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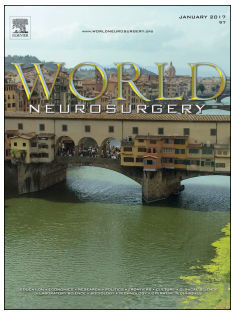

The Role of Circulating MicroRNA in Glioblastoma Liquid Biopsy

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Introduction

Glioblastoma multiforme (GBM) is the most common and aggressive primary malignancy of the central nervous system in adults. The Central Brain Tumor Registry of the United States reports that GBM accounts for 47.7% of primary malignant brain tumors.[1] Despite advancement in treatment, GBM is considered incurable, fatal, and has a 5-year survival rate of 5.6%;[1] although more recent studies suggest 5 year survivals in the 20-30% range with tumor treating fields (TTF) and the autologous tumor lysated pulsed dendritic cell vaccine (DcVax-L).[2,3]

The current standard used to monitor disease progression and therapeutic response has been magnetic resonance imaging (MRI), which is usually obtained pre- and post-operatively. Patients are monitored about every 2 to 3 months. Scans are usually repeated until progression is detected, but sometimes there is an inability to detect tumor progression using imaging,[4] or there is difficulty in differentiating true tumor progression from pseudoprogression.[5] The only reliable method to determine the true nature of radiographic changes in GBM is to perform a brain biopsy, which has its risks. There is a need for a more cost efficient and reliable method of tumor detection, such as circulating biomarkers. The analyzation of potential biomarkers in different malignancies have been an area of great interest and noncoding RNAs have been at the forefront. MicroRNAs (miRNAs) are a large family of short non-coding RNAs that control gene expression at the post-transcriptional level and has been shown to be detectable in different bodily fluids making it a likely candidate.[6]

Biogenesis of miRNAs

MiRNAs are short, single-stranded, non-coding RNAs that are encoded in the genome and post-transcriptionally control gene expression. As noted in figure 1, miRNAs are synthesized by RNA polymerase II as a 30-base pair, double-stranded, stem-loop structure known as primary miRNA precursors (primiRNA). The ribonuclease Drosha binds with DGCR8, a double stranded RNA binding protein, forming the Microprocessor complex that is responsible for the cleavage of primiRNA in the nucleus.[7,8] The primiRNA is released from the Microprocessor as a small hairpin-loop structure known as pre-miRNA. Pre-miRNA is then exported to the cytoplasm by the Ran-GTP-dependent Exportin-5 (XPO5) and processed by Dicer, a helicase-RNase III hybrid.[9] Dicer cuts the pre-miRNA near the terminal loop generating a miRNA duplex that is then dissociated (See Figure 1).

The guide strand of the miRNA duplex is loaded onto an Argonaut (Ago) protein, forming an active RNA Interference Effector Complex (RISC).[10] The 5' end of the miRNA contains a 2-7 nucleotide domain called "the seed" that is vital in the recognition of its target gene.[11] Once bound to its target gene, the miRNA: RNA complex is involved in regulation of the mRNA species via degradation, sponging, splicing, and other mechanisms.

Regulation of miRNAs

The half-life of miRNAs varies widely, but are generally several days; however, some miRNAs have a more rapid turnover.[12] It has been shown that there are certain proteins that can prolong the half-life of miRNAs. For example, evidence suggests that the overexpression of Argonaute proteins increase the stability of miRNA, decelerating their degradation.[13]

Transcription factors, such as p53, MYC, ZEBs, and MYOD1 have been shown to either upregulate or downregulate the expressions of different miRNAs.[14] The expression of miRNAs can alter due to modifications such as methylation or mutations to their promoters. Post-transcriptionally, their expression can also change if there are alterations in the steps of their biogenesis. Mutations or epigenetic modifications that cause an alteration in the activity of key enzymes, such as Dicer or Drosha, can downregulate their expression.[15] For example, in the presence of hypoxic stress, *EGFR* suppresses the development of certain miRNAs that are tumor-suppressor-like in character.[16] *EGFR* was found to associate through phosphorylation with AGO2 protein, reducing the binding of AGO2 to Dicer, and thus suppressing the biogenesis of mature miRNAs.[17]

Role of miRNA in RNA Degradation

MiRNAs usually decrease gene expression through its interaction with their 3' untranslated region.[10] MiRNAs typically do not completely silence their target gene, instead they reduce their expression via different mechanisms such as RNA degradation, induced de-adenylation, reduced ribosome occupancy, sequestration of mRNA, as well as many other mechanisms.[18] It is important to note, that not all interactions involving miRNAs result in reduction of gene expression.

Role of miRNA in RNA Sponging / Competing

The activity of miRNA is modulated by distinctive mechanisms; including sponge and non-sponge modulators. Sponge modulators are mRNAs or noncoding RNAs, which share miRNA binding sites with other RNAs that are targeted by the miRNA.[19] Some endogenous circular RNAs (ceRNA) function as miRNA sponges that impair miRNA activity through sequestration, thus increasing the expression of the miRNA target gene.[20] For example, circNT5E promotes glioma progression by sponging miR-422a and subsequently upregulating levels of NT5E, SOX4, PI3KCA, p-Akt, and p-Smad2.[21] Some artificial sponges are designed as specific competitive inhibitors of miRNA seed families.[22] Artificial miRNA sponges have been used to study the simultaneous effects of several miRNAs.[23]

Non-sponge modulators include RISC components, such as AGO proteins.[24] For example, the binding of a RISC complex to a mRNA target may have the capacity to supplant other RNA binding proteins that are repressive in nature. Such is the case of miRNA-466l antagonizing tristetrapolin in TLR-triggered macrophages, leading to an increase in expression in cytokine interleukin 10.[25]

Role of MiRNA in RNA Splicing

Abnormal alternative splicing plays a role in cancer formation. Intron removal is catalyzed by the spliceosome, a macromolecular complex consisting of small nuclear RNAs and proteins. Studies have reported miRNAs as indirect regulators of alternative splicing by targeting the expression of splicing factors.[26] Serine/arginine-rich splicing factors (SRSFs) are among a family of key splicing factors.[27,28] As a prototypical splicing factor, SRSF1 (SF2/ASF) binds to exonic enhancers and stimulate alternative splicing.[29] There is increased levels of expression of SRSF1 in glioma and higher expressions are associated with worse prognoses.[29] Meseguer et al. noticed that miR-10a and miR-10b target SRSF1.[30] Another study observed that SF2/ASF is also regulated by a negative feedback loop in which miR-7 processing is involved.[28] SR proteins also auto-regulate in a negative feedback loop. Increased levels of SR proteins promote the increase of unproductive spliced variants of their own transcript.[31] MiRNAs target other splicing regulators. MiR-124 downregulates the RNA-binding protein PTBP1, a repressor of nervous system-specific splicing.[32] Furthermore, miR-10a and miR-10b target TRA2β in GBM cells, thus promoting tumor proliferation.[33] TRA2β is a nuclear splicing regulator that stimulates the insertion of alternative exons.[34] The microprocessing of intronic miRNAs also promote the splicing of host introns. For example, the microprocessing of miR-211 promotes the splicing of melastatin.[35]

Role of miRNA in N6-Methyladenosine (m6A)

Of the many modifications detected in mRNAs, m6A is the most prevalent.[36,37] The m6A modification occurring within the RNA motif known as RRACH;[38,39] influences the processing of mRNA from its splicing and translation to its degradation.[40]

RNA methylation regulators include proteins which write (or add methyl groups), read, and erase the methyl groups.[41] Writer proteins include the methyltransferase like 3/14 (METTL3/METTL14) complex, Wilms' tumor 1-associating protein (WTAP), VIRMA and RBM15 make up the m6A methyltransferase complex.[42-44] Of the non-heme Fe II/ α-KG-dependent dioxygenase AlkB family proteins, the fat mass and obesity associated gene (FTO) and ALKBH5 are known to demethylate the m6A sites.[45,46] Reader proteins, such as those of the YTH domain-containing family (YTHDF), can recognize the m6A sites and through its interactions, carry out specific functions including nuclear transport, translation and decay of mRNAs.[40,43]

A large proportion of 3'UTRs contain m6A sites preceding miRNA sites.[47] Although miRNA mediated regulation and degradation of mRNA appears to be a primary mechanism by which miRNA regulate mRNA translation and protein expression, new data has suggested that some miRNA may bind a RRACH motif on the 3'UTR of mRNA, initiating FTO mRNA m6A demethylation and nascent protein translation (See Figure 2). Although it is unclear how similar miRNA bind to and regulate the rheostat of mRNA degradation versus translation, it appears that miRNA regulation of mRNA processing is a critical step in protein translation and cellular regulation.[48]

Export of miRNAs into the Blood via Exosomes

MiRNAs can stably exist in bodily fluids via: exosomes, loaded on HDL, or bound by AGO2 proteins outside of vesicles.[49-52] First discovered in 1983, extracellular vesicles (EV) are divided into three major subtypes including exosomes.[53-57] Exosomes are membrane vesicles of endosomal origin that release upon the fusion of multivesicular bodies and the plasma membrane of the cell.[55,56] The inward invagination of endosomal membranes lead to multiple intraluminal vesicles that form multivesicular bodies.[57] Endosomal Sorting Complexes Required for Transport (ESCRTs) are essential in the formation of multivesicular bodies and are associated with their secretion as exosomes.[58,59]

Exosomes can target neighboring cells in surrounding tissues or distant organs through circulation (See Figure 3). GBM cells can produce exosomes containing mRNA, miRNA and proteins that can be taken up by normal host cells and may be functional in their new environment.[60,61] Exosomal miRNAs perform one of two functions upon release into the cytoplasm. They can act as regulators of target genes or as ligands that can activate the immune system by binding to toll-like receptors (TLR),[62] activating a prometastatic inflammatory.[63] GSCs-derived exosomes that overexpress miR-21 can also be internalized by endothelial cells and promote angiogenesis through the increase of miR-21 and VEGF secretion[64] and miR-26a, which promotes angiogenesis and proliferation.[65] Another example is miR-1246 which is highly enriched in hypoxic exosomes derived from glioma and plays a role in the induction of M2 macrophage polarization.[66]

Potential of miRNAs for Liquid Biopsy

There is currently a lack of reliable diagnostic techniques for GBM that can be used for early detection and monitoring.[67] Although PET scans and spectroscopy have been attempted, their specificity is not high and these techniques are not commonly used.[68,69] On MRI, it is often difficult to differentiate between tumor progression, pseudoprogression, and radionecrosis.[4] Pseudoprogression occurs in 10-30% of GBM patients within the first 12 weeks of treatment, especially in GBMs with MGMT promotor methylation.[70] It is induced by a local inflammatory response to treatment that manifests radiographically with an increase in contrast enhancement that seems suggestive of tumor progression. [49,71] Radionecrosis, on the other hand, is the direct effect of radiation therapy to local tissue and the blood brain barrier that may also mimic tumor progression on imaging.[72] These are situations that usually resolve without further treatment, but could potentially lead to unnecessary procedures or premature discontinuation of standard treatment.

In light of the expense of imaging and the occasional inability to determine disease progression, alternative means should be further explored. If non-invasive liquid biopsy techniques could prove reliable in discerning treatment response, clinicians would have a new tool in patient management which could be less expensive and more accurate than our current imaging techniques. Variations in miRNA expressions have been detected in several diseases, including GBM.[73-76] Detecting miRNAs in biological fluids is relatively easier and safer than

detecting proteins in brain tissue. Their stability, relative convenience of extraction, detection, and quantification makes it possible for circulating miRNAs to be used effectively as biomarkers.[77]

As listed in tables 1-2, various studies have identified potential miRNAs in the blood and CSF of patients that could be used as biomarkers.[66, 78-112] Each miRNA plays an epigenetic role in the regulation of tumorigenesis and progression. MiRNAs appear to allow cells to adapt to their environment, permitting them to survive hypoxia and tumor therapy, including radiation and chemotherapy. For example, miR-21, which has been shown to be upregulated in GBM patients, plays a role in tumor progression, angiogenesis and resisting apoptosis.[113-115] MiR-10b, miR-106a-5p, miR-185, miR-210 play roles in tumor progression and invasion and are upregulated in sera of GBM patients.[116-120] MiR-29, miR-127, miR-137, miR-197, miR-205, miR-485 are downregulated and play tumor suppressor roles in GBM. [121-126] MiR-221/miR-222, MiR-223, miR-125b-2 plays roles in tumor sensitivity to temozolomide (TMZ). [127-129] MiR-128 and miR-301a play roles in glioma sensitivity to radiation.[130, 131]

MiRNAs are remarkably stable in plasma and serum, as they are resistant to RNase activity, and can be exposed to various storage conditions such extreme pH and multiple freeze-thaw cycles.[132-134] MiRNAs can be found either free within serum or CSF, or locked within lipid membranes know as exosomes. Exosomes are a subset of extracellular membrane vesicles ranging from 30 to 150 nanometers in diameter. They carry several specific molecules, such as DNA, RNA (miRNA), and proteins from a host to a recipient cell.[135] Exosomal miRNAs have emerged as promising biomarkers as they are extremely stable under various conditions such as different temperature storage.[132] There is an abundance of extracellular vesicles in plasma, with a concentration of approximately 10^{10} EVs per milliliter of plasma.[136] The profiling of EVs need to be further pursued as they may be involved in future mechanisms of GBM diagnosis.[136]

These characteristics make them appealing potential biomarkers for a liquid biopsy, but most importantly, many studies have reported the diagnostic significance of various miRNAs. Zhang et al., observed that serum miR-145-5p was significantly downregulated in the samples from patients with GBM compared to control samples. They reported that miR-145-5p might be a reliable diagnostic marker for GBM with an AUC of 0.895, sensitivity of 84.6% and specificity of 78.0%.[96] Roth et al. quantitatively analyzed 1158 mature miRNAs in 20 blood samples from GBM patients and found miR-128 and miR-342-3p to be significantly dysregulated compared to control samples. Using machine learning, they obtained a miRNA signature that could differentiate between the blood samples of patients with GBM and healthy controls with an accuracy of 81%, sensitivity of 79% and specificity of 81%.[102]

MiRNAs also may be found in and have diagnostic potential when isolated from CSF samples. Baraniskin et al., reported overexpression of miR-15b and miR-21 in the CSF samples of patients with glioma compared to control subject. When combined in expression analysis, there was a sensitivity of 90% and specificity of 100% in distinguishing patients with glioma and control subjects as well as patients with primary CNS lymphoma.[107] A recent study also demonstrated that the miR-1246 levels in the CSF of GBM patients is higher compared with

levels found in patients with low grade gliomas. Most importantly, the levels of miR-1246 in the CSF decline post-resection in GBM patients.[66]

Several exosomal miRNAs have been reported as potential prognosis biomarkers. One study proved that extracellular vesicles from CSF derived from individuals with GBM exhibited significant increase in levels of miR-21 versus non-oncological patients with a sensitivity of 87%, specificity of 93% and AUC of 0.91.[112] In another study, Santangelo et al. reported the diagnostic accuracy of a serum panel for GBM consisting of exosomal miR-21, miR-222, and miR-124-3p; yielding an AUC of 0.87. The expression of these miRNAs sharply decreased after tumor resection in patients with high grade gliomas.[94] In another study, miR-320 and miR-574-3p were also significantly elevated in exosomes isolated from sera of 75 GBM patient and were associated with a GBM diagnosis.[93]

MiRNAs have been associated with histopathological grades of gliomas. Wang et al. analyzed nine cell-free miRNAs from the plasma of 50 GBM patients and matched healthy individuals, but only three were shown to be significantly dysregulated. MiR-21 was significantly upregulated in GBM patients compared to healthy individuals with ROC curve (AUC) value of 0.9300, 90% sensitivity, and a 100% specificity. MiR-128 and miR-342-3p were significantly downregulated in the plasma of GBM patients with AUC of 1.000, 90% sensitivity and a 100% specificity.[78] They were also able to depict an association with the expression levels of miR-342-3p with histopathological grades of gliomas. The dysregulated levels of expression of this miRNA, along with miR-21 and miR-128, returned to nearly normal postoperatively.[78]. Xiao et al. demonstrated that the up-regulation of miR-182 might be correlated with clinical glioma progression. Using circulating cell-free miR-182, they could distinguish between patients with high-grade glioma from healthy individuals with AUC of 0.815.[85] Yue et al., noted that the expression of miR-205 in the serum of glioma patients demonstrated a stepwise decrease with ascending pathological grade. After tumor resection, the levels of expression increased and once again decreased with recurrence.[101] Lan et al., reported that serum exosomal miR-301a was significantly upregulated in high grade glioma and tumor resection resulted in decreased levels of expression. Recurrence of the tumor would once again increase its level of expressions.[92] This suggests that these specific miRNAs may have potential use as non-invasive biomarkers for a liquid biopsy.

MiRNAs could also potentially play a role in monitoring treatment response. Seigal et al. noted higher levels of miR-10b and miR-21 expression in the sera of GBM patients undergoing treatment with bevacizumab compared to levels detected from their pretreatment sera.[137] They also noted that the level of expressions from these miRNAs negatively correlated with tumor diameters in patients treated with bevacizumab. They did not see this correlation in patients treated with TMZ.[137] Dysregulation of some miRNAs may reflect the antitumor effect of therapy and therefore, the monitoring of these circulating miRNAs could eventually predict the effects of therapy.[138]

It is noteworthy to point out that dysregulated miRNAs have been associated with prognosis in GBM patients. Xiao et al. demonstrated that higher levels of expression of miR-182

in the plasma is related with worse patient survival.[85] Zhang et al. found that miR-145-5p strongly correlates with KPS scores, IDH1 mutation, radioresistance as well as the extent of tumor resection.[96] Wang et al., described the correlation between low levels of serum miR-485-3p expression and poor progression-free survival and overall survival.[103] Lan et al. also noticed am association with levels of exosomal miR-301a in the serum with lower KPS scores.[92] In one study, Zhao et al reported dysregulated miRNAs in sera of patients that had potential prognostic value. They reported that increased levels miR-20a-5p, miR-106a-5p, miR-222-3p and decreased levels of miR-182 and miR-145-5p were significantly associated with lower probability of 2-year overall survival. They also observed that increased levels of miR-20a-5p and miR-17-5p correlated with increased hazard of death.[83] Srinivasan et al developed a ten-miRNA expression signature to predict survival in GBM patients and found that there was overexpression of miR-17-5p, miR-20a, and miR-106a in samples of patients with shorter or median survival.[139] These conflicting reports could be due to differences in definition of survival terms.[140] Yue et al., observed a correlation with low levels of miR-205 and lower KPS score. Patients with elevated expression of miR-205 in their serum had a longer overall survival compared to patients with lower levels.[101] These results suggest that miRNAs could serve as prognostic predictors of glioblastoma.

Challenges for Application of miRNAs as Biomarkers

Despite the stability, ease of detection, and encouraging results of circulating miRNAs, many challenges remain before their widespread adoption in clinical use. There are many different potential miRNA biomarkers, different fluids (blood, serum, plasma, urine CSF) as well as methodologies for their isolation and detection. There are currently no standard protocols for sample collection and preparation or methods of assessment and application for circulating biomarkers. The heterogeneity between studies makes it a challenge to compare data between different study groups and to summate their data to assess the value of miRNAs as biomarkers.[140] Even the diversity of the study population may impact miRNA levels. Various studies have shown a difference in race specific miRNA expressions in other pathological states and may influence miRNA expression in patients with brain tumors.[141-143] One study evaluated the effect of clinical parameters such as gender and fasting on regulating expression profiles in healthy individuals and they observed that gender-associated abundance variations are small.[144] Additionally, patient's physiological expression levels vary according to overall lifestyle, meal ingestion and circadian rhythm.[145]

The type of bodily fluid acquired for miRNA isolation is a biological variable that may impact the miRNA profile. It is important to note that miRNAs have been found to be dysregulated in various types of samples such as serum/plasma, exosomes, CSF, urine, saliva, and tissues. As noted in tables 1 and 2, both cell-free and exosomal MiR-21 have been found upregulated in the plasma and CSF of patients with GBM. Unfortunately, there is also discrepancies between levels of expression among the various types of bodily fluids.[146] For example, Zhi et al., noticed the upregulation of expressions of miR-106a-5p and miR-181b-5p in

the serum of patients with GBM, while previous reports support the downregulation of these miRNAs in tissue samples.[84] One study has shown that miR-128 levels were upregulated in the peripheral whole blood of GBM patients, although downregulated GBM tissues and sera.[102,105] MiR-128 levels in whole blood is probably falsely elevated due to miRNA release from red blood cell lysis suggesting that the coagulation processes may influence the total amount of miRNA present in samples.[147] Even in studies focusing on CSF, there are discrepancies from samples extracted from lumbar or cisternal origin. The exosomal miRNA signature panel developed by Akers yielded a sensitivity of 67% and specificity of 80% in differentiating GBM patients from healthy controls when detected in samples of cisternal CSF. The sensitivity and specificity of lumbar CSF were 28% and 95%, respectively, further complicating an analysis of that miRNA's utility in diagnositics.[106]

The outcomes of miRNA analysis can be influenced by various factors in sample processing ranging from sample collection, preservation, handling and miRNA detection. The RNA extraction techniques differ from study to study. One study compared isolation methods of exosomal RNA and observed variations in miRNA content and amount because of methodological differences.[147] Kopkova et al. advocated for standardization as they compared different commercially available RNA extraction kits and found that their usage significantly influenced results.[148] Furthermore, there is currently no commercially available method for CSF miRNA quantification. Many kits used in studies analyzing miRNAs in CSF were designed specifically for isolating miRNA from cells, tissue or serum/plasma samples. These issues suggest need for standardization before widespread clinical adoption.

There is also a wide range of techniques that can be used for initial screening of RNA detection such as NanoString, Next Generation Sequencing, and TaqMan. Afterwards, miRNA expression levels are usually confirmed with quantitative RT-PCR. While qRT-PCR is the most conventional method to estimate levels of miRNA expression in specimen, it does have some limitations. Quantitative determination of low amounts of miRNA is the main challenge for qPCR, leading to low sensitivity and accuracy.[149] RT-qPCR is not able to precisely distinguish samples with lower than ten miRNA copies.[148] Furthermore, among studies using qRT-PCR, there is great variability as most of them use relative quantification with different molecules for normalization. Different miRNA expression normalizers can lead to significantly different quantifications of expression levels. [140,145] A global consensus to establish a gold standard of miRNA expression data normalization needs to occur before miRNAs get closer to clinical utilization.

Most studies present the limitation of the small sizes of their cohorts, therefore they require further validation of their findings in larger cohorts. Larger studies are required to determine the effectiveness of miRNAs as biomarkers. Since there is heterogeneity in GBM, it will be difficult to predict GBM from only one miRNA. MiRNA also overlap with other conditions and there are discrepancies among samples. MiR-21 levels have been shown to be increased in brain tissue, serum and exosomes of patients with GBM. It is also upregulated in breast, hepatocellular, colorectal, ovarian and other several types of cancers.[138] This suggest that

miRNA is not specific of a cancer type, and thus a miRNA panel may have increased prognostic and diagnostic accuracy for GBM. The ideal clinical model must have high sensitivity and specificity, but must also have a suitable cost-effectiveness. Cost benefit models should be developed to determine the number of miRNAs included in a panel, thus the panel can be easily translated in a clinical setting.

Conclusion

GBM is the most aggressive primary brain tumor. Identifying appropriate biomarkers could aid clinicians in the determination of diagnosis, prognosis and treatment monitoring. MiRNAs derived from peripheral bodily fluids have high potential as GBM biomarkers. However, there needs to be a consensus on optimization and standardization of their analytical process to overcome the lack of reproducibility and specificity of studies. Only then, can we get closer to utilizing miRNAs in the clinical setting.

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Figure 1: MicroRNA biogenesis. In the nucleus, miRNA genes are transcribed by RNA polymerase II (RNA Pol II) into primary miRNAs (pri-miRNAs). They are cleaved in the nucleus by Drosha and DGCR8 into precursor miRNAs (pre-miRNAs). They are exported from the nucleus by Ran-GTP-dependent Exportin-5. In the cytoplasm, pre-miRNAs are cleaved by Dicer into miRNA duplexes. The guide strands of the duplexes are loaded onto Argonaut 2(Ago 2) proteins, as part of active RNA Interference Effector Complexes (RISCs), resulting in mature miRNAs. The other strands of the duplexes are subject to degradation.

Figure 2: MicroRNA Binding and Effects. DNA is transcribed into messenger RNA (mRNA) by RNA polymerase I (RNA Pol I). N6-methyladenosine modifications can occur on an adenosine of an RNA motif known as RRACH that is regulated by writers such as the m6A methyltransferase complex composed of methyltransferase like 3/14 (METTL3/METTL14) complex, Wilms' tumor 1-associating protein (WTAP), VIRMA and RBM15. MiRNA may bind a RRACH motif on the 3'untranslated of mRNA, initiating fat mass and obesity associated gene (FTO) mRNA m6A demethylation. ALKBH5 also demethylates m6A sites. Readers, such as YTH domain-containing family proteins (YTHDF) can carry out specific functions such as translation, degradation and splicing

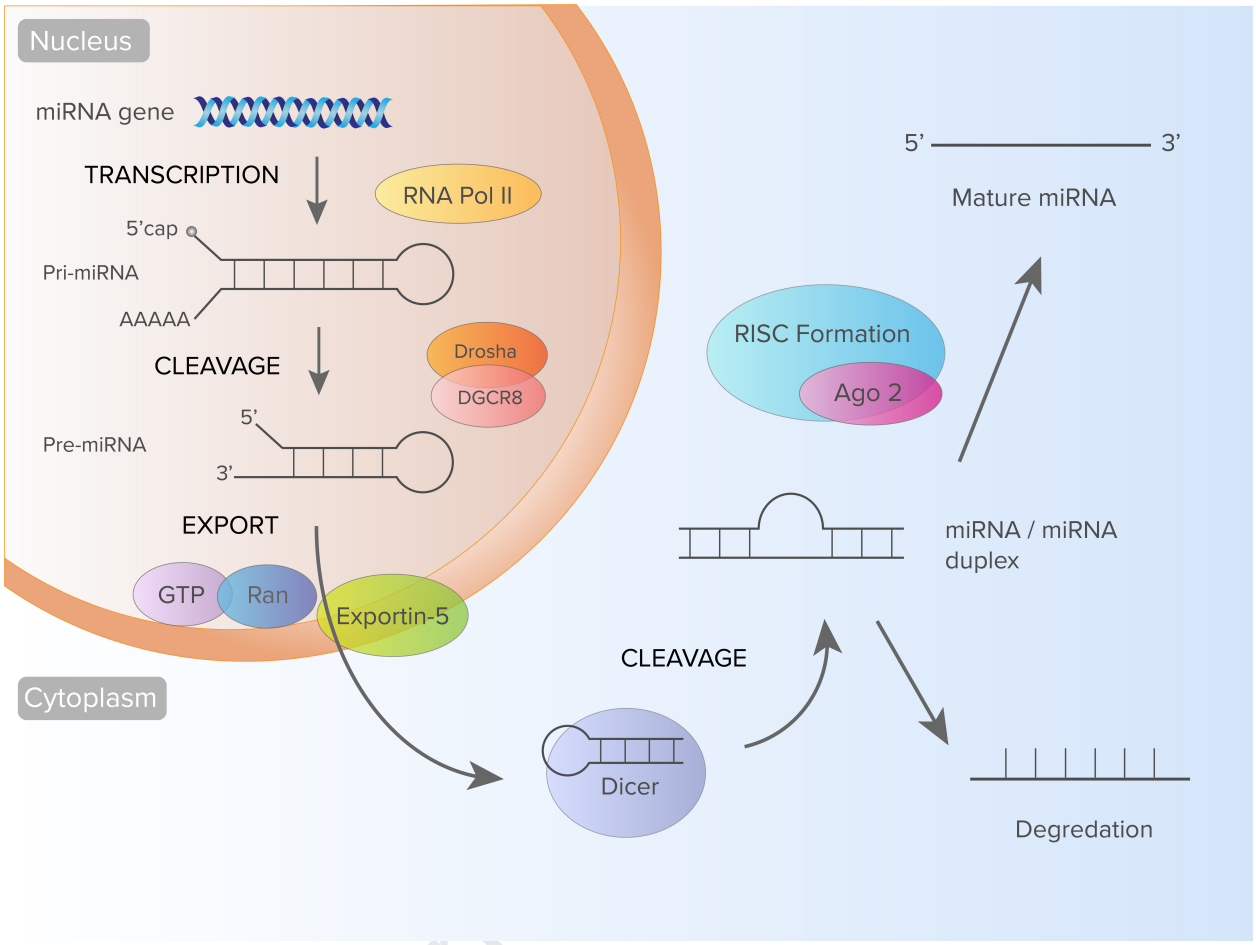
Figure 3: Exosomal Transfer of MicroRNA. MiRNA genes are transcribed in the nucleus of donor cells as primary miRNAs that are then exported to the cytoplasm. There, they are processed by Dicer into pre-miRNA and the RISC complex into mature miRNA. Mature miRNA can be directly transferred to circulation via microvesicles (MVs) shed by the plasma membrane. Mature miRNAs and even some pre-miRNAs, can also be sorted by Endosomal Sorting Complexes Required for Transport (ESCRTs) into endosomes that will become multivesicular bodies (MVBs). GTPases Rab 27a and Rab 27b dock MVBs at the plasma membrane and assist with its release as exosomes. These membrane-derived carriers fuse with recipient cells; allowing extracellular miRNAs transported via these carriers, to alter the gene expression of recipient cells.

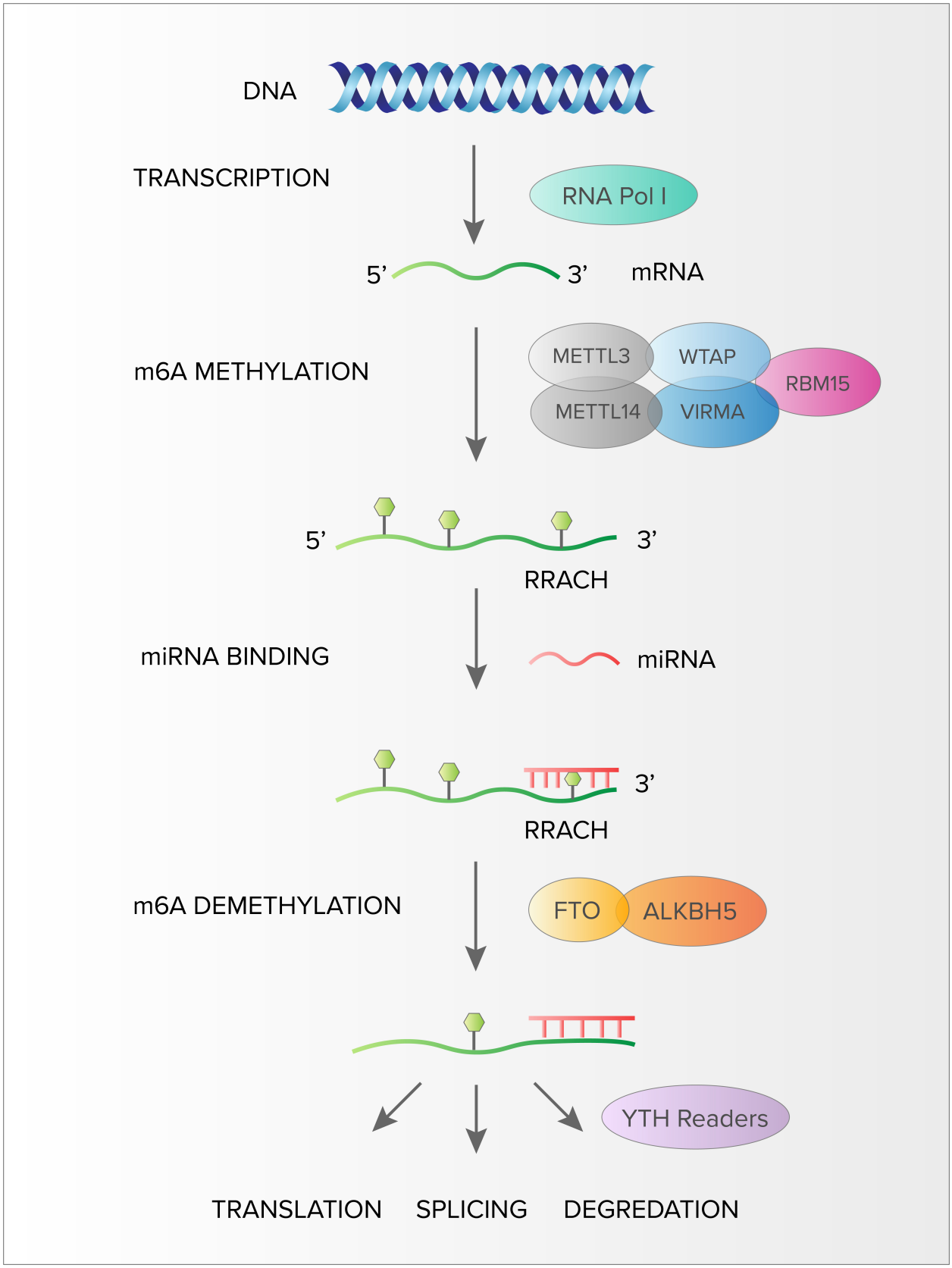
Table 1: MiRNAs Identified in the Blood

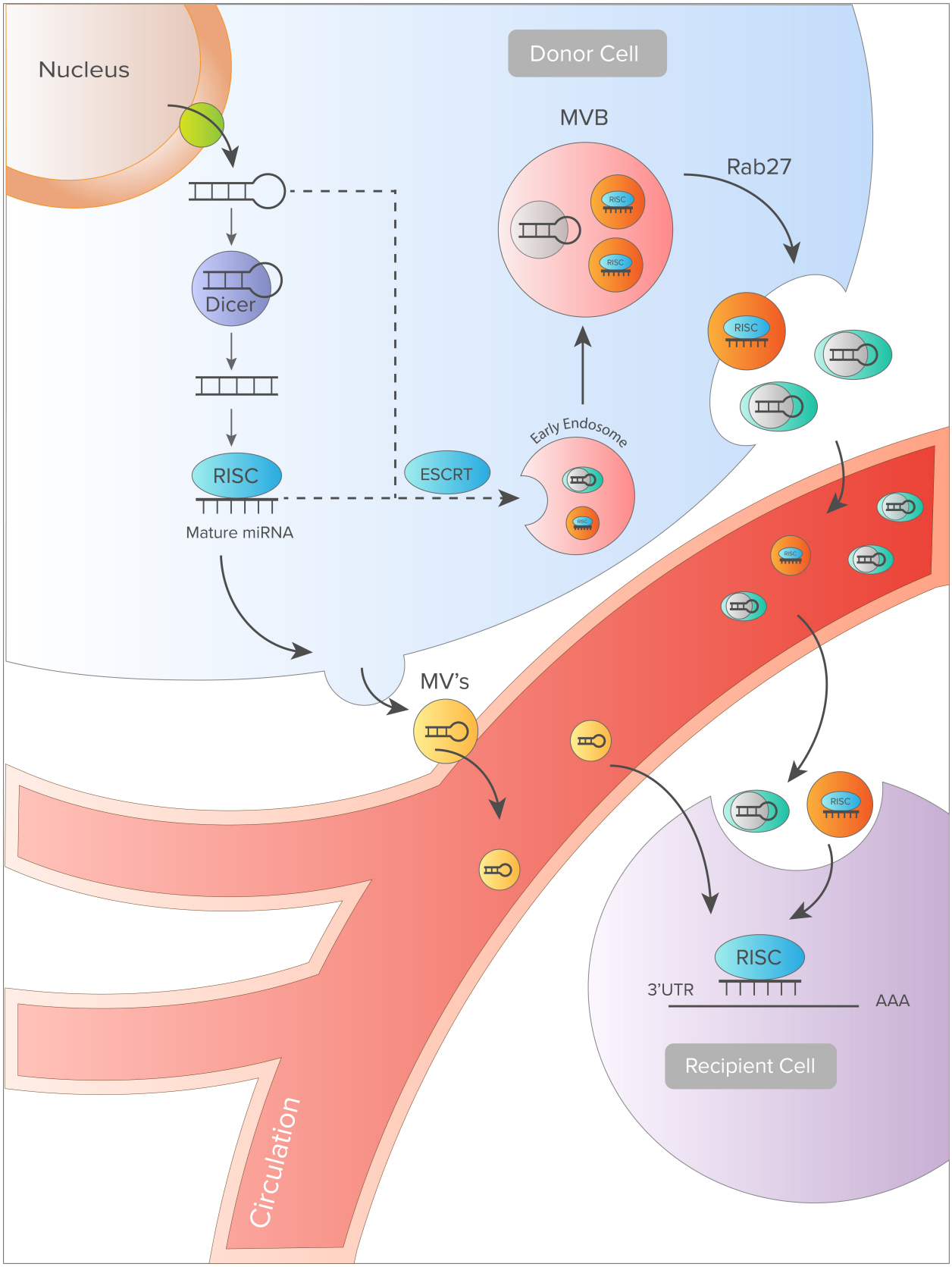
MiRNAs	Regulatory Status	References
miR-21	Upregulated	78-82
miR-15b, miR-21	Upregulated	82
miR-20a-5p, miR-106a-5p, miR-222-3p	Upregulated	83
miR-106a-5p, miR-181b-5p	Upregulated	84
miR-182	Upregulated	85
miR-185	Upregulated	86
miR-210	Upregulated	87
miR-221/222	Upregulated	88
miR-340, miR-576-5p, miR626	Upregulated	89
miR-454-3p	Upregulated	90
miR-514a-3p, miR-592	Upregulated	91
Exosomal miR-301a	Upregulated	92
Exosomal miR-320 and miR-574-3	Upregulated	93
Exosomal miR-21, miR-222 and miR-124-3p	Upregulated	94
miR-23a, miR-133a, miR-150, miR-197, miR-548b	Downregulated	95
miR-302c-3p, miR-484, MiR-493-3p, miR-514a-3p, miR-592, miR-1260a	Downregulated	78
miR-182 and miR-145-5p	Downregulated	83
miR-145	Downregulated	96
miR-29	Downregulated	97
miR-125b	Downregulated	98,99
miR-137	Downregulated	100
miR-205	Downregulated	101
miR-310, let-7g-5p, miR-7-5p	Downregulated	89
miR-342-3p	Downregulated	78, 102
miR-383-5p	Downregulated	91
miR-497	Downregulated	95, 99
miR-485-3p	Downregulated	103
miR-628-3p	Downregulated	102
miR-203	Downregulated	104
miR-128	Upregulated in whole blood, but downregulated in plasma	102, 105

Table 2: MiRNA Identified in CSF

MiRNAs	Regulatory Status	References
miR-10b, miR-21	Upregulated	106-109
miR-15b, miR-21	Upregulated	107,108
miR-21-5p, miR-218-5p, miR-193b-3p, miR-331-3p, miR374a-5p	Upregulated	106, 110
miR-106, miR-17, miR-27a, miR-130, miR-25, miR-23a	Upregulated	108
miR125b, miR-223, miR-451, miR-711, miR-935	Upregulated	111
Exosomal miR-21	Upregulated	112
miR-1246	Upregulated	66
miR548c-3p, miR520f-3p, miR27b-3p and miR-30b-3p	Downregulated	106
miR-128	Downregulated	108


Nucleus
miRNA gene
TRANSCRIPTION
RNA Pol II
5'cap
Pri-miRNA
AAAAA
CLEAVAGE
Drosha
DGCR8
5'
Pre-miRNA
3'
EXPORT
GTP
Ran
Exportin-5
Cytoplasm
5'
3'
Mature miRNA
RISC Formation
Ago 2
miRNA / miRNA duplex
CLEAVAGE
Dicer
Degredation


DNA
TRANSCRIPTION
RNA Pol I
5'
3'
mRNA
m6A METHYLATION
METTL3
WTAP
RBM15
METTL14
VIRMA
5'
3'
RRACH
miRNA BINDING
miRNA
3'
RRACH
m6A DEMETHYLATION
FTO
ALKBH5
YTH Readers
TRANSLATION
SPLICING
DEGREDATION


Nucleus
Donor Cell
MVB
Rab27
Dicer
RISC
Mature miRNA
ESCRT
Early Endosome
MV's
Circulation
RISC
3'UTR
AAA
Recipient Cell

Abbreviation List:
Ago: Argonaut
AKT: protein kinase B
AMPK: 5′AMP-activated protein kinase
Bim: Bcl-2-like protein 11
ceRNA: endogenous circular RNAs
CSF: Cerebrospinal fluid
DcVax-L: lysated pulsed dendritic cell vaccine
EGFR: Epidermal growth factor receptor
ESCRT: Endosomal Sorting Complexes Required for Transport
EV: extracellular vesicle
FTO: fat mass and obesity associated gene
GBM: Glioblastoma multiforme
GLI1: glioma-associated oncogene homolog 1
GOLPH3: Golgi Phosphoprotein 3
GSCs: glioma stem cells
HOXD10: homeobox D10:
IDH1: isocitrate dehydrogenase 1
KPS: Karnofsky Performance Scores
LKB1: liver kinase B1
lncRNA: long noncoding RNA
M6A: N6-methyladenosine
MAPK: mitogen-activated protein kinase
METTL: methyltransferase like protein
MGMT: O^6-methylguanine-DNA methyltransferase
miRNA, miR-: MicroRNAs
MMP: Metalloprotease
MRI: magnetic resonance imaging
mTOR: mammalian target of rapamycin
NFkB: nuclear factor kappa-light-chain-enhancer of activated B cells
OLIG: Oligodendrocyte transcription factor
PDGFRA: platelet-derived growth factor receptor A
PKC: protein kinase C
PPAR-γ: peroxisome proliferator-activated receptor-γ
PRC1/2: polycomb repressor complex 1/2
primiRNA: primary miRNA precursors
PTEN: phosphatase and tensin homology
RISC: RNA Interference Effector Complex
ROD1: regulator of differentiation 1
SRSFs: Serine/arginine-rich splicing factors
SOX9: SRY-Box 9
TGIF2: Transforming growth factor-beta-induced 2
TMZ: temozolomide
TLR: toll-like receptors
TTF: tumor treating field
UTR: untranslated region

VEGFA: Vascular Endothelial Growth Factor A
Vps20: vacuolar protein sorting-associated protein 20
WTAP: Wilms' tumor 1-associating protein
XPO: Exportin
YTHDF: YTH domain-containing family proteins