PKC which inturn triggers numerous regulatory molecules to affect angiogenesis, infiltration, proliferation and survival events in tumors [31]. The downstream effectors of PKC isoenzymes are cellular cycle regulators- p53 and p21, regulators of cellular growth & proliferation -RAS-RAF1 and glycogen synthase kinase 3 (GSK3), cell motility regulators-integrins, cell survival regulators-BCL2 and BAD, as well as inflammation regulator NFκB [47,48].

4. Rationalizing nanoparticles for miRNA delivery in glioblastoma multifome.

4.1 The blood-brain barrier and challenges to drug delivery

Tangled transmembrane complex of non-fenestrated endothelial cells, microglia, pericytes, astrocytes and basement membrane assembled with structural proteins such as collagen, laminin, junctional adhesion molecule-1, occludin, claudins, and cytoplasmic proteins (zonula occludens-1 & 2) form extensive tight junctions of protective blood-brain barrier (BBB) surrounding the brain. Primary role of BBB is to strictly deny paracellular entry, majorly of systemically circulating molecules, more prominently hydrophilic agents, owing to their exogenous origin [49]. The process of piocytosis which is some non-specific, non-saturable, non-carrier-mediated vesicular transportation however may felicitate vesicular uptake of bulk fluids and their solute components within the cells, from the site of their microenvironment. The passage of these hydrophilic solutes is usually energy-independent processes so their level in brains are insignificant unless transported as substrates of any transporters (such as GLUT 1,3,4,5,6 and 8: glucose transporters for hydrophilic glucose molecules) [50]. In order to exploit this; the glucose- RGD (Glu- RGD) derivative was synthesized as ligand which favored selective targeting and accumulation of paclitaxel (PTX) liposomes in glioma cells (up-to 4.72- fold higher uptake than pure drug) [51].

In parallel to this, SLC7A5-13, and SLC7A15 [L-type amino acid transporters (LATs)] and SLC7A1-4 and SLC7A14 [cationic amino acid transporters (CATs)] which belong to solute carrier family 7 (SLC7) as well as heteromeric amino acid transporter (HAT) such as LAT1 (SLC7A5); exhibit unusual high expression levels during cancer progression, and were thus visualized as channels delivering essential amino acids into the tumor cells via some unknown cancer-associated reprogrammed metabolic networks, thereby promoting normal cancer growth into high-grade tumors with metastasis [52,53]. The, overexpressed multi-drug transporters like P-glycoprotein at brain capillary endothelial cells and astrocytic end-feet, further complicate the situation by ensuring expulsion of exogenous molecules/drugs out of brain i.e reduction of drug bioavailability. Hence, approaches for combining chemo-therapeuticals with inhibitory substrates of such efflux conferring transporters are critical to reduce dose of drugs, increase the bioavailability as well as to encounter multidrug resistance incidents of GBM [54].

Apart from the protective role as well as secretion of both barrier-promoting as well as barrier-disrupting factors, BBB, by virtue of its paracrine interaction with astrocytes, pericytes and ECs, enables interlinked network of neuronal signals. The paracrine interaction of BBB with astrocytes co-regulates the blood flow. While astrocytes also regulate matrix metalloproteinase

for the purpose of breakdown of the basement membrane during neuronal inflammation, so that entry of immune cells would be felicitated inside the brain [55]. Similarly, functions of pericytes which enwrap blood microvessels cells, is to maintain BBB homeostasis, regeneration of stroma, process of angiogenesis and neo-vascularization, regulation of antigen presenting cells during brain infections, differentiation of neural stem cell attributes, as well as EC proliferation [56]. In addition, lecticans, tenascins, hyaluronic acid, proteoglicans as well as hyaluronan constitutes extracellular matrix (ECM) which serves as base component of BBB structures, and also interacts with other matrix proteins such as laminin and endothelial integrin, to ensure establishment of harbored endothelium networks as well as regulation of paracellular diffusion [56,57]

4.2 Impact of glioblastoma multifome progression on the blood-brain barrier

In the progression of cancerous states, including GBM, the changes in morphology, permeability and functionality of BBB, render it more suitable terminology i.e blood-brain tumor barrier (BBTB) [58]. During initial (1^{st} phase) progression of cancer, capillaries maintain continuous and non-fenestrated i.e. integrity, morphology and functionality of BBB is not affected significantly. But, as the cancer progress into 2^{nd} growth phase, cancer cells start invading surrounding tissues, grow-up into around few (2-3) mm of volume, followed by alteration in permeability of the BBB due to neovascularization. In this angiogenesis mediated neovasculature formation, newly formed capillaries (12 nm size) which are of continuous and fenestrated morphology, start allowing very selective and spherical nutrient molecules up-to 12 nm to cross through this newly formed barrier i.e., BBTB. While, in 3^{rd} or final stage; characteristic features such as enlargement of capillary fenestration size usually upto 48 nm, reduction in micro-vessel basement membrane thickness, loss of junctional proteins, increased inter-endothelial gaps up-to 1 μm, taunts the full integrity of BBTB [59]. The peptides based biomolecules are being released from tumor cells of CNS into blood serum which can be exploited as affluent source of potentially predictive cancer specific biomarkers. CTCs, ctDNA and MVs have been sampled from different biofluids in GBM patients [60,61].

4.3 Nanotechnology enabled miRNA targeted delivery in glioblastoma multifome

The complexity of CNS and presence of physiological barriers possesses major scientific challenges, in conventional delivery approaches for newly synthesized drugs or hydrophilic molecules or phytoconstituents in neurological disorders and cancers, such as glioma (GBM) [62–65]. Furthermore, In contrast to the neuronal delivery of synthetic chemical analogues, miRNA-based therapeuticals, bear additional challenges due to their susceptibility towards enzyme and PH mediated degradation even before reaching the target sites [66]. Hence in lieu of above, it would be judicious to develop an efficient and biocompatible carrier system which would felicitate entry of nucleic acid loads to specific areas within the brain, without being subjected to degradation. The nanotechnology fabricated carriers e.g polymeric systems, lipid based carriers, liposomes and metallic nanoparticles hold the capacity and efficiency to deliver the miRNA based loads [67–69]. Inaddition, the surface of metallic nanoparticles, such as spherical silver and gold NPs, have been attributed to their catalytic properties; hence exploited for functionalization purposes of peptide analogues [70].The integration of nanotechnology to deliver rationalized pharmaceutical compositions, is the most favorable approach which significantly reduces the burden of drug regimen by reducing the course of treatment, coupled with improved potential therapeutic outcomes and minimal side effects [71–73]. The efficient delivery of tumor suppressor microRNA–conjugated systems using nanotechnological processed carriers in brain is very promising field. The miRNA nanoparticles based drug delivery systems therefore provides new means to rationalize selection of appropriate approaches to enhance overall permeability of loaded biomolecules or drugs, with enhanced retention impacts i.e. by exploiting nanoparticulated drug delivery leading into enhanced permeation and retention (EPR) of loaded miRNA preferentially within the cancer affected regions in brain for the effective clinical management of GBM [74–76]. Therefore, various novel drug delivery approaches for miRNA delivery in cases of GBM had been developed in the recent past.

In order to efficiently knock down miR-21 (oncomir) expression in GBM, anti-miR-21 LNA loaded Multi-valentfolate (FA)-conjugated 3WJ RNP were injected into glioblastoma cells in vitro to induce rescue of tumor suppressors; PTEN and PDCD4 culminating into apoptosis [77]. The delivery of miR-21 antisense oligonucleotide (anti-miR-21) via lipid and polymer constituting unique single hybrid systems; lipid-polymer nanoparticles (LPNs), efficiently targeted glioblastomas [78]. PLGA-Nanoparticle encapsulated antisense miR-21 delivered prior to TMZ treatment of cells, significantly brought down the number of viable cells ($p < 0.001$).

Similarly cell cycle arrest at G2/M phase increased (1.6-fold) upon treatment with TMZ in U87 MG cells [79]. The delivery of anti-miR-21 oligo-nucleotides via Chlorotoxin (CTX)-coupled SNALP promoted silencing of miR-21, elevation of PTEN and PDCD4, activation of caspase 3/7, culminating in reduced tumor size/ proliferation, apoptosis and improvement of animal survival [80]. Nadiya M Teplyuk et al., 2016 applied convection- enhanced delivery (CED) to continuously deliver anti- miR- 10b- containing nanoparticles for two weeks, via intracranial osmotic pumps, significant reduction in intracranial GBM progression and cell proliferation with increase in apoptosis was observed. All of these properties were evident from decreased staining for proliferation markers PCNA and KI67 (cleaved caspase 3 staining) [81].

Polymeric nanogels (NGs)-miR-34a nano-polyplexes upregulated miR-34a while downregulating its target oncogenes like c-MET, CDK6, Notch1 and Bcl-2 in Human U-87 MG GBM cells resulting in inhibition of proliferation/migration of cells in vitro, and also tumor suppression in GBM-bearing SCID mice [82]. Dendritic polyglycerolamine (dPG-NH_2)–miR-34a polyplex reduced expression of oncogenes like C-MET, CDK6, Notch1 and BCL-2 in U-87 MG human cells leading to inhibition of cell cycle progression, proliferation and migration of GBM cells in vitro, and reduction of tumor growth in GBM inoculated SCID mice [83]. CXCR4 receptor- stimulated lipoprotein- like nanoparticle (SLNP) loaded with microRNA- 34a in the core, reduced expression of Y- box 2 (sex- determining region) and Notch1, thereby Inhibited GICs stemness and chemoresistance and significantly prolonged the survival of GICs- bearing mice [84]. An evident increment in apoptosis and decrement in proliferation was observed when GBM tissue slices were treated with nanoparticle loaded with miR-29b, resulting in reduction in expression of COL1A2, COL3A1, COL4A1, ELN, ITGA11, MMP24, and SPARC, to mediate an anticancer effect [85].

In gliomas, miR221 is liked with invasiveness and temozolomide resistance. The, delivery of temozolomide (TMZ) in association of anti-miR221 PNA, loaded on mesoporous silica nanoparticles (MSNPs), induced apoptosis in TMZ resistant T98G cells [75]. The combination of anti-miR-21 oligo-nucleotides via Chlorotoxin (CTX)-coupled SNALP with Sunitinib (a tyrosine kinase inhibitor) decreased tumor cell proliferation & enhanced apoptosis in C57BL/6 mice [80]. The delivery of 5-FU and antisense miR-21 constituting synergistic pharmaceutical compositions via PAMAM based carriers exhibited prominent antiproliferative effect in human

glioma U251 cells [86]. In association with TMZ, both antagomiR-21 and antagomiR-10b, delivered into U87MG and Ln229 GBM cells via PLGA based and cRGD-targeting nano-carriers, not only efficiently arrested cell cycle at G2/M phase but also sensitized cells, even to low concentrations of TMZ [87]. The, combo-delivery of miR-148a and miR-296-5p containing pharmaceutical compositions not only inhibited stem cell phenotype of human GBM cells in vitro but also increased long-term survival in athymic nude NCR Nu/Nu and response to γ radiation therapy [88]. Among metallic nanoparticles, Iron oxide owing to its magnetic properties is being exploited for external magnet controlled localized release of drugs as well as hyperthermia mediated apoptosis of tumors, including GBM [89,90]. While, silver and gold nanoparticles, owing to their anti-infectious properties as well as unique SPR (Surface Plasmon Resonance) mediated analytical signal amplifications, are exploited not only for their restricted use as drug carriers but also for diagnosis of state of GBM [91–93]. Hence, unique single hybrid system of both gold and iron oxide nanoparticles (GIONs) was developed with functionalized β-cyclodextrin-chitosan (CD-CS) hybrid polymer and PEG-T7 peptide, for co-delivery of miR-100 and antimiR-21, the delivery of which in association of TMZ lead into synergistically enhanced anticancer potentials in U87-MG GBM cell-derived orthotopic xenograft models in mice [94]. Hence with inspiration of all related studies; nanotechnology platform enabled delivery of RNA interference therapeuticals assures promising therapeutic response to efficiently combat gliomas **(Table 1 & 2).**

5. Future prospects

The recent promising discoveries and evidences on critical role of miRNAs in the formation & progression of glioma, enable some of them to emerge as exclusive clinical hallmarks in diagnosis, prognosis as well as novel agents in mitigation of glioblastoma multiforme (GBM). Subsequently, with the progression of GBM; the integrity of BBB is compromised in a way that these miRNAs appeared to be released in a systemic circulation, which could be exploited for early diagnosis & identification of different stages of giloma. Advanced analytical techniques like northern blotting, qRT-PCR, microarrays and NGS needs to be standardized, validated and integrated to generate and process the complex raw data in establishing definitive clinical decisions. Furthermore, additional techniques such as enzymatic amplification may be required to identify those miRNAs which are specific hallmarks in GBM but give poor signaling. The unique SPR attributes of metallic nanoparticles would favor adequate amplification of these

detection signals. In the past assessment of promising role of nanotechnology based materials and processes with synthetic and spiked- samples validated as proof-of-principle. But the absence of internationally recognized regulatory procedures as well as stringent quality control strategies limits the clinical translation of these strategies. The above mentioned unmet issues and challenges needs to be addressed in a very judicious and scientific way which would regulate the integration of nanotechnological techniques for site specific delivery of miRNAs in clinical settings.

6. Conclusion

Herein, we reviewed the biological nature of GBM tumors, the devastating outcome for patients, the failure of chemo and radio therapy and recent progress in establishing the role of miRNAs as diagnostic markers as well as therapeutic agents (alone or in combination with chemotherapeutic molecules) in mitigation of GBM. The deeper understanding of miRNAs and their capacity to modulate signaling pathways, which govern tumorigenicity opens new avenues for improving the life expectancy and QoL of GBM patients. But these miRNAs exhibit low stability, solubility and achieve non-site selective targeting, which paved way for nanotechnological based novel delivery approaches. Over the last few years, progress and advancement in nanotechnology-driven strategies to fabricate novel material/s for targeted drug delivery of biological macromolecules (such as peptides, RNA, miRNAs etc) and chemotherapeuticals enabled by the state of the art, controlled, functionalized and target centric modus operandi, accomplishes specific functions to cater the need required for. Different types of nanoparticles, such as silver nanoparticles, colloidal gold, silica nanoparticles, liposome, polymeric carriers, bioinspired systems, niosomes, limicubes, NLC, SLN etc, have been rationalized for optimal benefit in different pathological states including neuronal cancers. These delivery systems not only protect miRNAs from degradation by nucleases but also increases their half-life in the blood or serum. Inaddition, numerous clinical trials reported combination therapies, likely be the most promising method for GBM treatment, and that nanotechnology allows conjugation of miRNAs with anti-cancer drugs and that too with synergistic effects. Thus the successful treatment of GBM lies with the effective nano-tech enabled delivery of miRNAs across the BBTB either alone or in combinations.

Conflict of interest

The authors declare no conflict of interest

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Figure legends:

Fig. 1: Modulation of EGFR signaling pathways in gliobalstoma multiforme with miRNAs

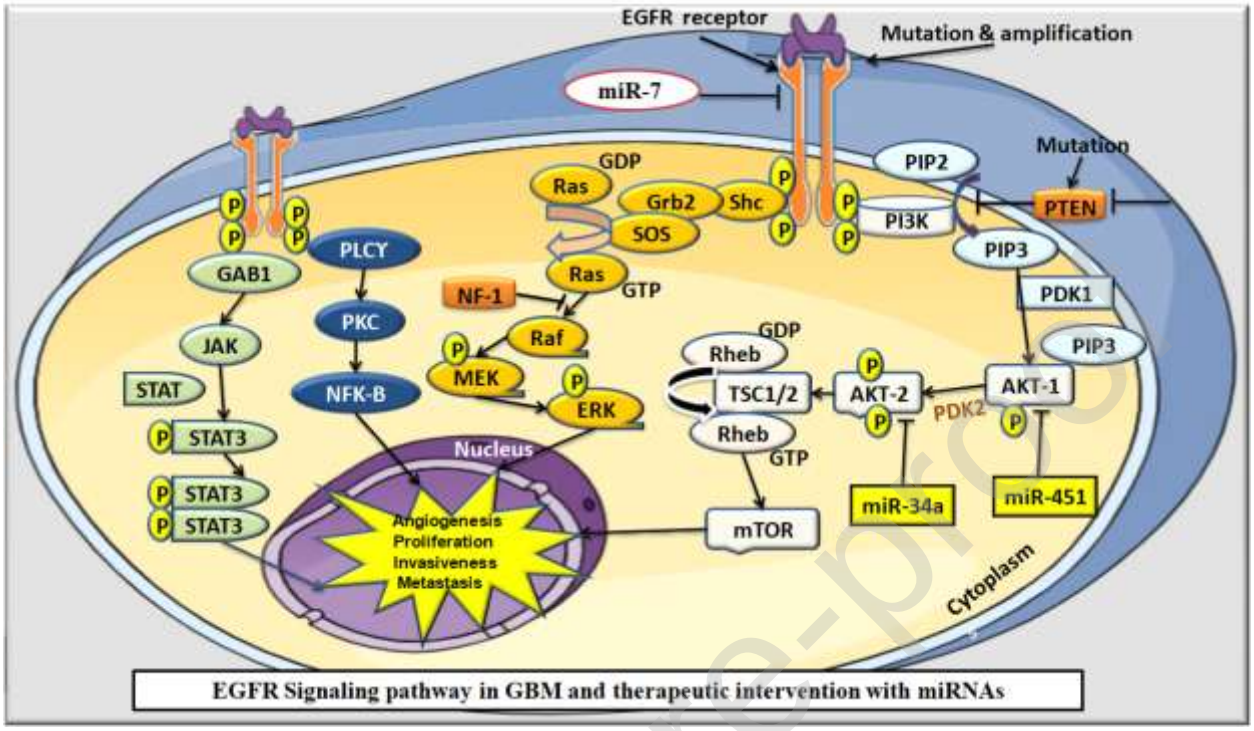

Table 1: Nanotechnology enabled miRNA targeted delivery in Glioblastoma Multiforme (In vitro studies)

S.NO	Nanoformulation/ s type	Rationale	Model (animal/cell line)	Outcomes	References
1.	anti-miR-21 LNA loaded Multi-valentfolate (FA)-conjugated 3WJ RNP	miR-21 knock down	U87EGFRvIII or Gli36 cells	Glioblastoma cell apoptosis	[77]
2.	Chlorotoxin (CTX)-coupled SNALP-anti-miR-21 oligonucleotides	miR-21 silencing, PTEN and PDCD4 upregulation, caspase 3/7 activation	U87 human GBM and GL261 mouse glioma cells	Decrease in tumor cell proliferation	[95]
3.	PACE-antimiR-21 nanoparticles & PLA-HPG-antimiR-21 nanoparticles	miR-21 suppression, PTEN upregulation	U87 cells	Apoptosis of human GBM cells	[96]
4.	PLGA nanoparticle encapsulated antisense miR-21	knock down endogenous miR-21	U87 MG, LN229, and T98G cells	Overexpression of the miR-21 target genes PTEN (by 67%) and caspase-3 (by 15%) upon cotreatment with TMZ	[79]
5.	5-FU-Loaded PAMAM-antisense- miR-21	Down-regulation of miR-21	Human glioma U251 cells	as-miR-21 significantly improved the cytotoxicity of 5-FU and dramatically increased the apoptosis of U251 cells	[86]
6.	LPNs loaded with pemetrexed and anti-miR-21	Co-delivery of anti-miR-21 & pemetrexed	U87MG human glioblastoma cells.	improved accumulation of LPNs in the nucleus of U87MG cells.	[78]
7.	Nanoparticle loaded miR-29b	marked decrease in proliferation	GBM tissue slices	miR-29b inhibits the expression of COL1A2, COL3A1,	[85]

		and significant increase in apoptosis after miR-29b treatment		COL4A1, ELN, ITGA11, MMP24, and SPARC, which mediate an anticancer effect.	
8.	NG-miR-34a nano-polyplexes	upregulation of miR-34a and downregulation of its target oncogenes like c-MET, CDK6, Notch1 and Bcl-2 .	Human U-87 MG GBM cells	Inhibition of cell proliferation and migration	[82]
9.	dPG-NH_2–miR-34a polyplex	lower expression of C-MET, CDK6, Notch1 and BCL-2 genes	U-87 MG human cells	Inhibition of cell cycle progression/ proliferation and migration of GBM cells	[83]
10.	Cy5-MSNPs loaded with anti-miR221 PNA	down-regulation of miR221	temozolomide-resistant T98G cell line.	Effective induction of apoptosis (70.9% of apoptotic cells)	[75]
11.	cRGD-targeted PLGA nanoparticles encapsulating antagomiR-21 and antagomiR-10b	Co-inhibition of miR-21 and miR-10b increased cell cycle arrest at G2/M phase upon TMZ treatment.	U87MG and Ln229 GBM cells	Improvement in sensitivity of these cells to lower concentrations of TMZ	[87]
12.	nano-miRs containing miR-148a + miR-296-5p	Delivery of miR-148a + miR-296-5p	GBM1A cells	Inhibit the stem cell phenotype of human GBM cells in vitro	[88]

Abbreviations: Mesoporous silica nanoparticles (MSNPs); temozolomide (TMZ); peptide nucleic acids (PNAs); poly(amine-co-esters) (PACE); poly(lactic acid) and hyperbranched polyglycerol (PLA-HPG); lipid-polymer hybrid nanoparticles (LPNs); Chlorotoxin (CTX); polymeric nanogels (NGs); Poly(lactic-co-glycolic acid) (PLGA); Locked Nucleic Acids (LNAs)

Table 2: Nanotechnology enabled miRNA targeted delivery in Glioblastoma Multiforme (In vivo studies)

S.NO	Nanoformulation/stype	Rationale	Model (animal/cell line)	Outcomes	References
1	ApoE coated PACE-antimiR -21 NPs & PLA-HPG- antimiR-21 nanoparticles	miR-21 inhibition,	Male RNU rats (Convection-enhanced delivery)	Increase in PTEN expression	[96]
2.	anti-miR-21 LNA loaded Multi-valentfolate (FA)-conjugated 3WJ RNP	PTEN and PDCD4 upregulation	Athymic nu/nu outbred mice	Tumor growth regression	[77]
3.	Chlorotoxin (CTX)-coupled SNALP-anti-miR-21 oligonucleotides	miR-21 silencing	Adult male C57BL/6 mice	Decreased tumor cell proliferation & enhanced apoptosis in combination with tyrosine kinase inhibitor;Sunitinib	[80]
4.	Co-delivery of miR-148a and miR-296-5p	Delivery of miR-148a + miR-296-5p	athymic nude NCR Nu/Nu mice	Long-term survival	[88]
5.	Nanogels (NGs) -miR-34a nano-polyplexes		GBM-bearing SCID mice	Inhibited tumor growth	[82]
6.	cRGD-targeted and non-targeted PLGA nanoparticles encapsulating antagomiR-21 and antagomiR-10b	Co-inhibition of miR-21 and miR-10b	nude mice (nu/nu)	Increase in cellular chemosensitivity towards lower doses of TMZ	[87]
7.	dPG-NH_2–miR-34a polyplex	Upregulaion of miR-34a	SCID mice	Inhibition of tumor growth	[83]
8.	FH38- miR34a- SLNPs	Reduces sex- determining region Y- box 2 and Notch1 expression	GICs- derived orthotopic mice models	Inhibits GICs stemness and chemoresistance and significantly prolongs the survival of	[84]

				GICs- bearing mice.	
9.	anti- miR- 10b- conta ining nanoparticles	Downregulation of miR-10b (oncogenic miRNA)	Intracranial human GSC-derived xenograft and murine GL261 allograft models in athymic and immunocomp etent mice.	Attenuated growth and progression of established intracranial GBM	[81]
10.	CD-CS coated GIONs co-loaded with miR-100 and antimiR-21. surface functionalization with PEG-T7 peptide using CD-adamantane host-guest chemistry.	Downregulate miR-21 and upregulate miR-100	U87-MG GBM cell-derived orthotopic xenograft models in mice.	Significant increase in survival of mice co-treated with T7-polyGIONs loaded with miR-100/antimiR-21 plus systemic TMZ	[94]

Abbreviations: Chlorotoxin (CTX)-coupled (targeted) stable nucleic acid lipid particle (SNALP); glioma-initiating stem-like cells (GSC); gold-iron oxide nanoparticles (polyGIONs); β-cyclodextrin-chitosan (CD-CS); cationic poly(amine-co-ester) (PACE); poly(lactic acid) and hyperbranched polyglycerol (PLA-HPG).; peptide nucleic acid (PNA); convection-enhanced delivery (CED)