



**HAL**  
open science

## MicroRNA-based therapeutics and nanomedicine for brain tumour treatment

Shubaash Anthiya, Audrey Griveau, Claire Loussouarn, Patrick Baril, Martin Garnett, Jean-Paul Issartel, Emmanuel Garcion

► **To cite this version:**

Shubaash Anthiya, Audrey Griveau, Claire Loussouarn, Patrick Baril, Martin Garnett, et al..  
MicroRNA-based therapeutics and nanomedicine for brain tumour treatment. 2019. inserm-  
01691531v1

**HAL Id: inserm-01691531**

**<https://inserm.hal.science/inserm-01691531v1>**

Preprint submitted on 10 Jul 2019 (v1), last revised 24 Jan 2018 (v2)

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **MicroRNA-based therapeutics and nanomedicine for brain tumour treatment**

2

3 Shubaash ANTHIYA<sup>1,2\*</sup>, Audrey GRIVEAU<sup>1\*</sup>, Claire LOUSSOUARN<sup>1</sup>, Patrick BARIL<sup>3</sup>,  
4 Martin GARNETT<sup>2</sup>, Jean-Paul ISSARTEL<sup>4</sup> and Emmanuel GARCION<sup>1,✉</sup>

5

6 <sup>1</sup>CRCINA, INSERM, Université de Nantes, Université d'Angers, 49933 Angers, France.

7 <sup>2</sup>School of Pharmacy, University Park, University of Nottingham, Nottingham NG7 2RD, U.K.

8 <sup>3</sup>Centre de Biophysique Moléculaire, CNRS UPR4301, Université d'Orléans, 45071, Orléans,  
9 France.

10 <sup>4</sup>INSERM U1216, Grenoble Institut des Neurosciences, Université Joseph Fourier, CHU  
11 Michallon, 38043, Grenoble, France.

12

13 \*These authors contributed equally to this work.

14

15

16

17 ✉Corresponding author:

18 Dr Emmanuel Garcion

19 [emmanuel.garcion@univ-angers.fr](mailto:emmanuel.garcion@univ-angers.fr)

20 CRCINA, INSERM U1232 Team 17,

21 Design and Application of Innovative Local treatments in Glioblastoma (GLIAD),

22 Université d'Angers,

23 IBS - CHU,

24 4 Rue Larrey,

25 F-49933 Angers, France

26 +33 (0) 2 44 68 85 43

27 **Abstract (119/120 words)**

28 miRNAs are key cell regulatory elements code by the genome. Although their signature in  
29 cancer is incompletely known and not yet an element of diagnosis, the role of oncomiRs become  
30 increasingly documented. Alongside cancer intrinsic signals in which they exert essential  
31 functions, miRNAs are also responding to environmental cues. Developing novel approaches to  
32 target miRNA-pathways represents a pivotal issue in research and may result in a major  
33 breakthrough for human health. Due to their submicron size and versatile physicochemical  
34 properties the emergence of innovative nanomedicines may be here largely advantageous for the  
35 development of targeted clinical strategies. With a special focus on glioblastoma, the present  
36 review addresses cancer functions of miRNAs and raises the question of an adapted  
37 nanomedicine.

38

39 **Keywords:** glioblastoma, RNAi, drug delivery, brain targeting, argonaute

40

41

## 42 **Glioblastoma - Current Treatments and Therapeutic Failure**

43 Malignant glioblastomas (GBM) are aggressive grade IV primary tumours of the central nervous  
44 system [1, 2]. The standard treatment regime consists of a maximal surgical resection followed  
45 by external radiotherapy and temozolomide chemotherapy and the patients present a median  
46 survival of about 15 months [2]. The addition of a tumour treating field (TTF) along with the  
47 maintenance temozolomide has significantly increased the overall median survival to 20.5  
48 months along with better quality of life due to the locoregional nature of the treatment [3]. Other  
49 treatment options for GBM were discussed by Ben Williams and Stephen Western, (see:  
50 [https://virtualtrials.com/pdf2017/treatment\\_options\\_gbm\\_2017.pdf](https://virtualtrials.com/pdf2017/treatment_options_gbm_2017.pdf)). All the available treatments  
51 are only palliative without a cure [2, 3]. The therapeutic failure imposes two main problems  
52 inherent to glioblastoma and often shared by other cancers: i) the identification of relevant drug  
53 targets and ii) the resistance to treatment, that may itself result from the infiltrating behaviour of  
54 GBM cells, the high expression of survival proteins, but also non-optimal delivery of drugs.  
55 Among new targets potentially capable of revolutionizing the therapeutic approach of GBM,  
56 microRNAs (or miRNAs or miRs), discovered twenty year ago, represent an important line of  
57 research [4, 5]. Not only they allow a better understanding of the GBM pathophysiology, but  
58 they also offer the possibility of developing new therapeutic strategies based on their inhibition,  
59 replacement or modulation of their activity. In this context, due to their small size and to their  
60 versatility of structure, the emergence of new nanomedicines may represent an important issue  
61 for establishing new treatment modalities and to optimize the biodistribution and activity of  
62 conveyed miRNA-modulating drugs [6, 7].

63

64

## 65 **MicroRNA Biogenesis and functions**

66 Human microRNA biogenesis and its various functions are presented in **Figure 1** [8, 9].  
67 MicroRNA genes are transcribed mainly from the nuclear genome (Primary-miRNA, pri-miR)  
68 [5], processed and transported out of the nucleus (Precursor-miRNA, pre-miR) [10, 11], trimmed  
69 in the cytoplasm (Duplex miRNA, miR-5p/miR-3p) [12] and loaded on to argonaute proteins to  
70 form RISC complex (Mature miRNA). Either or both single-stranded mature miRNA-strands can  
71 be loaded onto individual RISC [13, 14]. MiRNA-guided RISC binds to target messenger-RNAs  
72 (majorly in the 3'-untranslated region, at specific sites called miRNA recognition elements  
73 (MREs)) and blocks their expression by mRNA destabilization and translational repression [15-  
74 17]. The RISC complex also exerts nuclear [18, 19] and possibly mitochondrial functions [20].  
75 They can be secreted outside the cell, either as free or extracellular vesicles loaded form, to  
76 function like endocrine signalling molecules [20-22] or transported to adjacent cells via  
77 connexins [23].

78

## 79 **Glioblastoma and miRNA**

80 MicroRNA are an important class of gene-expression regulators involved in almost all major  
81 functions of the cell, including hallmarks of cancer, GBM pathophysiology, treatment-resistance  
82 and plasticity, see **Figure 2** [22, 24, 25]. Differential expression of miRNAs has been observed  
83 between GBM tumour mass and control brain tissue, glioma sub-types [26, 27] and even  
84 between different subpopulations of the same tumour [22]. These differences in miRNA  
85 expression can be used as diagnostic and prognostic biomarkers [22, 28]. MirBase-version-21  
86 enlists 1881 precursors and 2588 mature human miRNAs [29], of which several of them are  
87 identified as preferentially tumour-suppressors (ts-miRs) or oncogenic miRNAs (oncomiRs) or

88 both depending on the context [30]. Recent developments with clinically-related miRNAs are  
89 discussed below.

90 MiR-128 is highly expressed in normal brain tissue, but down-regulated in GBM [31]. There  
91 exists an inverse correlation between miR-128 expression and grade of glioma [32]. MiR-128  
92 reduces tumour growth (EGFR, PDGFR [33]), stemness (BMI1 and E2F3 [34, 35]), invasion  
93 (DCX, RELN [36]), induces apoptosis [37] and senescence [38]. miR-128 was expressed at low  
94 levels in proneural-GSCs (Glioma Stem-like Cancer cells) and no expression in more aggressive  
95 mesenchymal-GSCs [35]. However, overexpressing miR-128 inhibited tumour growth in all  
96 molecular-subtypes of GSCs [35]. miR-145 is implicated in the reduction of cancer stemness and  
97 invasion abilities by controlling several genes involved in self-renewal (OCT4, SOX2,  
98 NANOG), a junction adhesion molecule (JAM-A) which has the potential to rescue stemness by  
99 the activation of pAKT [39] and EMT (e.g. ZEB2) [30, 40]. It also targets efflux pump, ABCB1  
100 (or P-gp) [41], cell cycle regulators CDK6 and SP1 thereby involved in chemo-sensitization [42].  
101 Both miR-128 [32] and miR-145 [43] targets p70S6 kinase1, an important down-stream effector  
102 of PI3k/ AKT/ mTOR pathway, highly expressed in glioma and promotes angiogenesis by  
103 activates hypoxia-inducible factor 1 (HIF- $\alpha$ ) [32]. MiRNA-7 sensitized GBM to TRAIL-  
104 mediated apoptosis (TNF-related apoptosis-inducing ligand) by directly downregulating XIAP  
105 (X-linked inhibitor of apoptosis protein) [44]. MiR-16 inhibited tumour proliferation and  
106 invasion by targeting multiple genes BCL2, CDK6 (Cyclin-dependent kinase-6), cyclin D1,  
107 cyclin E1 and SOX5 at protein level, however seed base-pairing with isolated target sites yielded  
108 only faint but statistically significant downregulation [45]. Similarly, overexpression of  
109 oncomiR-10b did not show any downregulation of predicted targeted genes even at protein-level,  
110 however, the therapeutic impact was evident upon blocking its expression [46]. Abolishing miR-

111 10 expression by genome engineering did not affect normal brain cells while eradicating tumour  
112 [47]. MiR-21-5p is widely studied oncomiR, while relatively less expressed miR-21-3p  
113 oncogenic role was recently identified [13]. MiR-21-3p targets PHACTR4 (Phosphatase and  
114 actin regulator 4) resulting in rapid cell proliferation and oncogenesis; This study indicates the  
115 importance of studying the miRNA functions without strand-bias [13]. MiR-21-5p decreases  
116 apoptosis by targeting FASLG (FAS ligand) [48]. miRNA-21 and miR-29a loaded exosomes are  
117 released by tumours cells which activates Toll-like receptors (TLR7 and TLR8) in macrophages  
118 causing inflammation and promoting tumour proliferation and metastasis [49]. miR-215 targets  
119 epigenetic regulator KDM1B (Lysine Demethylase 1B) and provides GBM growth adaptation  
120 under hypoxic condition [50], while reintroducing hypoxia suppressed miR-124 induces cell  
121 death by suppressing TEAD1 (Transcriptional Enhancer Factor 1), MAPK14/p38 $\alpha$  and SERP1  
122 (Stress-associated Endoplasmic Reticulum Protein 1) [51]. Shea *et al* reviewed an extensive list  
123 of miRNAs and its role in GBM [52].

124 Apart from bona-fide ts- and oncomiRs, other miRNAs can also be beneficial for developing  
125 GBM therapeutics. For example, miRNA-181d-5p and miR-409-3p targets MGMT (O<sup>6</sup>-  
126 methylguanine-DNA methyltransferase) and hence can be used as chemo-sensitizing agents [53].  
127 Repeated treatment with miR-138-mimic-lipoplex partially reduced the expression of key  
128 immune-checkpoints proteins in T-cells (CTLA-4, PD-1 and FoxP3), resulting in significant T-  
129 cell mediated tumour regression in brain-tumour model expressing PD-L1 ligand [54].

130 Taken together, a single miRNA can regulate multiple genes involved in distinct functions of the  
131 cell, which makes miRNA-based strategies a very attractive candidate for cancer treatment. A  
132 bona-fide tumour-suppressor miRNAs have a great potential to act as a single agent miRNA-  
133 therapeutic for treating cancer [42], but it can also act as sensitizing agent for conventional

134 treatments [41]. Similarly, suppression of a single oncomiR can reactivate multiple tumour-  
135 suppressor genes leading to tumour regression or even tumour eradication in the case of miR-10b  
136 [47]. Most of the studies use overexpression to show the tumour-suppressor role of genes that are  
137 targeted by onco-miRs. However, overexpressed mRNA of the gene would act as a miRNA-  
138 sponge quenching the oncomiR function and hence the observed tumour-suppression may not be  
139 the direct effect of the overexpressed gene. However, caution must be taken while choosing  
140 miRNAs for therapeutic purpose as many of the miRNAs can display tumour-suppressor and  
141 oncogenic duality [55]. For example, miR-302/367 cluster have shown to have potent tumour-  
142 suppressor roles in GBM [56] while they are also been strongly implicated in both oncogenic and  
143 stemness enrichment [57, 58]. On the other hand, blocking oncogenic miR-21 expression in  
144 immune cells instead of tumours might block T-cell activation and their anti-tumour immune  
145 response [59]. Hence, it is important understand the holistic context of miRNA activity [55] and  
146 to direct the miRNA therapeutics towards the target cells to maximise successful outcome. The  
147 following section will address various tools and modalities available for microRNA-modulation.  
148



## 149 **MicroRNA Modulation Strategies**

150 MicroRNAs modulation can be exploited therapeutically by affecting either their biogenesis or  
151 mode of action through non-specific (affecting multiple miRNAs) or specific (directly affecting a  
152 single miRNA) strategies, see **Figure 1**.

153

### 154 *Interventions on microRNA biogenesis*

155 Globally reduced levels of mature miRNA is a common feature of cancer and usually associated  
156 with aggressive metastatic phenotypes, drug resistance and poor prognosis [60-62]. Mutation in  
157 p53 gene is a common occurrence in cancer. Mutant-p53 sequesters cofactors of Drosha (e.g.  
158 p68, p72) and interferes with the nuclear cleavage of certain pri-miRNAs leading to EMT  
159 (epithelial to mesenchymal transition), cell migration and survival [62]. Hypoxia downregulates  
160 the expression of both Drosha and Dicer thereby reducing miRNA biogenesis resulting in tumour  
161 progression and infiltration [63]. Targeting either HIF1a (Hypoxia-inducible factor 1-alpha) or  
162 combinatory blocking of transcription factors ETS1 (Avian Erythroblastosis Virus E26  
163 Oncogene Homolog-1) and ELK1 (ETS Transcription Factor) rescued miRNA biogenesis and  
164 reduced tumour growth [63]. Exportin-5 (XPO5) acts as a tumour suppressor by increasing the  
165 levels of mature miRNA in the cytoplasm as it mediates the nucleocytoplasmic export of pre-  
166 miR [60]. Activation of ERK blocks XPO5 activity by phosphorylation [61]. Pharmacologically  
167 targeting of ERK [61] or treating with epigenetic regulators [60] induce XPO5 expression,  
168 sensitized cancer cells to taxols and reduce tumour progression [60, 61]. Taken together,  
169 restoring miRNA biogenesis in cancer is beneficial in most instances, while abrogating  
170 microRNA biogenesis might have some beneficial effects. For example, knockdown of Ago2 by  
171 siRNA in myeloid leukaemia cells induces cell apoptosis [64]. However, such treatments might

172 exhibit several side effects in clinical setting as it would affect the homeostasis of miRNA  
173 biogenesis in the entire body. If any of the drugs proves to be selectively affecting the behaviour  
174 of cancer cells and if the side effects are temporary and reversible, then these miRNA-biogenesis  
175 modulating drugs can be beneficial to use over conventional chemotherapeutic drugs as they do  
176 not possess direct DNA damaging property. Similarly, drugs that selectively inhibit the  
177 biosynthesis of specific microRNAs can be used. For example, a synthetic peptides blocks pre-  
178 miR-21 [65] and TargapremiR-210 blocks pre-miR-210 biogenesis [66], see **Figure 1**.  
179 Alternatively, chemicals that activate the expression of microRNAs can be used. Phenformin, an  
180 anti-diabetic drug, increases the expression of let-7, miR-124 and miR-137 resulting in the  
181 inhibition of GBM stemness and growth [67]. Silibinin treatment upregulated miR-494, which  
182 downregulated ADAM10 and BMI1 resulting in inhibition of tumour growth and stemness in  
183 head and neck cancer. Blocking the protease activity of ADAM10 (A Disintegrin And  
184 Metalloprotease Domain 10), an enzyme responsible for Neuroligin-3 (NLGL3) secretion,  
185 potently inhibits *in vivo* glioma growth [68]. Hence, silibinin could be useful for GBM treatment.  
186 Erismodegib, a phase III medulloblastoma drug, upregulates tumour suppressor miR-128, anti-  
187 EMT miR-200 family and suppresses anti-apoptotic miR-21 in glioma-initiating cells [69]. 5-  
188 aza-2'-deoxycytidine and curcumin upregulate miR-145 expression [42, 70]. Curcumin but also  
189 enhanced the activity miR-145 by blocking the expression of LncRNA-ROR, a ceRNA that  
190 quenches miR-145 activity [71]. Klinger *et al* points out the importance and desperate need of  
191 clinical trials with curcumin for glioblastoma and other brain tumours [72]. These literatures  
192 indicate the possibility of repurposing FDA approved drugs and other natural products based on  
193 the beneficial miRNA modulation. Also, these small-molecule drugs have good pharmacokinetic

194 properties compared to nucleic acid therapeutics and can then be synergistically used for cancer  
195 therapy helping to reduce or replace genotoxic chemotherapeutic interventions.

196

### 197 ***Gene therapy Interventions***

198 CRISPR/Cas9 Genome editing enables permanent changes in specific locations on the genome.

199 Cas9 is a bacterial endonuclease guided by a dual-RNA system comprising a CRISPR RNA

200 (crRNA) and a trans-activating crRNA (tracrRNA). Jinek *et al* engineered a single guide RNA

201 (sgRNA) to replace the dual RNA [73]. sgRNA/Cas9 nucleoprotein complex creates double

202 strand breaks (DBS) in a specific genome location complimentary to sgRNA and a “NGG”

203 triple-nucleotide protospacer adjacent motif (PAM) [73]. These DBS are fixed by one of the two

204 DNA repair mechanisms, error-prone non-homologues end-joining (NHEJ) or template-mediated

205 homology directed repair (HDR). NHEJ creates insertion/deletion mutations (INDEL). Such

206 mutations in the stemloop structure of a specific pri-miRNA disrupts its biogenesis and this

207 strategy is used for selective knock-out of onco-miRNAs [47, 74]. Ablation of miR-10

208 expression in GBM caused death of tumour cells not normal cells [47]. Similarly, a missing gene

209 (e.g. ts-miRNA) can be introduced by harnessing HDR mechanism, by supplying a DNA

210 template; however, CRISPR/Cas9 mediated gene insertion is relatively less common due to the

211 challenges associated with the co-delivery of DNA-template [75, 76]. Cas9-mediated HDR were

212 used to modify mesenchymal stem cells (MSCs) with electroporation to overcome the limitations

213 of using lentiviral vectors [76]. Hirosawa *et al* developed miR-Cas9-ON and OFF systems for

214 cell-type specific genome editing controlled by endogenous miRNA levels [77]. This study also

215 used Cas9 to cause several DBS using a sgRNA which targeting Alu1 region [77]. As majority

216 of human miRNA expression profile and their promoters are mapped, the specificity and safety

217 can be increased by including multiple miRNA-target sites in the Cas9-gene [77]. Similar  
218 miRNA responsive system using CymR as a transcriptional repressor was used in RNAi-  
219 Inducible Luciferase Expression System (RILES) [78]. Zika virus shown GSC-specific  
220 proliferation and oncolytic [79]. This ability can be further used for delivery of miRNA or the  
221 replication of the virus could be limited to GBM by intentionally including miRNA target sites in  
222 the vital genes. T-VISA-miR-34a plasmid system that overexpresses the encoded miRNA is also  
223 modified to specifically express the miRNA only in the cells that express high levels of hTERT,  
224 hence targeting cancer cells [80]. This system has a prolonged miRNA activity at the therapeutic  
225 level, one week compared to 2 days for miRNA mimics approach [80]. MicroRNA-sponges are  
226 artificial sequences encoded in a plasmid that potently inhibit miRNA function like the natural  
227 ceRNAs [81].

228

### 229 ***Oligonucleotide Therapy***

230 MicroRNA-mimics are double stranded RNAs (dsRNAs) used for replacing missing endogenous  
231 miRNA. Yu *et al* showed that free metabolically stabilized single-stranded-siRNA can function  
232 like dsRNA. When stereotactically injected into mouse brain ss-siRNA combined with AGO2  
233 and inhibited target genes without any carrier system [82]. On the other hand, anti-sense  
234 oligonucleotides (ASOs) are single stranded RNAs (ssRNAs) that compromise mature miRNAs  
235 functions by tight base-pairing which also cause the release of miRNA from RISC complex and  
236 blocks its interaction with its target mRNAs [83]. They are referred by different terminologies  
237 such as antagomiRs, anti-miRs, anti-microRNA oligonucleotides (AMOs) or Locked Nucleic  
238 Acids (LNA). Small RNA zippers are a new class of ssDNA-LNA designed to block a miRNA  
239 by connecting its 3'-end sequences of a mature miRNA to the 5'-end sequences of the adjacent

240 miRNA molecule [84]. Most of the clinical trials with ODNs are conducted with naked RNA, i.e.  
241 without a carrier system. ODNs are chemically modified to achieve desired properties to meet  
242 the special requirements of RNA-delivery into target cells [82, 85]. Most often, they contain a  
243 mix of chemical modifications optimised for required function [82, 85-87]. To be clinically  
244 active, these synthetic oligonucleotides (ODNs) must survive endo and exonucleases in the body,  
245 avoid immune activation, avoid sequestration by reticuloendothelial system, have longer  
246 circulation time, have access to its target site (passive diffusion), preferentially taken up by the  
247 target cells (active targeting) and have access to the cytoplasm which is the site of action [85].  
248 Additional requirements for an effective, functional of miRNA-mimics includes successful  
249 RISC-loading, passenger strand separation, target interaction, translational suppression and  
250 release [85].

251 After they are released, the targeting ability of the miRNA mimics might require RNA editing  
252 enzymes like ADAR (Adenosine deaminase acting on RNA) and APOBEC1 (apolipoprotein  
253 B mRNA editing enzyme, catalytic polypeptide-like-1) to function like natural miRNAs [88].  
254 ADAR2 acts on dsRNA and mediates adenosine-to-inosine (A-to-I) editing which changes  
255 miRNA target specificity and is essential for the stability and normal functioning of certain  
256 miRNAs [88]. ADAR2 is downregulated in GBM resulting in hypo-edition of miRNAs [88].  
257 This problem can be overcome if the supplied miR-mimics already contain inosine at appropriate  
258 positions. Some other problems associated with miRNA-resistance includes the expression of  
259 competitive endogenous RNAs (ceRNAs) [71] and competitive endogenous RNA-binding  
260 proteins (ceRBPs) can bind to miRNA-target sites (MRE) preventing access to RISC and hence  
261 block miR-mediated gene-silencing [89]. In a 3D *in-vivo* setting, gap-junction (connexin)  
262 mediated intracellular transport of miRNA [23] could dilute the miRNA concentration in the

263 target cells, but also could help to spread the gene-silencing activity to neighbouring cells [23].  
264 All these points must be considered to achieve successful miRNA function. Chemical  
265 modifications are discussed in **Table 1** [83, 85-87, 90] and clinical trials are discussed in **Box 1**.  
266 Body fluids contain abundant extracellular miRNA stabilized by exosomes and argonaute protein  
267 complexes and they are biologically active after cellular entry [20, 21, 91] indicating that  
268 chemical modifications can be beneficial but not a requirement to achieve RNAi effects when  
269 combined with RNA-stabilizing nanocarriers derived from both natural and synthetic sources.

270

### 271 **Natural and Bio-mimetic Nanomedicine**

272 Exosomes and microvesicles are naturally secreted miRNA loaded vesicles, which are known to  
273 act as natural delivery and signalling systems for cell to cell communication [92]. These vesicles  
274 can be charged with miRNA either by transfecting huge amount of exogenous miRNA in to the  
275 packaging cells or by directly transfecting the exosomes by electroporation or chemical methods  
276 [92-94]. Alternatively, mesenchymal stem-cells (MSC) or tumour cells can be modified ex-vivo,  
277 i.e. overloading it with excess of a miRNA, either by miRNA-mimics transfection or over-  
278 expression which enrich the secreted exosomes with of those miRNA [95]. These modified  
279 MSCs can be injected periodically to delivery miRNA. Also, GBM cells have been used for the  
280 delivery of exosomal miRNA [56]. The opportunities and aptness of argonaute as a miRNA  
281 delivery system is discussed in **Box 2**.

282

### 283 **Bio-mimetic Delivery Systems**

284 Bacterial-mediated RNAi delivery (transkingdom RNAi) has also been explored where the  
285 microRNA of interest can be expressed in bacteria through a plasmid encoding its hairpin loop

286 structure as described for *in vitro* siRNA delivery [96]. The virus-like particles (VLPs) via  
287 bacteriophage MS2 can deliver a miRNA. MS2 VLPs are biocompatible, biodegradable, stable  
288 and the synthesis is simple [97].

289

## 290 **Synthetic Nanoparticles**

291 Spherical nucleic acids (SNA) consist of a core gold nanoparticle (AuNP) with its surface  
292 densely packed with oligonucleotides attached via thiolate-Au interaction. The passenger strand  
293 bearing a thiol (SH) group could react with gold nanoparticles to deliver miRNA or siRNA. The  
294 guide strand would be separated from the passenger strand inside the cells by the RNAi  
295 machinery [98]. These nanoparticles show high transfection efficiency, low toxicity and ability  
296 to cross blood brain barrier (BBB) without a targeting ligand [98].

297 Cell penetrating peptides (CPP) and other cationic peptides can be complexed with nucleic acids  
298 to form high efficiency delivery systems. Introducing thiol group in the peptide helps to create  
299 bioreducible polymeric peptide that can specifically release in the intracellular environment and  
300 deliver both siRNA and plasmid DNA [99]. A liposome containing siRNA-CPP complexes and  
301 magnetic nanoparticles were used where magnetic field was used to concentrate the  
302 nanoparticles at the site tumour and later the release of siRNA-CPP was triggered using an  
303 electric field [100]. This strategy is interesting for selective release of RNAi molecules using  
304 external triggers. Lipid nanocapsules (LNCs) are a class of LNPs with an oily hydrophobic core  
305 stabilized by surfactants. Oligonucleotides like LNAs can be delivered with a cationic peptide  
306 grafted on LNC's surface [101].

307 Polyethylenimine (PEI) is a gold standard cationic polymer used in the nucleic acid delivery. Its  
308 use in clinic is limited by its toxicity [102]. The toxicity issue was overcome by either

309 chemically modifying the more toxic primary and secondary amines into less toxic secondary  
310 and tertiary amines. High molecular weight (HMW) and branched cationic polymers show high  
311 toxicity while the low molecular weight (LMW) and linear polymers show poor nucleic acid  
312 binding and transfection efficiency [102]. One way to overcome this issue is by using hybrid  
313 polymers, e.g. linear PEI-chitosan hybrid nanoparticles showed better transfection efficiency and  
314 improved safety profiles [102]. Another way is by cross-linking LMW polymers with  
315 bioreducible disulphide linkages. For example, thiol cross-linked LMW-PEI polyplexes  
316 conjugated with brain targeting rabies virus glycoprotein (RVG) were useful for the delivery of  
317 miR-124a [103]. These nanoparticles displayed low toxicity and brain targeting capabilities.

318 Endosomal escape and release from nanocarrier are two major factor that impacts the efficiency  
319 of synthetic nanocarriers. The polymeric delivery systems can destabilize the late endosomal  
320 (LE) compartment by proton-sponge effect during its acidification (pH 5 – 6) [104]. Therefore,  
321 endosomal escape of polyplex should accompany the escape of oligonucleotides from the  
322 polymers for a functional activity. Similarly, the fate of ~70 % of siRNA delivered by lipid  
323 nanoparticles reach late endosomes (LE), packed into exosomes for exocytosis [105] and the  
324 remaining would be degraded after fusion with lysosome [104, 106]. Only a small fraction (1 –  
325 2%) of the oligonucleotides reach the cytoplasm that account for the functional activity. This  
326 cytoplasmic release was predicted to happen either by direct fusion of liposomal delivery  
327 systems with cell membrane or destabilization of the endosomal bilayer causing the  
328 oligonucleotides to leak out into the cytoplasm [106, 107]. Hence, strategies that allow the intra-  
329 cellular release of oligonucleotides after internalization should be addressed more seriously to  
330 achieve highly functional delivery systems [108].

331



## 332 **Crossing the BBB and Locoregional Delivery**

333 Blood Brain Barrier (BBB) prevents the access of drugs and nanoparticles from the blood stream  
334 to reach the brain. BBB is a huge challenge to overcome to enable therapeutically significant  
335 amount of cancer drugs to reach the intracranial tumour. Several strategies have been devised to  
336 overcome BBB including ligand-mediated transcytosis [109], temporary physical or chemical  
337 disruption of BBB [110], convection enhanced delivery [111]. Yu *et al* created an optimised bi-  
338 specific antibody targeting human transferrin receptor that enabled BBB crossing in mice and  
339 monkey, while the other arm of the antibody targeted an intra-brain enzyme  $\beta$ -secretase hence  
340 achieving dual targeting [109]. Optimal affinity between ligand and receptor is an important  
341 factor to increase brain uptake, as it prevents receptor degradation thereby making it available for  
342 multiple rounds of transcytosis [109]. In a clinical trial, Carpentier *et al* implanted an ultrasound  
343 (US) transducer in the skull of GBM patients and achieved safe, reversible, loco-regional BBB  
344 opening and observed no dose-limiting toxicities [110]. US-mediated BBB opening enabled the  
345 crossing of gadolinium contrast agent (1kDa) and hydrophilic carboplatin (0.3kDa) [110]. As this  
346 study indicates that the intensity of ultrasound can be safely increased further, this technique can  
347 also be used to facilitate brain entry of ASOs (8kDa), miRNAs (~15kDa) or nanoparticles  
348 (>100kDa).

349 siR-LODER is a PLGA based biodegradable polymeric implant loaded with siRNA polyplexes  
350 that releases siRNA-polyplexes in the local environment for extended period [2 – 5 months]  
351 [112]. The phase I study reported good progress and Silenseed Ltd. is starting a Phase II clinical  
352 trials with siG12D-LODER (siRNA against mutated KRAS oncogene) in combination with  
353 gemcitabine for pancreatic cancer patients (NCT01676259). Such long-term release systems can  
354 be very helpful for loco-regional delivery of RNAi molecules for GBM treatment.

355 **Concluding Remarks and Future Perspectives**

356 A single microRNA regulates multiple genes involved in multiple functions which make them  
357 attractive for cancer therapeutics. MicroRNA modulation strategies can either act as a stand-  
358 alone therapeutic or it can be used in combination with conventional therapies as a sensitizing  
359 agent. Most of the microRNA modulation strategies use plasmid DNA or oligonucleotides and  
360 hence require the aid of special delivery strategies to be effective in clinical setting. Cell delivery  
361 of the nucleic acids is an important technical problem. Improvement in chemical modifications  
362 allow the use of RNAi molecules with greater efficacy under *in vivo* conditions. Simpler delivery  
363 strategies are more likely enter clinical trials, hence naked RNA delivery is attractive. Synthetic  
364 nanoparticles often bring promising results under *in vitro* and *in vivo* conditions, however fail to  
365 deliver in human applications due to stability, delivery, toxicity and targeting issues. Use of  
366 patient derived exosomes would be more biocompatible and safe for clinical applications. The  
367 potential of AGO2 to excel as a RNAi delivery system is high. If argonaute-mediated miRNA  
368 delivery is properly explored, AGO2 can be a breakthrough for RNAi, like CRISPR-Cas9 is for  
369 genome editing. AGO2 will not however solve the delivery concerns with ASOs, hence other  
370 strategies are vital.  
371

372 **References**

- 373 1. Louis, D.N. et al. (2016) The 2016 World Health Organization Classification of Tumors of the  
374 Central Nervous System: a summary. *Acta Neuropathol* 131 (6), 803-20.
- 375 2. Stupp, R. et al. (2005) Radiotherapy plus concomitant and adjuvant temozolomide for  
376 glioblastoma. *N Engl J Med* 352 (10), 987-96.
- 377 3. Stupp, R. et al. (2015) Maintenance Therapy With Tumor-Treating Fields Plus Temozolomide  
378 vs Temozolomide Alone for Glioblastoma: A Randomized Clinical Trial. *JAMA* 314 (23), 2535-  
379 43.
- 380 4. Lee, R.C. et al. (1993) The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with  
381 antisense complementarity to *lin-14*. *Cell* 75 (5), 843-54.
- 382 5. Lee, Y. et al. (2004) MicroRNA genes are transcribed by RNA polymerase II. *Embo j* 23 (20),  
383 4051-60.
- 384 6. Nel, A.E. et al. (2009) Understanding biophysicochemical interactions at the nano-bio  
385 interface. *Nature Materials* 8 (7), 543-557.
- 386 7. Peer, D. et al. (2007) Nanocarriers as an emerging platform for cancer therapy. *Nature*  
387 *Nanotechnology* 2 (12), 751-760.
- 388 8. Bartel, D.P. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116  
389 (2), 281-97.
- 390 9. Finnegan, E.F. and Pasquinelli, A.E. (2013) MicroRNA biogenesis: regulating the regulators.  
391 *Crit Rev Biochem Mol Biol* 48 (1), 51-68.
- 392 10. Han, J. et al. (2006) Molecular basis for the recognition of primary microRNAs by the  
393 Droscha-DGCR8 complex. *Cell* 125 (5), 887-901.
- 394 11. Lund, E. et al. (2004) Nuclear export of microRNA precursors. *Science* 303 (5654), 95-8.
- 395 12. Wilson, R.C. et al. (2015) Dicer-TRBP complex formation ensures accurate mammalian  
396 microRNA biogenesis. *Mol Cell* 57 (3), 397-407.
- 397 13. Ge, Y. et al. (2016) Strand-specific in vivo screen of cancer-associated miRNAs unveils a  
398 role for miR-21( \*) in SCC progression. *Nat Cell Biol* 18 (1), 111-21.
- 399 14. Frank, F. et al. (2010) Structural basis for 5'-nucleotide base-specific recognition of guide  
400 RNA by human AGO2. *Nature* 465 (7299), 818-22.
- 401 15. Bartel, D.P. (2009) MicroRNAs: target recognition and regulatory functions. *Cell* 136 (2),  
402 215--33.
- 403 16. Eichhorn, S.W. et al. (2014) mRNA destabilization is the dominant effect of mammalian  
404 microRNAs by the time substantial repression ensues. *Mol Cell* 56 (1), 104-15.
- 405 17. Jonas, S. and Izaurralde, E. (2015) Towards a molecular understanding of microRNA-  
406 mediated gene silencing. *Nat Rev Genet* 16 (7), 421-33.
- 407 18. Weinmann, L. et al. (2009) Importin 8 is a gene silencing factor that targets argonaute  
408 proteins to distinct mRNAs. *Cell* 136 (3), 496-507.
- 409 19. Nishi, K. et al. (2013) Human TNRC6A is an Argonaute-navigator protein for microRNA-  
410 mediated gene silencing in the nucleus. *RNA* 19 (1), 17-35.
- 411 20. Makarova, J.A. et al. (2016) Intracellular and extracellular microRNA: An update on  
412 localization and biological role. *Prog Histochem Cytochem* 51 (3-4), 33-49.
- 413 21. Arroyo, J.D. et al. (2011) Argonaute2 complexes carry a population of circulating  
414 microRNAs independent of vesicles in human plasma. *Proc Natl Acad Sci U S A* 108 (12), 5003-  
415 8.

416 22. Godlewski, J. et al. (2017) MicroRNA Signatures and Molecular Subtypes of Glioblastoma:  
417 The Role of Extracellular Transfer. *Stem Cell Reports* 8 (6), 1497-1505.

418 23. Zong, L. et al. (2016) Gap junction mediated miRNA intercellular transfer and gene  
419 regulation: A novel mechanism for intercellular genetic communication. *Sci Rep* 6, 19884.

420 24. Zhao, J. (2016) Cancer stem cells and chemoresistance: The smartest survives the raid.  
421 *Pharmacol Ther* 160, 145-58.

422 25. Hanahan, D. and Weinberg, R.A. (2011) Hallmarks of cancer: the next generation. *Cell* 144  
423 (5), 646-74.

424 26. Lages, E. et al. (2011) MicroRNA and target protein patterns reveal physiopathological  
425 features of glioma subtypes. *PLoS One* 6 (5), e20600.

426 27. Piwecka, M. et al. (2015) Comprehensive analysis of microRNA expression profile in  
427 malignant glioma tissues. *Mol Oncol* 9 (7), 1324-40.

428 28. Ye, X. et al. (2017) Identification of microRNAs associated with glioma diagnosis and  
429 prognosis. *Oncotarget* 8 (16), 26394-26403.

430 29. Kozomara, A. and Griffiths-Jones, S. (2014) miRBase: annotating high confidence  
431 microRNAs using deep sequencing data. *Nucleic Acids Res* 42 (Database issue), D68-73.

432 30. Cioce, M. et al. (2016) Mir 145/143: tumor suppressor, oncogenic microenvironmental factor  
433 or ...both? *Aging (Albany NY)*.

434 31. Adlakha, Y.K. and Saini, N. (2014) Brain microRNAs and insights into biological functions  
435 and therapeutic potential of brain enriched miRNA-128. In *Mol Cancer*, p. 33.

436 32. Shi, Z.-M. et al. (2012) MiR-128 inhibits tumor growth and angiogenesis by targeting  
437 p70S6K1. *PLoS One* 7 (3), e32709.

438 33. Papagiannakopoulos, T. et al. (2012) Pro-neural miR-128 is a glioma tumor suppressor that  
439 targets mitogenic kinases. *Oncogene* 31 (15), 1884-1895.

440 34. Shan, Z.N. et al. (2016) miR128-1 inhibits the growth of glioblastoma multiforme and  
441 glioma stem-like cells via targeting BMI1 and E2F3. *Oncotarget* 7 (48), 78813-78826.

442 35. Rooj, A.K. et al. (2017) MicroRNA-Mediated Dynamic Bidirectional Shift between the  
443 Subclasses of Glioblastoma Stem-like Cells. *Cell Rep* 19 (10), 2026-2032.

444 36. Evangelisti, C. et al. (2009) MiR-128 up-regulation inhibits Reelin and DCX expression and  
445 reduces neuroblastoma cell motility and invasiveness. *FASEB J.* 23 (12), 4276-4287,  
446 10.1096/fj.09-134965.

447 37. Godlewski, J. et al. (2008) Targeting of the Bmi-1 Oncogene/Stem Cell Renewal Factor by  
448 MicroRNA-128 Inhibits Glioma Proliferation and Self-Renewal. *Cancer Res.* 68 (22), 9125-  
449 9130.

450 38. (2014) MicroRNA 128a Increases Intracellular ROS Level by Targeting Bmi-1 and Inhibits  
451 Medulloblastoma Cancer Cell Growth by Promoting Senescence.

452 39. Alvarado, A.G. et al. (2016) Coordination of self-renewal in glioblastoma by integration of  
453 adhesion and microRNA signaling. *Neuro Oncol* 18 (5), 656-66.

454 40. Li, C. et al. (2017) The lincRNA-ROR/miR-145 axis promotes invasion and metastasis in  
455 hepatocellular carcinoma via induction of epithelial-mesenchymal transition by targeting ZEB2.  
456 *Sci Rep* 7 (1), 4637.

457 41. Zheng, H. et al. (2014) Fas signaling promotes chemoresistance in gastrointestinal cancer by  
458 up-regulating P-glycoprotein. *Oncotarget*.

459 42. Zhu, X. et al. (2014) miR-145 sensitizes ovarian cancer cells to paclitaxel by targeting Sp1  
460 and Cdk6. *Int J Cancer* 135 (6), 1286-96.

461 43. Xu, Q. et al. (2012) MiR-145 directly targets p70S6K1 in cancer cells to inhibit tumor  
462 growth and angiogenesis. *Nucleic Acids Res* 40 (2), 761-74.

463 44. Zhang, X. et al. (2017) Identification of miRNA-7 by genome-wide analysis as a critical  
464 sensitizer for TRAIL-induced apoptosis in glioblastoma cells. *Nucleic Acids Res* 45 (10), 5930-  
465 5944.

466 45. Tian, R. et al. (2017) Differential expression of miR16 in glioblastoma and glioblastoma  
467 stem cells: their correlation with proliferation, differentiation, metastasis and prognosis.  
468 *Oncogene*.

469 46. Guessous, F. et al. (2013) Oncogenic effects of miR-10b in glioblastoma stem cells. *J*  
470 *Neurooncol* 112 (2), 153-63.

471 47. El Fatimy, R. et al. (2017) Genome Editing Reveals Glioblastoma Addiction to MicroRNA-  
472 10b. *Mol Ther* 25 (2), 368-378.

473 48. Shang, C. et al. (2015) MiR-21 up-regulation mediates glioblastoma cancer stem cells  
474 apoptosis and proliferation by targeting FASLG. *Mol Biol Rep* 42 (3), 721-7.

475 49. Fabbri, M. et al. (2012) MicroRNAs bind to Toll-like receptors to induce prometastatic  
476 inflammatory response. *Proc Natl Acad Sci U S A* 109 (31), E2110-6.

477 50. Hu, J. et al. (2016) MiR-215 Is Induced Post-transcriptionally via HIF-Drosha Complex and  
478 Mediates Glioma-Initiating Cell Adaptation to Hypoxia by Targeting KDM1B. *Cancer Cell* 29  
479 (1), 49-60.

480 51. Mucaj, V. et al. (2015) MicroRNA-124 expression counteracts pro-survival stress responses  
481 in glioblastoma. *Oncogene* 34 (17), 2204-14.

482 52. Shea, A. et al. (2016) MicroRNAs in glioblastoma multiforme pathogenesis and therapeutics.  
483 *Cancer Med* 5 (8), 1917-46.

484 53. Khalil, S. et al. (2016) miRNA array screening reveals cooperative MGMT-regulation  
485 between miR-181d-5p and miR-409-3p in glioblastoma. *Oncotarget* 7 (19), 28195-206.

486 54. Wei, J. et al. (2016) MiR-138 exerts anti-glioma efficacy by targeting immune checkpoints.  
487 *Neuro Oncol* 18 (5), 639-48.

488 55. Svoronos, A.A. et al. (2016) OncomiR or Tumor Suppressor? The Duplicity of MicroRNAs  
489 in Cancer. *Cancer Res* 76 (13), 3666-70.

490 56. Fareh, M. et al. (2017) Cell-based therapy using miR-302-367 expressing cells represses  
491 glioblastoma growth. *Cell Death Dis* 8 (3), e2713.

492 57. Gao, Z. et al. (2015) The miR-302/367 cluster: a comprehensive update on its evolution and  
493 functions. *Open Biol* 5 (12), 150138.

494 58. Guo, Y. et al. (2017) miR-302/367/LATS2/YAP pathway is essential for prostate tumor-  
495 propagating cells and promotes the development of castration resistance. *Oncogene*.

496 59. He, W. et al. (2017) MiR-21 is required for anti-tumor immune response in mice: an  
497 implication for its bi-directional roles. *Oncogene* 36 (29), 4212-4223.

498 60. Li, Y. et al. (2016) Downregulation and tumor-suppressive role of XPO5 in hepatocellular  
499 carcinoma. *Mol Cell Biochem* 415 (1-2), 197-205.

500 61. Sun, H.L. et al. (2016) ERK Activation Globally Downregulates miRNAs through  
501 Phosphorylating Exportin-5. *Cancer Cell* 30 (5), 723-736.

502 62. Gurtner, A. et al. (2016) Dysregulation of microRNA biogenesis in cancer: the impact of  
503 mutant p53 on Drosha complex activity. *J Exp Clin Cancer Res* 35, 45.

504 63. Rupaimoole, R. et al. (2014) Hypoxia-mediated downregulation of miRNA biogenesis  
505 promotes tumour progression. *Nat Commun* 5, 5202.

506 64. Naoghare, P.K. et al. (2011) Knock-down of argonaute 2 (AGO2) induces apoptosis in  
507 myeloid leukaemia cells and inhibits siRNA-mediated silencing of transfected oncogenes in  
508 HEK-293 cells. *Basic Clin Pharmacol Toxicol* 109 (4), 274-82.

509 65. Shortridge, M.D. et al. (2017) A Macrocyclic Peptide Ligand Binds the Oncogenic  
510 MicroRNA-21 Precursor and Suppresses Dicer Processing. *ACS Chem Biol* 12 (6), 1611-1620.

511 66. Costales, M.G. et al. (2017) Small Molecule Inhibition of microRNA-210 Reprograms an  
512 Oncogenic Hypoxic Circuit. *J Am Chem Soc* 139 (9), 3446-3455.

513 67. Jiang, W. et al. (2016) Repurposing phenformin for the targeting of glioma stem cells and the  
514 treatment of glioblastoma. *Oncotarget* 7 (35), 56456-56470.

515 68. Venkatesh, H.S. et al. (2017) Targeting neuronal activity-regulated neuroligin-3 dependency  
516 in high-grade glioma. *Nature advance online publication*.

517 69. Fu, J. et al. (2013) NPV-LDE-225 (Erismodegib) inhibits epithelial mesenchymal transition  
518 and self-renewal of glioblastoma initiating cells by regulating miR-21, miR-128, and miR-200.  
519 *Neuro Oncol* 15 (6), 691-706.

520 70. Zaman, M.S. et al. (2010) The functional significance of microRNA-145 in prostate cancer.  
521 *Br J Cancer* 103 (2), 256-64.

522 71. Liu, T. et al. (2017) Curcumin suppresses proliferation and in vitro invasion of human  
523 prostate cancer stem cells by ceRNA effect of miR-145 and lncRNA-ROR. *Gene*.

524 72. Klinger, N.V. and Mittal, S. (2016) Therapeutic Potential of Curcumin for the Treatment of  
525 Brain Tumors. *Oxid Med Cell Longev* 2016, 9324085.

526 73. Jinek, M. et al. (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive  
527 bacterial immunity. *Science* 337 (6096), 816-21.

528 74. Chang, H. et al. (2016) CRISPR/cas9, a novel genomic tool to knock down microRNA in  
529 vitro and in vivo. *Sci Rep* 6, 22312.

530 75. Chen, Z.H. et al. (2017) Targeting genomic rearrangements in tumor cells through Cas9-  
531 mediated insertion of a suicide gene. *Nat Biotechnol* 35 (6), 543-550.

532 76. Meca-Cortes, O. et al. (2017) CRISPR/Cas9-Mediated Knockin Application in Cell Therapy:  
533 A Non-viral Procedure for Bystander Treatment of Glioma in Mice. *Mol Ther Nucleic Acids* 8,  
534 395-403.

535 77. Hirosawa, M. et al. (2017) Cell-type-specific genome editing with a microRNA-responsive  
536 CRISPR-Cas9 switch. *Nucleic Acids Res* 45 (13), e118.

537 78. Ezzine, S. et al. (2013) RILES, a novel method for temporal analysis of the in vivo regulation  
538 of miRNA expression. *Nucleic Acids Res* 41 (20), e192.

539 79. Zhu, Z. et al. (2017) Zika virus has oncolytic activity against glioblastoma stem cells. *J Exp*  
540 *Med*.

541 80. Li, L. et al. (2012) Targeted expression of miR-34a using the T-VISA system suppresses  
542 breast cancer cell growth and invasion. *Mol Ther* 20 (12), 2326-34.

543 81. Ma, S. et al. (2017) Combination of AAV-TRAIL with miR-221-Zip Therapeutic Strategy  
544 Overcomes the Resistance to TRAIL Induced Apoptosis in Liver Cancer. *Theranostics* 7 (13),  
545 3228-3242.

546 82. Yu, D. et al. (2012) Single-stranded RNAs use RNAi to potently and allele-selectively inhibit  
547 mutant huntingtin expression. *Cell* 150 (5), 895-908.

548 83. Ariyoshi, J. et al. (2015) Development of Novel Antisense Oligonucleotides for the  
549 Functional Regulation of RNA-Induced Silencing Complex (RISC) by Promoting the Release of  
550 microRNA from RISC. *Bioconjug Chem* 26 (12), 2454-60.

551 84. Meng, L. et al. (2017) Small RNA zippers lock miRNA molecules and block miRNA  
552 function in mammalian cells. *Nat Commun* 8, 13964.

553 85. Khvorova, A. and Watts, J.K. (2017) The chemical evolution of oligonucleotide therapies of  
554 clinical utility. *Nat Biotechnol* 35 (3), 238-248.

555 86. Nikan, M. et al. (2016) Docosahexaenoic Acid Conjugation Enhances Distribution and  
556 Safety of siRNA upon Local Administration in Mouse Brain. *Mol Ther Nucleic Acids* 5 (8),  
557 e344.

558 87. Elkayam, E. et al. (2017) siRNA carrying an (E)-vinylphosphonate moiety at the 5' end of the  
559 guide strand augments gene silencing by enhanced binding to human Argonaute-2. *Nucleic  
560 Acids Res* 45 (6), 3528-3536.

561 88. Paul, D. et al. (2017) A-to-I editing in human miRNAs is enriched in seed sequence,  
562 influenced by sequence contexts and significantly hypoadapted in glioblastoma multiforme. *Sci  
563 Rep* 7 (1), 2466.

564 89. Degrauwe, N. et al. (2016) The RNA Binding Protein IMP2 Preserves Glioblastoma Stem  
565 Cells by Preventing let-7 Target Gene Silencing. *Cell Rep* 15 (8), 1634-47.

566 90. Esposito, C.L. et al. (2016) A combined microRNA-based targeted therapeutic approach to  
567 eradicate glioblastoma stem-like cells. *J Control Release* 238, 43-57.

568 91. Li, L. et al. (2012) Argonaute 2 complexes selectively protect the circulating microRNAs in  
569 cell-secreted microvesicles. *PLoS One* 7 (10), e46957.

570 92. Wahlgren, J. et al. (2016) Delivery of Small Interfering RNAs to Cells via Exosomes.  
571 *Methods Mol Biol* 1364, 105-25.

572 93. Lunavat, T.R. et al. (2016) RNAi delivery by exosome-mimetic nanovesicles - Implications  
573 for targeting c-Myc in cancer. *Biomaterials* 102, 231-8.

574 94. Haraszti, R.A. et al. (2017) Loading of Extracellular Vesicles with Chemically Stabilized  
575 Hydrophobic siRNAs for the Treatment of Disease in the Central Nervous System. *Bio Protoc* 7  
576 (20).

577 95. Lee, H.K. et al. (2013) Mesenchymal stem cells deliver synthetic microRNA mimics to  
578 glioma cells and glioma stem cells and inhibit their cell migration and self-renewal. *Oncotarget* 4  
579 (2), 346-61.

580 96. Ahmed, O. et al. (2015) Delivery of siRNAs to cancer cells via bacteria. In *Methods Mol  
581 Biol* (2014/10/17 edn), pp. 117-29.

582 97. Ashley, C.E. et al. (2011) Cell-specific delivery of diverse cargos by bacteriophage MS2  
583 virus-like particles. *ACS Nano* 5 (7), 5729-45.

584 98. Kouri, F.M. et al. (2015) miR-182 integrates apoptosis, growth, and differentiation programs  
585 in glioblastoma. *Genes Dev* 29 (7), 732-45.

586 99. Yoo, J. et al. (2017) Bioreducible branched poly(modified nona-arginine) cell-penetrating  
587 peptide as a novel gene delivery platform. *J Control Release* 246, 142-154.

588 100. Yang, Y. et al. (2016) Thermal and magnetic dual-responsive liposomes with a cell-  
589 penetrating peptide-siRNA conjugate for enhanced and targeted cancer therapy. *Colloids Surf B  
590 Biointerfaces* 146, 607-15.

591 101. Griveau, A. et al. (2013) Silencing of miR-21 by locked nucleic acid-lipid nanocapsule  
592 complexes sensitize human glioblastoma cells to radiation-induced cell death. *Int J Pharm.*

593 102. Tripathi, S.K. et al. (2012) Linear polyethylenimine-graft-chitosan copolymers as efficient  
594 DNA/siRNA delivery vectors in vitro and in vivo. *Nanomedicine* 8 (3), 337-45.

595 103. Hwang do, W. et al. (2011) A brain-targeted rabies virus glycoprotein-disulfide linked PEI  
596 nanocarrier for delivery of neurogenic microRNA. *Biomaterials* 32 (21), 4968-75.

597 104. Gujrati, M. et al. (2016) Multifunctional pH-Sensitive Amino Lipids for siRNA Delivery.  
598 *Bioconjug Chem* 27 (1), 19-35.

599 105. Sahay, G. et al. (2013) Efficiency of siRNA delivery by lipid nanoparticles is limited by  
600 endocytic recycling. *Nat Biotechnol* 31 (7), 653-8.

601 106. Juliano, R.L. and Carver, K. (2015) Cellular uptake and intracellular trafficking of  
602 oligonucleotides. *Adv Drug Deliv Rev* 87, 35-45.

603 107. Gilleron, J. et al. (2013) Image-based analysis of lipid nanoparticle-mediated siRNA  
604 delivery, intracellular trafficking and endosomal escape. *Nat Biotechnol* 31 (7), 638-46.

605 108. Gilleron, J. et al. (2015) Identification of siRNA delivery enhancers by a chemical library  
606 screen. *Nucleic Acids Research*.

607 109. Yu, Y.J. et al. (2014) Therapeutic bispecific antibodies cross the blood-brain barrier in  
608 nonhuman primates. *Sci Transl Med* 6 (261), 261ra154.

609 110. Carpentier, A. et al. (2016) Clinical trial of blood-brain barrier disruption by pulsed  
610 ultrasound. *Sci Transl Med* 8 (343), 343re2.

611 111. Halle, B. et al. (2016) Convection-enhanced delivery of an anti-miR is well-tolerated,  
612 preserves anti-miR stability and causes efficient target de-repression: a proof of concept. *J*  
613 *Neurooncol* 126 (1), 47-55.

614 112. Zorde Khvalevsky, E. et al. (2013) Mutant KRAS is a druggable target for pancreatic  
615 cancer. *Proc Natl Acad Sci U S A* 110 (51), 20723-8.

616 113. Bartel, D. (2004) MicroRNAs Genomics, Biogenesis, Mechanism, and Function. *Cell* 116  
617 (2), 281--297.

618 114. Toscano-Garibay, J.D. and Aquino-Jarquin, G. (2014) Transcriptional regulation  
619 mechanism mediated by miRNA-DNA\*DNA triplex structure stabilized by Argonaute. *Biochim*  
620 *Biophys Acta* 1839 (11), 1079-83.

621 115. Wang, Z. (2011) The principles of MiRNA-masking antisense oligonucleotides technology.  
622 *Methods Mol Biol* 676, 43-9.

623 116. Weil, S. et al. (2017) Tumor microtubules convey resistance to surgical lesions and  
624 chemotherapy in gliomas. *Neuro Oncol* 19 (10), 1316-1326.

625 117. Hong, X. et al. (2015) Gap junctions modulate glioma invasion by direct transfer of  
626 microRNA. *Oncotarget* 6 (17), 15566-77.

627 118. Prud'homme, G.J. et al. (2016) Neuropilin-1 is a receptor for extracellular miRNA and  
628 AGO2/miRNA complexes and mediates the internalization of miRNAs that modulate cell  
629 function. *Oncotarget* 7 (42), 68057-68071.

630 119. Kang, T. et al. (2016) Synergistic targeting tenascin C and neuropilin-1 for specific  
631 penetration of nanoparticles for anti-glioblastoma treatment. *Biomaterials* 101, 60-75.

632 120. Ferreira, R. et al. (2014) Argonaute-2 promotes miR-18a entry in human brain endothelial  
633 cells. *J Am Heart Assoc* 3 (3), e000968.

634 121. van der Ree, M.H. et al. (2016) Miravirsin dosing in chronic hepatitis C patients results in  
635 decreased microRNA-122 levels without affecting other microRNAs in plasma. *Aliment*  
636 *Pharmacol Ther* 43 (1), 102-13.

637 122. Van Der Ree, M. et al. LO7 : A single subcutaneous dose of 2mg/kg or 4mg/kg of RG-101,  
638 a GalNAc-conjugated oligonucleotide with antagonist activity against MIR-122, results in  
639 significant viral load reductions in chronic hepatitis C patients. *Journal of Hepatology* 62, S261.

640



641 **Legend - Figure 1: MicroRNA Biogenesis and MicroRNA Modulating Strategies**

642 (a) **Canonical Biogenesis of miRNA:** (adapted and modified with permission [113]) MiRNA  
643 genes are transcribed by RNA polymerases in the nucleus forming large primary-miRNA (> 500  
644 bases) which harbours one or more stem-loop structures [5]. The RNA-binding protein (Di  
645 George syndrome Critical Region gene 8, DGCR8/Pasha) and a RNase III endonuclease  
646 (Drosha) recognizes the stemloop structures and releases the stem-loop Precursor miRNA (Pre-  
647 miRNA, ~70 nucleotides) [10]. Pre-miRNA is then transported out of the nucleus through  
648 nuclear pore complexes (NPC) by binding to Exportin-5 (XPO5) and Ran-GTPase [11]. The pre-  
649 miRNA has two arms (5' and 3') each might encode an active miRNA sequence named as miR-  
650 X-5p (Red strand) or miR-X-3p (Black strand) respectively. Once in the cytoplasm, the pre-  
651 miRNA loads into a pre-RISC (RNA induced silencing complex) consisting of Dicer, TAR RNA  
652 binding protein (TRBP), one of the argonautes (AGO1 - AGO4) and chaperones (Heat Shock  
653 Proteins, HSP70/HSP90) [8, 9, 12]. Dicer cleaves the stem-loop structure creating a ~23bp  
654 miRNA duplex which loads into an argonaute (AGO) [8, 9, 12]. Either miR-5p or miR-3p strand  
655 loads into the AGO [13, 14]. Binding of seed sequence (2 – 7 nucleotides at the 5'-end of the  
656 miRNA, highlighted in green) to the 3'-untranslated region (3'-UTR) of the target mRNA results  
657 in the inhibition of protein synthesis either by messenger RNA (mRNA) destabilization (>75%)  
658 and translational repression [16, 17].

659 (b) **Other functions of miRNA and RISC:** RISC complex also exerts nuclear functions by  
660 shuttling between cytoplasm and nucleus via Exportin 1 and Importin 8 [18, 19, 114] and  
661 possibly mitochondrial functions [20]. Secreted miRNA, AGO2 or extracellular vesicles loaded  
662 form [20-22].

663 **(c) MicroRNA Modulation Strategies:** Genome editing [47, 74]; Small Molecule miRNA  
664 Inhibitors (e.g. TargapremiR) [66]; MicroRNA mimics and ss-siRNA [82, 85]; Antisense  
665 Oligonucleotides (ASO) [84]; MicroRNA Sponges (Gene Therapy) [81]; MicroRNA Masks  
666 [115].

667 **(d) Can recombinant-AGO2 with targeting ligands acts as a RNAi delivery system?**

668 **Legend - Figure 2: Role of miRNA in the Hallmarks of Cancer and Treatment Resistance**

669 Standard glioblastoma treatment involves radio and chemotherapy which damage DNA and  
670 create double strand breaks (DSBs). Numbers 1 to 7 show various resistance mechanisms leading  
671 to treatment failure. (1) Efflux pumps reduce intracellular drug concentration [41]; (2)  
672 Detoxifying enzymes inactivate the drug [24]; (3) DNA repair reverses lethal DSBs and DNA  
673 damages; MGMT is a DNA repair enzyme targeted by miR-181d and miR-409 [53]; (4, 5)  
674 Damaged DNA induces cell death signals and stop cell cycle progression. For detailed list of  
675 resistance genes and mechanisms, see review [24]; (6) Tumour-microtubes and connexin  
676 mediated invasion through exchange of resistance factors (e.g. miRNAs) helps cancer cells to  
677 adapt to genotoxic treatments and surgery [116, 117]; (7) ceRNA inhibiting ts-miRNA [71]; (8)  
678 Small molecule drugs can be used to increase the expression of tumour-suppressor miRNAs and  
679 block onco-miRs [42, 66, 67, 69, 71]; (9) Role of ts-miRNAs (miR-128 [32-38], miR-145 [30,  
680 39-43], miR-7 [44], miR-16 [45]) and oncomiRs (miR-21-5p [48] and miR-21-3p [13]) in  
681 controlling other hallmarks of cancer. (10) Exosomal miRNAs (especially miR-21 and miR-29a)  
682 can directly bind to Toll-like receptors (TLR7 and TLR8) in the endosomal compartment of  
683 macrophages resulting in pro-metastatic inflammation [49]; (11) Vascular endothelial cells  
684 express neuropilin1 (NRP1) which acts a receptor for AGO2-miRNA complexes [118-120].  
685 Functional significance of AGO2-miRNA complexes in GBM is yet unknown. (12) Legend  
686 explaining the symbols.

687 **Box 1** Clinical Trials with AMOs and MicroRNA mimics

688 Miravirsen was the first AMO in human clinical trials. Miravirsen development was  
689 discontinued due to poor performance in liver, attributed to the lack of uptake enhancers like  
690 targeting ligands or nanocarriers [121]. RG-101, the next generation GalNAc conjugated  
691 antagomiR against miR-122 is under phase II clinical trial conducted by Regulus Therapeutics  
692 (EudraCT Number: 2015-001535-21). The targeted RG-101 has shown significant reduction in  
693 the viral load with a single subcutaneous injection at 2 and 4 mg/kg of dosing [122]. These  
694 clinical trials emphasise the importance of cellular uptake enhancers is required for the activity  
695 of ODNs *in vivo*. MRX34 by Mirna Therapeutics is the only miRNA therapeutic in clinical trials  
696 and uses SMARTICLE® lipid based formulation. Phase I clinical trial (NCT01829971)  
697 evaluating maximum tolerated dose was completed. The clinical trial with MRX34 and all other  
698 pipeline R&D programmes were voluntarily discontinued by the Mirna Therapeutics due to  
699 serious immune related adverse events. This clinical study emphasises on the safety of RNA-  
700 delivery systems is paramount for achieving success in clinic.

701

## 702 **Box 2: Argonaute as miRNA Delivery Systems**

703 Extracellular microRNAs are extremely stable and abundantly found in all body fluids, either  
704 bound to argonaute proteins (especially AGO2) or encapsulated in extracellular vesicles (EV)  
705 [20, 21, 91]. Also, AGO2 stabilizes many of the miRNAs in exosomes [91]. EV-associated  
706 circulating miRNA and its role in cell-to-cell communication is increasingly becoming evident  
707 while the functions of AGO2-associated miRNAs are explored very less. Ferreira *et al* showed  
708 that AGO2 increased miRNA uptake in selective human endothelial cells (EC), derived from  
709 cerebral arteriovenous malformation (AVM), normal and glioma endothelium, without the need  
710 of any transfection agent [120]. AGO2 also protected miRNA from degradation after cellular  
711 entry and they were functionally active under both *in-vitro* and *in-vivo* conditions [120]. In an  
712 intracranial glioma model, intravenously injected AGO2-miR-18a complexes inhibited  
713 angiogenesis by specific internalization by brain endothelium which is in direct contact with  
714 blood stream [120]. The specificity of AGO2-miR uptake strongly indicates the involvement of  
715 receptor mediated endocytosis. Prud'homme *et al* showed neuropilin-1 (NRP1) acts as a receptor  
716 mediating translocation of free miRNAs and productive uptake of AGO2-miRNA complexes  
717 [118]. NRP1 is highly expressed in vascular endothelial cells, GBM and other cancer cells which  
718 makes them ideal targets for AGO2-mediated miRNA delivery and might explain the results  
719 from Ferreira *et al* [118-120]. The AGO2-mediated RNAi delivery can be expanded to other  
720 tissues by attaching ligands on its surface or producing recombinant AGO2-ligand fusion  
721 proteins, see **Figure 1**. Also, AVM-EC can be used for AGO-miRNA delivery as it is known to  
722 secrete AGO2. Unlike other delivery systems where the guide strand must be load into  
723 endogenous AGO to be functional, AGO2-RNAi would be functional once reaching the  
724 cytoplasm. Pre-loading the exogenous AGO would minimize the toxicity associated with the

725 overloading and sequestering the endogenous RNAi machinery by exogenous RNAi molecules.  
726 Also, loading AGO2 with a mature guide strand eliminates the non-specific effects associated  
727 with the passenger strand. AGO2 protects the RNA from degradation, hence no chemical  
728 modifications are necessary; AGO2 is conserved across all humans and hence the native form of  
729 recombinant-AGO2 is probably safe for systemic delivery. Exploring the endocytosis and  
730 endosomal-release pathways of AGO2-miRNA complexes are needed. With proper exploration,  
731 AGO2 can become the solution for RNAi delivery as it has the potential to overcome several of  
732 the associated barriers.

### **Trends Box (900 characters, including spaces, required)**

- miRNA are essential entities supporting hallmark of GBM development and involved in resistance to conventional therapies.
- miRNA act both as intrinsic and extrinsic signals of GBM cells and communicate closely with their microenvironment.
- miRNA modulating strategies can act at multiple steps of their mode of action. Facing specific GBM issues, due to its location in the seat of our consciousness, they may strongly benefit from new biotechnology and nanotechnology developments.
- Strong evidences for AGO2 to act as RNAi carrier has emerged. With intense research, AGO2 can become a breakthrough for RNAi delivery.
- Essential information toward future applications in human GBM emerged from pioneer clinical trials using single stranded antagomiR targeting miR-122a, PLGA based biodegradable polymeric implants loaded with siRNA polyplexes or liposomal miR-34a mimic.

## Outstanding Questions Box

- Can miRNA solve the treatment resistance, notably radioresistance, in glioblastoma?
- Can small molecule drugs that modulate miRNA become a main stream medicine in GBM treatment? Why not? What are the challenges?
- Can argonaute become the solution for RNAi delivery?
- If argonaute can act as delivery system, can the same techniques be applied for CRISPR-Cas9 delivery or vice versa?
- Can miRNA mimics and anti-miRNA oligonucleotides use be during surgery? Can it reduce implantation of tumor cells dispersed during surgery? Can it help invoke an immune-response against GBM? Can it block neurotrophin release and tumor-microtubule mediated repopulation?
- How can we achieve long-term delivery and functional effects of microRNA in the brain?
- What are the minimal invasive, clinically relevant modes of administration and schedule?
- MicroRNA Replacement Therapy or Inhibition, which is more safe and effective for GBM treatment?
- What would be the best gene therapy method for selective expression of toxic protein under the control of glioblastoma-specific microRNA? How to minimize background expression of inducible systems?
- Can therapeutic weapons dedicated to miRNAs greatly benefit from innovative nanomedicines?
- What are the best microRNA pathways to target?
- How can we improve the safety of delivery systems?



Fig 2 - Role of miRNA in the Hallmarks of Cancer and Treatment Resistance

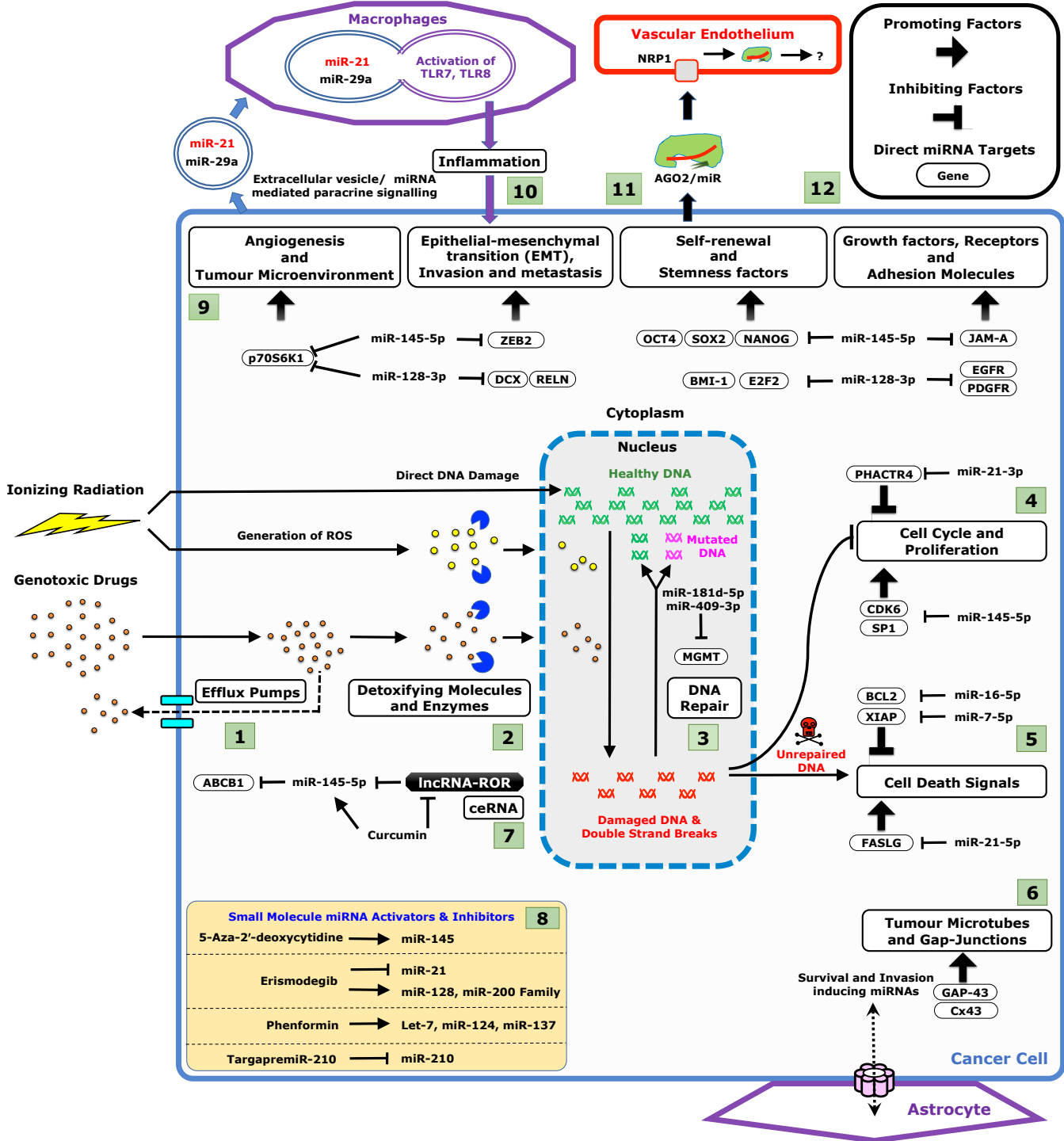
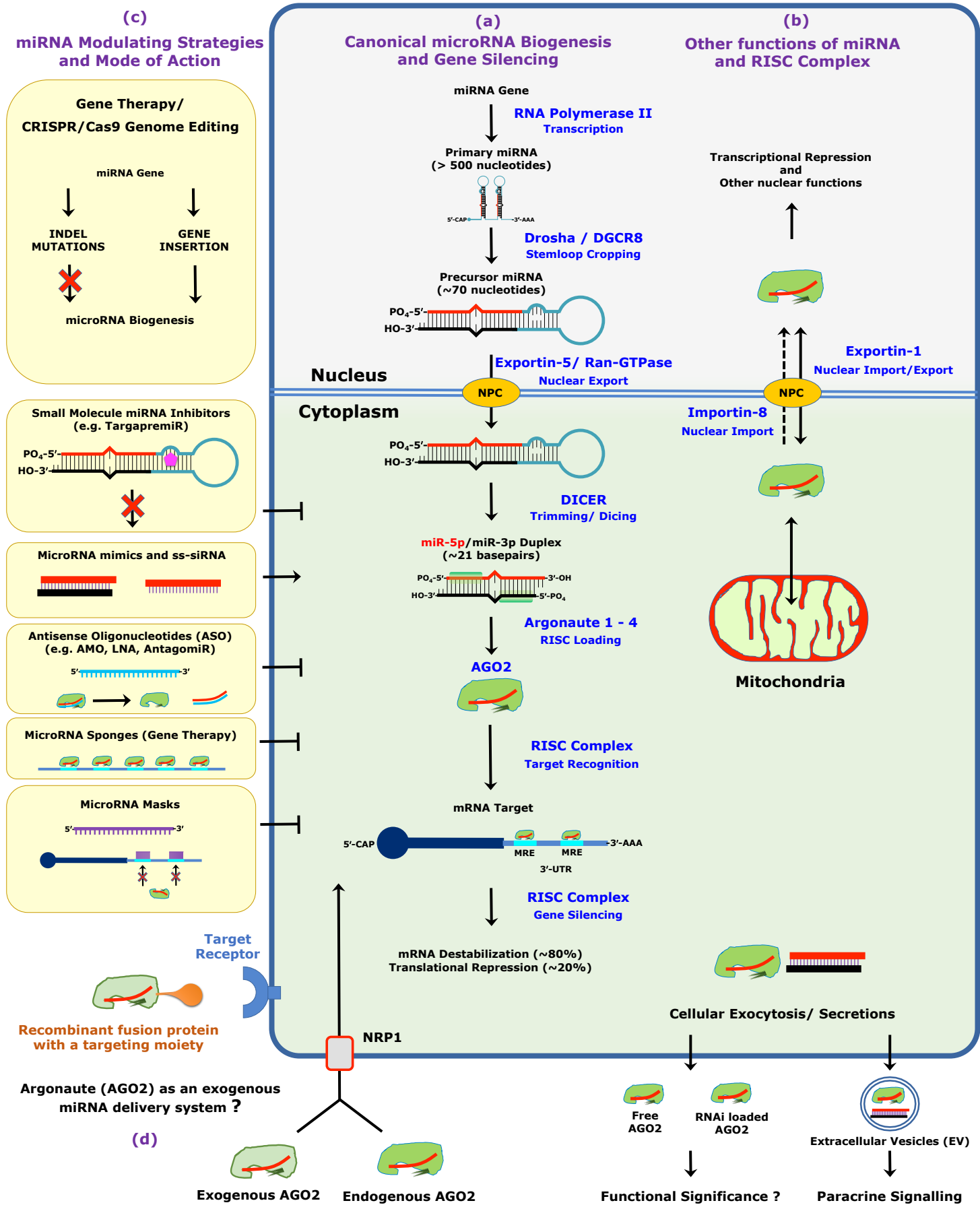


Figure 1



**Table 1 Important Chemical modifications**

<b>Location</b>	<b>Examples of Chemical Modification</b>	<b>References</b>
5'-end	(E)-vinylphosphonate	[87]
3'-end	Aptamer conjugates	[90]
3'-end	hydrophobic moieties	[86]
backbone	phosphorothioate (PS)	[86]
backbone	peptide nucleic acids (PNAs)	[85]
backbone	morpholino	[85]
ribose sugar	2'-O-Methyl	[86]
ribose sugar	2'-O-Fluoro	[86]
ribose sugar	2'-O, 4'-C methylene bridged locked nucleic acids (LNA)	[85]
ribose sugar	2'-O, 4'-C-ethylene-bridged nucleic acids (ENA)	[83]

## Glossary (325/ 450 words)

1. **AGO:** Argonaute is a core element of RNA-induced silencing complex (RISC) which comprises of a single-stranded guide RNA, argonaute and few other proteins. This complex binds to the target mRNA, interferes protein translation or mRNA cleavage resulting in gene silencing. Humans express four different AGOs (AGO1 – 4). AGO2 loaded with a guide RNA is called minimal-RISC as it can be functional *in-vitro* without the need of accessory proteins.
2. **ASOs:** Anti-sense oligonucleotides are synthetic single stranded molecules that binds to mature microRNA by complementary base-pairing. They act as microRNA inhibitors and described with different nomenclature including, antagomiRs, anti-miR, anti-microRNA oligonucleotides (AMO), Locked Nucleic Acid (LNA).
3. **ceRNA:** Competitive endogenous RNAs are natural miRNA-sponges, which includes certain long-non-coding RNAs (lncRNA) and circular RNAs (circRNA), that attenuate specific miRNA functions through their repeated decoy miR-binding sites.
4. **GBM:** Glioblastoma are aggressive, grade IV, malignant brain tumours with most frequent occurrence in adults and has poor prognosis (median survival less than 2 years). They fall under diffuse astrocytic and oligodendroglial phenotype with occasional mutation in IDH gene [Isocitrate dehydrogenase].
5. **Genome editing:** CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeat/CRISPR-associated protein) is a bacterial RNA-guided-endonuclease complex that acts as an immune system against invading plasmid and viral DNA. This system is simplified into sgRNA (single-guide RNA)/Cas9 for editing genomes. sgRNA guides Cas9 to specific locations with complementary DNA sequence. Cas9 creates site-specific double-strand breaks which is subsequently repaired either by homologous recombination (accurate, template-mediated) or nonhomologous end-joining (error-prone). This process is used as an opportunity to modify the genome at precise locations to obtain desired outcome (e.g. gene knock-in or knock-out).
6. **microRNA:** miRNAs are key regulatory elements code by the genome.
7. **miRNA mimics:** A synthetic double stranded molecule with identical sequence similarity to their natural microRNA counterpart. Used for microRNA-Replacement therapy and functional studies.
8. **TTF:** Tumour Treating Field is a low intensity electric field of intermediate frequency (~200kHz) which interferes with mitosis, hinders cell division and consequently induces cell death in dividing cells. TTF is produced by an external, non-invasive transducer-array (Optune®) which can be worn by patients like a cap.

## **Acknowledgements**

This work was supported by Inca (Institut National du Cancer) through the PL-BIO 2014-2020 INCA project MARENGO - “MicroRNA agonist and antagonist Nanomedicines for GliOblastoma treatment: from molecular programming to preclinical validation”. It is also related to the LabEx IRON “Innovative Radiopharmaceuticals in Oncology and Neurology” as part of the french government “Investissements d’Avenir” program. The authors are also thankful to the European support in the frame of the NanoFar consortium, an Erasmus Mundus Joint Doctorate (EMJD) program in nanomedicine and pharmaceutical innovation. European Commission (NanoFar EMJD), “*Le Conseil Général de Maine-et-Loire*” and Inca financially supported SA, AG and CL, respectively.