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microRNA Regulation of Glycolytic Metabolism in Glioblastoma Multiforme.

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### **Abstract**

Glioblastoma multiforme (GBM) is the most aggressive and common malignant brain and central nervous system tumour. A well-known hallmark of GBM, and many other tumours, is aerobic glycolysis. microRNAs (miRNAs) are a class of short non-protein coding sequences that exert post-transcriptional controls on gene expression and represent critical regulators of aerobic glycolysis in GBM. In GBM, miRNAs regulate the expression of glycolytic genes directly and via the regulation of metabolism-associated oncogenic pathways, such as the *PI3K/Akt* signalling pathway. The aim of this review is to establish links between miRNA expression levels, disease grade and prognosis, and the glycolytic phenotype of GBM. In this review, the involvement of 25 miRNAs in the regulation of glycolytic metabolism of GBM is discussed. Seven of these miRNAs have been shown to regulate glycolytic metabolism in other tumour types. Further eight miRNAs, which have been shown to be differentially expressed in GBM, were also reported to play a regulatory role in glycolysis in other cancer types. Such miRNAs could serve as potential glycolytic regulators in GBM but require functional validation. This review concludes with presenting a number of glycolytic regulatory miRNAs that have demonstrated their therapeutic potential either alone or as adjuvants in GBM, despite the major challenges that have to be solved before miRNA-based therapies can widely be used for the treatment of GBM patients.

**Key words:** microRNA, Glucose Metabolism, High Grade Brain Tumours.

**Abbreviations used:** Glioblastoma multiforme, GBM. microRNA, miRNA.

**Article category:** Mini-review

## **1. Introduction**

### **1.1 Glioblastoma multiforme**

Glioblastoma multiforme (GBM) represents 12-15% of all intracranial tumours.<sup>1</sup> GBM is the most common and aggressive form (World Health Organization (WHO) grade IV) of glioma, an umbrella term for tumours thought to originate from glial progenitors such as astrocytomas.<sup>2,3</sup> Primary GBM, which comprises 90% of all diagnosed GBM cases, arises *de novo*, whilst pre-existing low-grade glioma: grade I (pilocytic astrocytoma) and grade II (diffuse astrocytoma) can develop into high-grade glioma: grade III (anaplastic astrocytoma) that give rise to secondary GBM which constitutes the remaining 10% of GBM cases.<sup>3</sup> In general, GBM shows an increased incidence in Caucasian populations.<sup>4</sup> In the UK and the United States alone, the annual GBM incidence rate ranges between 4.64-5.26 per 100,000 people.<sup>5,6</sup> GBM treatment consists of maximal surgical resection followed by local radiotherapy in concurrence with adjuvant Temozolomide (TMZ) chemotherapy.<sup>7,8</sup> However, GBM prognosis remains poor with a median overall survival of 14 months and a 5-year survival rate of less than 10%.<sup>9,10</sup>

### **1.2 Regulation of glycolytic metabolism by oncogenic signalling in GBM**

GBM is characterised by upregulated aerobic glycolysis compared to normal brain.<sup>11,12</sup> Aerobic glycolysis, also known as the Warburg effect, is a catabolic process that, in the presence of oxygen, converts one glucose molecule into two lactate molecules.<sup>13</sup> Aerobic glycolysis is driven by several molecular mechanisms (reviewed in<sup>14</sup>). A major mechanism is the overexpression of glycolytic genes caused by somatic mutations in the encoding genes or in the oncogenes and tumour suppressor genes that regulate the expression of glycolytic genes. Comprehensive genomic characterisation<sup>15</sup> using 206 GBM samples performed by The Cancer Genome Atlas (TCGA) Network showed that genetic alterations were frequently found within the oncogenic phosphatidylinositol 3-kinase (*PI3K*)/ protein kinase B (*Akt*) pathway. The *PI3K/Akt* pathway plays an important role in the regulation of GBM glycolytic metabolism. The *PI3K/Akt* role in glycolysis was supported by Elstrom et al. (2004)<sup>16</sup> who observed differences in the glycolytic rates of various GBM cell lines which were then attributed to the differences in Akt activity levels in these cells. In their study, two GBM cell lines were grown in normal glucose conditions; LN18 cells with constitutive Akt activity, as measured by Akt phosphorylation, showed higher rates of aerobic glycolysis than LN229 cells with low Akt activity. The inhibition of the upstream regulator, PI3K, abolished Akt phosphorylation and reduced the glycolytic rate of LN18 cells while the overexpression of Akt in LN299 cells was sufficient to stimulate high rate of glycolysis.<sup>16</sup> Inter-tumour heterogeneity within the *PI3K/Akt* pathway was also suggested to be responsible for the different clinical outcomes of molecular targeted therapy. As such, it was proposed that GBM can be classified into five GBM subgroups with different molecular and clinical characteristics based on their distinct *PI3K/Akt* pathway signature.<sup>17</sup>

### **1.3 *PI3K/Akt* signalling in GBM**

Using 91 GBM samples, the TCGA study showed that within the *PI3K/Akt* pathway, the receptors tyrosine kinases (RTKs): hepatocyte growth factor receptor (encoded by *c-*

*Met*) and platelet-derived growth factor receptor- $\alpha$  (PDGFRA), are aberrantly activated in 4% and 13% of GBMs, respectively.<sup>15</sup> However, gain of function mutations and/or amplification in the epidermal growth factor receptor (*EGFR*) are the most common in GBM (45% of GBM cases).<sup>15</sup> Active *EGFR* signals via multiple effector pathways including *RAS* and *PI3K* signalling cascades. The cytoplasmic domain of *EGFR* recruits adaptor proteins to activate *RAS*.<sup>18</sup> Moreover, the activation of *RAS* signalling can be achieved through losing the expression of the *RAS* antagonist, neurofibromin 1 (*NF1*), which is observed in about 14% of GBM cases.<sup>15</sup> *RAS* activates *PI3K* while *PI3K* can independently be activated by the cytoplasmic domain of *EGFR*.<sup>19,20</sup> *PI3K* is aberrantly activated in 15% of GBMs.<sup>15</sup> Activated *PI3K* catalyses the phosphorylation of phosphatidylinositol (4,5)-bisphosphate (*PIP2*) into phosphatidylinositol (3,4,5)-trisphosphate (*PIP3*)<sup>21</sup>, which can be reversed by the phosphatase tensin and homologue<sup>22</sup> (*PTEN*; homozygous deletions and mutations are found in 36% of GBMs).<sup>15</sup> Following its recruitment into the plasma membrane by *PIP3*, *Akt* is phosphorylated by 3-phosphoinositide-dependent protein kinase 1 (*PDK1*).<sup>23,24</sup> *Akt* is found to be amplified in 2% of GBMs.<sup>15</sup> Activated *Akt* activates both the rapamycin sensitive mTOR complex 1 (mTORC1) and the rapamycin insensitive mTOR complex 2 (mTORC2). First, *Akt* phosphorylates the *SIN1* subunit of mTORC2, thus, induces the activation of mTORC2. In a positive feedback loop, mTORC2 phosphorylates and thereby fully activates *Akt*.<sup>25</sup> Second, *Akt* phosphorylates and inhibits *TSC2* thereby relieving the inhibitory effects of the *TSC1-TSC2* complex on mTORC1.<sup>26-28</sup> mTORC1 is also negatively regulated by the energy-sensing AMP-activated protein kinase (*AMPK*). The reduction in ATP causes an increase in the *AMP:ATP* ratio leading to the activation of *AMPK*.<sup>29,30</sup> *AMPK* mediates an activating phosphorylation of *TSC2* and an inhibitory phosphorylation of the mTORC1 subunit Raptor<sup>31,32</sup> (Figure 1a). In GBM, *PI3K/Akt* signalling upregulates *c-Myc*<sup>33</sup> and the hypoxia induced factor (*HIF*), under aerobic conditions and independent of hypoxia<sup>34</sup>; both of which upregulate glycolysis.<sup>35-38</sup>

#### 1.4 MicroRNAs

microRNAs (miRNAs) are a class of small non-coding RNA that regulate gene expression at the post-transcriptional level.<sup>39</sup> The primary transcripts of miRNA (pri-miRNA) are processed by Droscha, a nuclear RNase III enzyme, into 20-22 nucleotide RNA duplexes called precursor miRNAs (pre-miRNAs).<sup>40</sup> Pre-miRNAs are exported to the cytoplasm for further processing by Dicer, a cytoplasmic RNase III enzyme.<sup>41</sup> The result for each pre-miRNA is a mature miRNA strand that is loaded onto the miRNA-induced silencing complex (miRISC) and a passenger strand that is degraded.<sup>42</sup> Post-transcriptional gene silencing is arbitrated by the complementary binding of the mature miRNA strand within miRISC to the target mRNA 3'-untranslated region (3'-UTR).<sup>42</sup> In GBM, besides acting as biomarkers<sup>43</sup>, miRNAs regulate glucose metabolism by targeting mRNAs of the glycolytic genes and the signalling proteins that drive the expression of glycolytic genes.

This review aims to establish links between miRNA expression levels, disease grade and prognosis, and the glycolytic phenotype of GBM. First, the review will discuss the role of miRNAs in regulating GBM glycolytic metabolism by targeting glycolytic genes (Figure 1b) and via the *PI3K/Akt* pathway (Figure 1a). The review will then present

differentially expressed miRNAs in GBM which were reported to be involved in the regulation of glycolytic metabolism in other tumours. Such miRNAs could serve as potential glycolytic regulators in GBM, yet to be experimentally validated. Finally, the review will conclude with the discussion of the potential of targeting glycolytic metabolism with miRNA-based therapy in GBM.

## **2. miRNA regulation of glycolytic metabolism in GBM**

### **2.1 miRNA regulation of glycolytic transporters**

Akt controls the flux of glucose through glycolysis by regulating the expression and the membrane translocation of glucose transporter 1 and 3 (GLUT1 and GLUT3) which are upregulated in GBM.<sup>44,45</sup> miR-106a targets *SLC2A3* which encodes GLUT3.<sup>46</sup> miR-106a is downregulated in GBM compared to normal brain.<sup>46</sup> The low miR-106a expression is associated with shorter-term survival of GBM patients.<sup>46-48</sup> Moreover, the expression of miR-106a in high-grade glioma is lower than that in low-grade glioma, an expression pattern that is opposite to GLUT3.<sup>45,46,49</sup> Thus, miR-106a downregulation promotes glycolysis by releasing the suppression on GLUT3.

### **2.2 miRNA regulation of glycolytic enzymes**

Akt regulates glycolysis by enhancing the activity and the cellular localisation of the cancer-predominant isoform of the first glycolytic enzyme, hexokinase II (HKII).<sup>50</sup> The expression of glycolytic enzymes, is also regulated by miRNAs. miR-143 targets *HKII*<sup>51</sup> and is found to be downregulated in GBM compared to low-grade glioma and normal brain.<sup>51,52</sup> miR-143 expression is negatively correlated with *HKII* levels<sup>51</sup> which is associated with poor prognosis.<sup>53</sup> Another glycolytic enzyme, *PKM2*, is regulated by the miRNA, let-7a.<sup>54</sup> *PKM2* is the M2 isoform of pyruvate kinase (PK), the terminal glycolytic enzyme which converts phosphoenolpyruvate (PEP) to pyruvate.<sup>55</sup> *PKM2* has a relatively decreased enzymatic activity which leads to the accumulation of upstream glycolytic intermediates that can be channelled into the biosynthetic pathways.<sup>56</sup> *PKM2* is selectively expressed at low levels in GBM but is completely absent in normal brain.<sup>30</sup> *c-Myc*, which is also targeted by let-7a, upregulates the expression of the heterogeneous nuclear ribonucleoprotein A1 (*hnRNPA1*) splicing factor which, in turn, downregulates let-7a in a positive feedback loop.<sup>54</sup> *hnRNPA1* binds to the pri-let-7a and blocks its processing by Drosha.<sup>57</sup> In addition, *HnRNPA1* mediates the splicing of PK into the *PKM2* isoform as well as that of the Myc-interacting partner Max into the Delta Max isoform. Delta Max forms a complex with *c-Myc* to drive the transcription of the *c-Myc* target genes, including *hnRNPA1*.<sup>58-61</sup> As such, let-7a/*c-Myc*/*hnRNPA1*/*PKM2* regulatory loop ensures the downregulation of let-7a in order for *PKM2* to be expressed in GBM. Another miRNA which targets *PKM2*, miR-326, is downregulated in GBM compared to normal brain as a result of the decreased transcription of its host gene,  *$\beta$ -arrestin1*.<sup>30,62</sup> In GBM cells, the overexpression of miR-326 or the knock-down of its target, *PKM2*, reduced cellular proliferation, metabolic activity and ATP levels.<sup>30</sup> Such decrease in ATP levels was, however, rescued by transfecting GBM cells with *PKM2* mRNA lacking the 3'-UTR which renders them insensitive to miR-326.<sup>30</sup> Therefore, miR-326 mediates its effects on tumour metabolism by repressing *PKM2* expression.

### 2.3 miRNA regulation of RTKs

*c-Met* is a target of miR-410, which is downregulated in GBM compared to low-grade glioma and normal brain.<sup>63</sup> *c-Met* is also targeted by miR-144-3p which is downregulated in GBM.<sup>64</sup> miR-144-3p expression is inversely correlated with glioma grade and overall patient survival.<sup>64</sup> The expression of miR-34a, another negative regulator of *c-Met*, is also inversely correlated with glioma grade.<sup>65–68</sup> Moreover, miR-34a expression in GBM is suppressed by *PDGFRA*, which is targeted by miR-34a in a negative feedback loop.<sup>65</sup> The administration of imatinib, an inhibitor developed for BCR-ABL which can also inhibit PDGFR, KIT and ARG<sup>69,70</sup>, reversed the negative effect of *PDGFRA* on miR-34a expression.<sup>65</sup> Furthermore, miR-128, which targets *PDGFRA* and *EGFR*<sup>71</sup>, is downregulated in GBM relative to low-grade glioma.<sup>72–76</sup> *EGFR* is also targeted by miR-219-5p, which is downregulated in GBM.<sup>77,78</sup> In addition, *EGFR* is indirectly regulated by miR-21 which targets the *EGFR* transcriptional activator *STAT3*.<sup>79,79–82</sup> The expression of miR-21 is positively correlated with glioma grade and decreased patient survival.<sup>77,79,83–95</sup> Further links between miRNA and the glycolysis regulating *PI3K/Akt* signalling pathway in GBM were suggested by Kefas et al. (2008) and Webster et al. (2009) who proposed that *EGFR* is targeted by miR-7.<sup>96,97</sup> miR-7 shows a brain-specific expression, however, miR-7 shows a relatively decreased expression in GBM.<sup>98</sup> Although, pri-miR-7 levels are similar in both GBM and normal brain, pre-miR-7 levels are decreased in GBM. This suggests that changes of regulatory mechanisms that control the processing of pri-miR-7 to pre-miR-7 could be responsible for the decrease in miR-7 expression in GBM.<sup>96</sup>

### 2.4 miRNA regulation of the RAS and its antagonist, NF1

One of the effectors of the RTK signalling is the RAS pathway. RAS is antagonised by NF1 which is regulated by miR-9.<sup>99</sup> miR-9 is upregulated in GBM and is associated with poor prognosis.<sup>99,100</sup> Furthermore, *N-RAS* is regulated by miR-143<sup>101</sup>, which targets *HKII*<sup>51</sup>, and by miR-340, which is downregulated in GBM.<sup>102,103</sup> miR-340 expression is associated with poor prognosis.<sup>102,103</sup> Moreover, *K-RAS* is regulated by let-7a<sup>105</sup>, which regulates both *PKM2* and *c-Myc*<sup>54</sup>. *K-RAS* is also regulated by miR-134, which is found to be downregulated in GBM.<sup>104</sup>

### 2.5 miRNA regulation of *PI3K/Akt* and the *PI3K* antagonist, *PTEN*

miR-7, mentioned above to regulate *EGFR*, also targets *PI3K*. The overexpression of miR-7 was shown to downregulate *PI3K* expression in a dose-dependent fashion.<sup>98</sup> miR-542-3p, which targets *Akt* (specifically *Akt1*), is downregulated in GBM and is negatively correlated with glioma grade and is associated with poor prognosis.<sup>105</sup> Another *EGFR* regulator, miR-21, regulates the *PI3K* antagonist, *PTEN*.<sup>79</sup> miR-21 in GBM targets and downregulates *PTEN* while the knock-down of miR-21 leads to the upregulation of *PTEN*.<sup>79</sup> In GBM, *PTEN* is also targeted by miR-26a, which is upregulated by *c-Myc*.<sup>106</sup> However, copy number amplification mainly underlies the upregulation of miR-26a in GBM.<sup>72,107,108</sup> Another negative regulator of *PTEN* is miR-1908 which is upregulated in GBM relative to normal brain and low-grade glioma and is associated with poor prognosis.<sup>109</sup> The expression of *PTEN* is also repressed by miR-494-3p and miR10a/10b, which are upregulated in GBM.<sup>110,111</sup> Moreover, the high miR-10b expression levels correlates with poor prognosis in GBM patients.<sup>112</sup> Furthermore,

*PTEN* is targeted by miR-221/-222, clustered in Xp11.3, which is found to be upregulated in high-grade relative to low-grade glioma.<sup>72,113</sup>

## 2.6 miRNA regulation of AMPK/mTOR

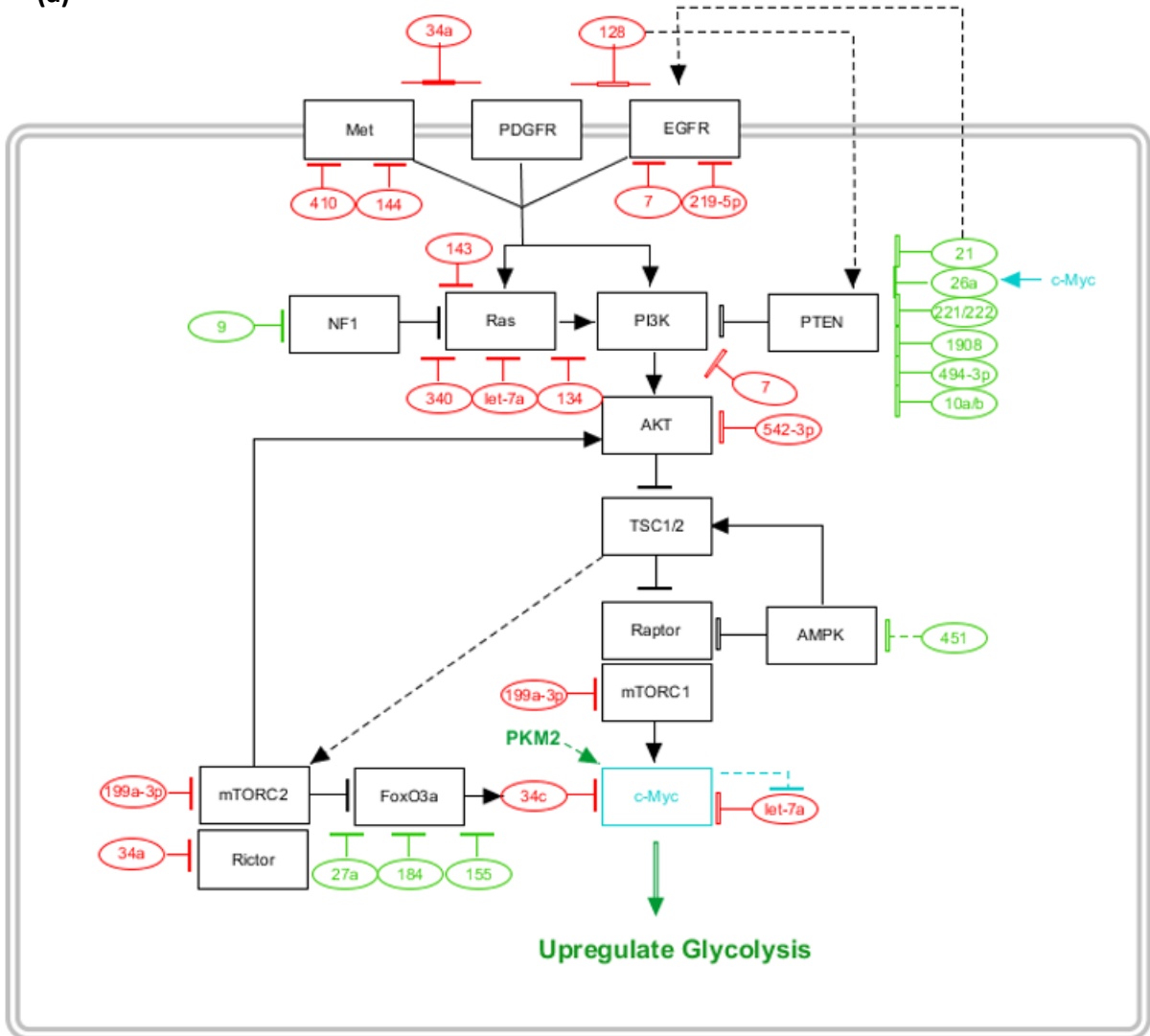
mTORC1, a positive regulator of *c-Myc*, is negatively regulated by AMPK which in turn is negatively regulated by miR-451.<sup>114</sup> The expression of miR-451 is found to be elevated in GBM patient samples which correlated with poor prognosis.<sup>114</sup> miR-451 targets *CAB39*, the binding partner for the protein kinase LKB1 which phosphorylates and activates AMPK.<sup>114</sup> The high expression levels of miR-451 are maintained by the activity of the transcription factor OCT1.<sup>115</sup> This forms a positive feedback loop where low AMPK activity caused by miR-451 upregulations allows OCT1 to further drive miR-451 expression.<sup>115</sup> Furthermore, the expression of *mTORC1* and *mTORC2* is suppressed by miR-199a-3p which is downregulated in GBM compared to normal brain.<sup>116</sup> However, the expression of miR-199a-3p was not significantly different between low-grade and high-grade glioma.<sup>116</sup> Moreover, the mTORC2 binding partner *Rictor* is targeted by miR-34a.<sup>66,117</sup> miR-34a expression, which is downregulated in GBM<sup>65-68</sup>, is negatively correlated with *Rictor* expression, which is associated with shorter patients' survival.<sup>66</sup>

## 2.7 miRNA regulation of FoxO3a/c-Myc

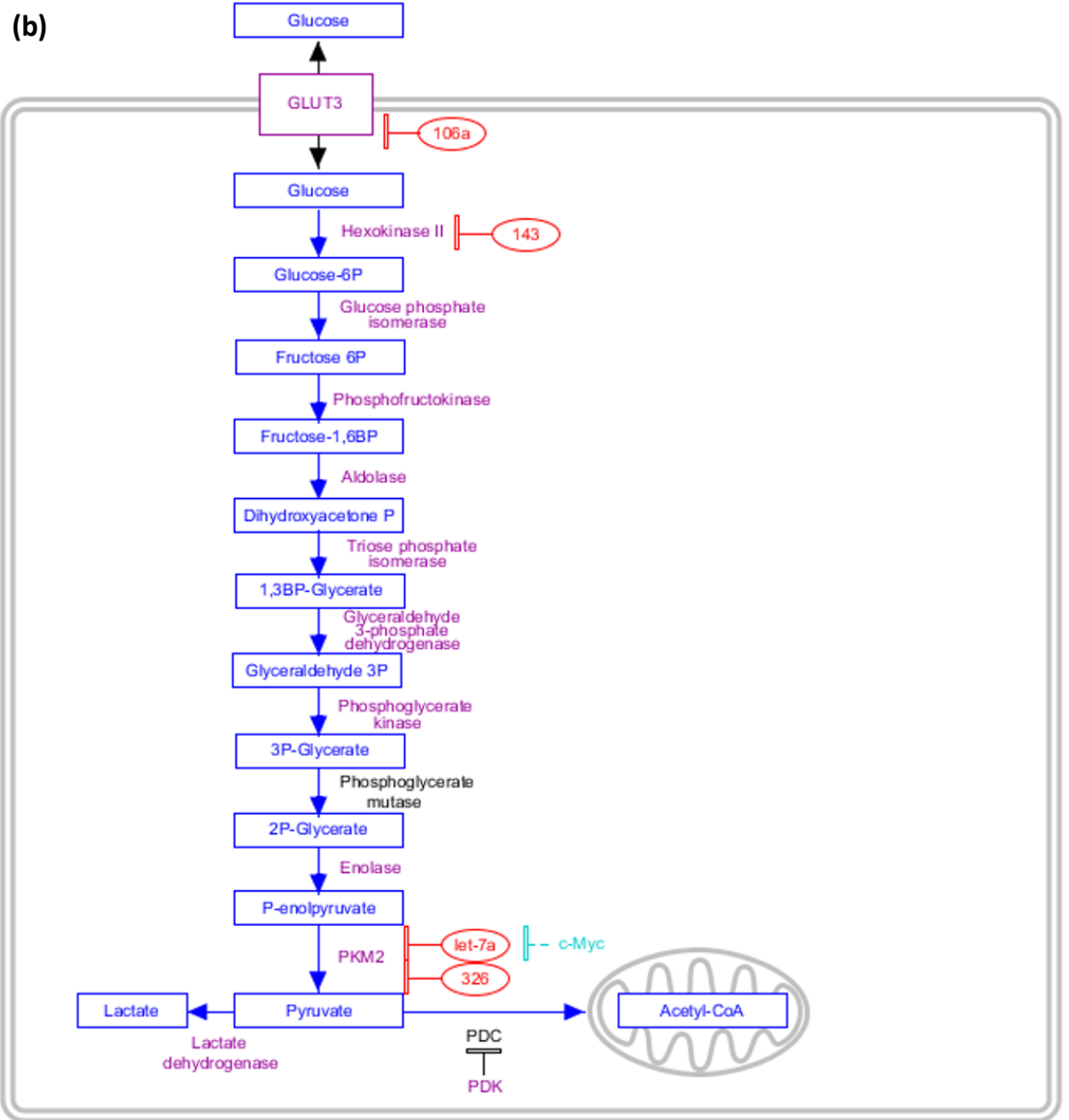
mTORC2 positively regulates *c-Myc* expression by suppressing FoxO3a. FoxO3a enhances the expression of miR-34c which directly targets *c-Myc*.<sup>33</sup> mTORC2 inhibits the phosphorylation of class IIa histone deacetylases (HDACs) rendering them inactive. As such, FoxO3a remains in its acetylated inactive form. Thus, the inactivation of FoxO3a relieves the miR-34c-mediated suppression on *c-Myc*.<sup>33</sup> In addition to its suppression by mTORC2, the expression of *FoxO3a* is suppressed by miR-mediated mechanisms in GBM. *FoxO3a* is negatively regulated by miR-27a, which is highly expressed in GBM relative to low-grade glioma and normal brain and is associated with faster disease progression and shorter patient survival.<sup>118</sup> miR-155 is another negative regulator of *FOXO3a* which is upregulated in GBM compared to normal brain.<sup>119</sup> The expression of miR-155 positively correlates with glioma grade and poor prognosis.<sup>120,121</sup>

Overall, each component of the *PI3K/Akt* pathway is tightly regulated by miRNAs. As such, miRNAs that suppress glycolytic metabolism directly (Figure 1b) or through the *PI3K/Akt* pathway (Figure 1a) are downregulated while those that promote glycolysis are upregulated in GBM as seen from the above discussion. The expression levels of these miRNAs are either (i) invariant across the different grades of glioma, suggesting that the expression change of a particular miRNA might signify a key early event in gliomagenesis, or (ii) can distinguish different glioma grades, thereby serving as a potential biomarkers of glioma progression.<sup>118,122</sup>

(a)







**Figure 1. miRNA regulation of glycolysis in GBM. (a) miRNAs regulating *PI3K/Akt* pathway in GBM. (b) miRNA regulating glycolytic enzymes in GBM.** Arrowheads designate positive regulation. Blunt ends designate negative regulation. Dashed lines indicate indirect effects. Green and red ovals indicate upregulated and downregulated miRNAs. P: phosphate. P-: phospho-. BP: bisphosphate. PDK: pyruvate dehydrogenase kinase. PKM2: pyruvate kinase type M2.

### 3. miRNA regulating aerobic glycolysis in other tumour types are also differentially expressed in GBM.

Many cancers appear to rely on aerobic glycolysis to fulfil their bioenergetic and anabolic requirements, evade apoptosis and counteract oxidative stress.<sup>123-127</sup> Here, we attempt to link several miRNAs that regulate the *PI3K/Akt* signalling in GBM, as mentioned above, to their documented metabolic regulatory role in different cancers; these include miR-144, miR-143/miR-155, miR-128, miR-34a, miR-340 and miR-26a as discussed below (Figure 2). miR-144, which is downregulated in GBM<sup>64</sup>, was found to

target *GLUT1* in lung cancer.<sup>128</sup> The overexpression of miR-144 in lung cancer cell lines has resulted in the reduction of glucose uptake and lactate production.<sup>128</sup> Furthermore, miR-143, which is downregulated in GBM<sup>51,52</sup>, has been identified as a direct regulator of *HKII* in head and neck squamous cell carcinoma (HNSCC) and in colon and lung cancer. Like in GBM, miR-143 expression is downregulated in these tumours.<sup>129-131</sup> Moreover, in breast cancer, miR-155 was shown to indirectly upregulate *HKII* by repressing the miR-143 transcriptional activator, CCAAT/enhancer binding protein (C/EBP)  $\beta$ .<sup>132</sup> miR-155 was also shown to promote *HKII* transcription by upregulating the expression of the *HKII* transcriptional activator, *STAT3*.<sup>132</sup> Similar to GBM, miR-155 expression is elevated in breast cancer and correlated with short survival and unfavourable clinical outcomes.<sup>121,133</sup> miR-128, which is downregulated in GBM<sup>72-76</sup>, was reported to target *PFK* in lung cancer.<sup>134</sup> miR-128 expression is downregulated in lung cancer and is associated with poor prognosis.<sup>134</sup> Another miRNA, miR-34a, which is downregulated in GBM<sup>65-68</sup>, is also expressed at low levels in breast cancer.<sup>135,136</sup> In breast cancer, miR-34a targets Lactate dehydrogenase A (*LDHA*).<sup>135,136</sup> In addition, in colon cancer, the PK alternative splicing proteins, *hnRNPI/hnRNAPA1/hnRNAPA2*, are targeted by miR-340, miR-124 and miR-137, which are downregulated in GBM.<sup>102,103,137</sup> In GBM, miR-137 downregulation is associated with poor prognosis.<sup>137-141</sup> In colon cancer, these three miRNAs, miR-340, miR-124 and miR-137, which target *hnRNPI/hnRNAPA1/hnRNAPA2*, are downregulated in order to promote the mutually exclusive alternative splicing of PK into the PKM2, which is a key glycolytic adaptation in cancer.<sup>142</sup> Finally, miR-26a, which is upregulated in GBM<sup>72,106-108</sup>, is also upregulated and can target pyruvate dehydrogenase protein X component (*PDHX*) in colon cancer.<sup>143</sup> This would, therefore, promote glycolysis and inhibit oxidative phosphorylation (OXPHOS) by suppressing the expression of *PDHX* in order to block the conversion of pyruvate into acetyl coenzyme A; thereby preventing the entry of pyruvate into the citric acid cycle.<sup>143</sup>

Other differentially expressed miRNAs in GBM have been shown to be involved in the regulation of glycolytic transporters in other tumours. miR-1291, for example, which targets *GLUT1*, is downregulated in several cancers including renal cell carcinoma (RCC) and GBM.<sup>144</sup> In bladder cancer, miR-195-5p, which targets *GLUT3*, is also downregulated.<sup>145</sup> Moreover, miR-195-5p overexpression was shown to decrease glucose uptake.<sup>145</sup> In GBM, miR-195-5p is downregulated and its decreased expression is associated with poor prognosis.<sup>83,146</sup> In tongue squamous cell carcinoma (TSCC), another glycolytic enzyme, PKM2, is targeted by miR-133a/133b, which are downregulated in TSCC and in GBM.<sup>147-149</sup> Moreover, miR-122, which also targets *PKM2*, is downregulated in hepatocellular carcinoma (HCC)<sup>150</sup> and GBM, where it correlates with shorter patients survival.<sup>151</sup> Moreover, the overexpression of miR-122 was shown to switch HCC cell metabolism from aerobic glycolysis to OXPHOS.<sup>150</sup> Furthermore, miR-124, which is downregulated in GBM<sup>139</sup>, has been found to also be downregulated in medulloblastoma (MB).<sup>152</sup> miR-124 was reported to regulate the transport of lactate into the extracellular space by targeting the lactate monocarboxylate transporter 1 (*MCT1*) in MB.<sup>152</sup> Of interest, miR-124 was reported to target *STAT3* in GBM.<sup>153</sup> Since *STAT3* is a transcriptional activator for *HKII* in colorectal and esophageal cancer<sup>154,155</sup>, miR-124 downregulation in GBM could be speculated as

another miR-mediated mechanism of *HKII* upregulation. Another glycolytic enzyme, PFK, which is targeted by miR-128 as mentioned above, is also targeted by miRNA-320 in lung cancer.<sup>156</sup> miR-320 expression is downregulated in both lung cancer<sup>156</sup> and GBM.<sup>157</sup> A final example of differentially expressed miRNAs in GBM that regulate glycolysis in other cancers is miR-375, which targets LDHB in maxillary sinus squamous cell carcinoma (MSSCC).<sup>158-160</sup> miR-375 is downregulated in MSSCC and GBM, and this associates with low survival rate.<sup>158-160</sup>

Together, these miRNAs which regulate glucose metabolism in different tumours can serve as potential glycolytic regulators in GBM. It must be noted, however, that despite their differential expression in GBM, which could suggest a similar metabolic regulatory role in GBM tumours, these miRNAs have not yet been described in relation to GBM glycolysis. Thus, carrying out functional validation studies in GBM would be necessary in order to establish such links between miRNA expression levels and their regulatory role in glucose metabolism.

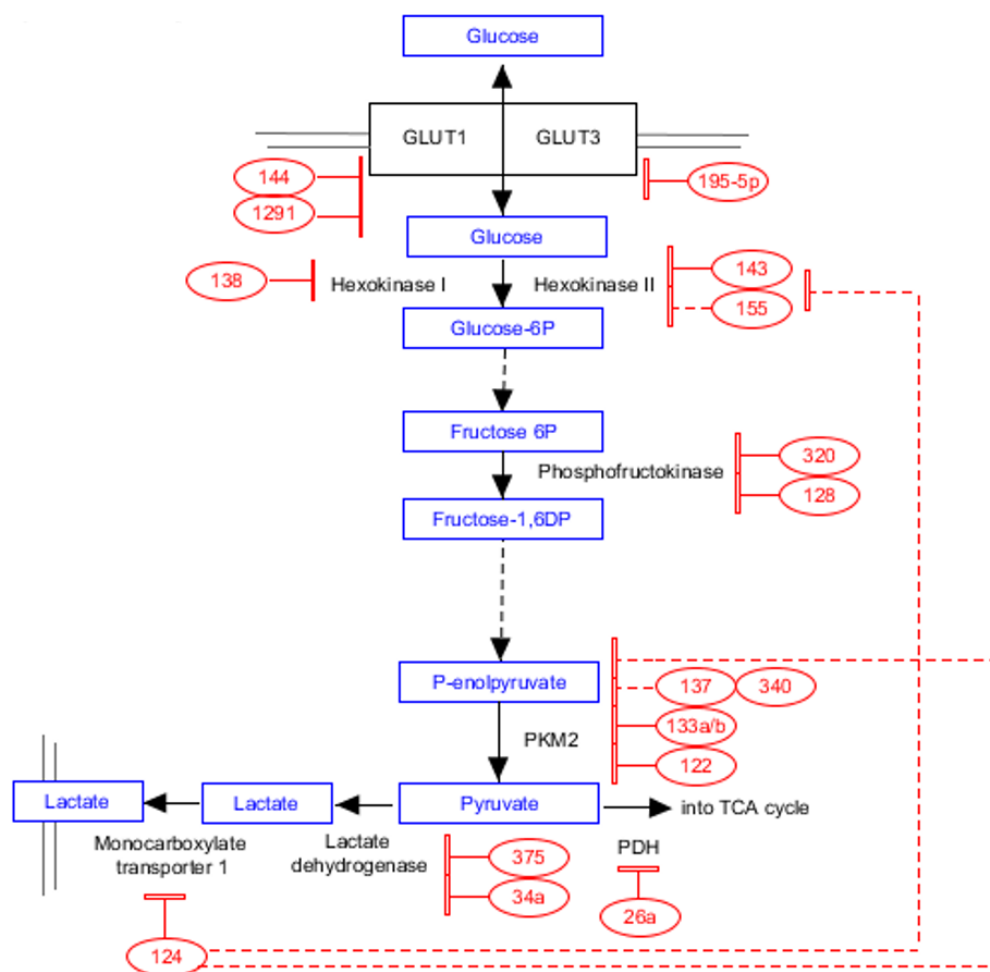


Figure 2. **Differentially expressed miRNA in GBM which are involved in the regulation of glycolysis in other tumours.** Downregulated miRNAs are shown in red ovals. Blunt ends designate negative regulation. Double lines represent cell membrane. Dashed red

lines denote indirect regulation. Dashed black lines indicate that several steps have been omitted.

#### **4. miRNA targeting as a therapeutic approach against GBM glycolytic metabolism.**

Targeting miRNAs, which can simultaneously target multiple genetic pathways, has the potential to disrupt glycolytic metabolism and overcome the limitations in current GBM therapy.<sup>161,162</sup> However, the potential of off-target effects, low stability and short half-life in plasma of miRNAs<sup>163</sup> and the lack of efficient delivery systems for miRNA-based therapy<sup>164</sup> present major challenges that would need to be solved before miRNA-based therapies can widely be used for the treatment of GBM patients.

In spite of the major challenges, a number of glycolysis regulating miRNAs discussed above have *in vitro* or/and *in vivo* demonstrated their therapeutic potential in GBM. Therapeutic targeting of miRNAs may be accomplished by: the inhibition of the overexpressed miRNAs or the replacement of downregulated miRNAs, as described below. In the first strategy, miRNA antagonists (antagomiRs or anti-miRs) are used to inhibit miRNA function. Anti-miRs are antisense oligonucleotides which are complementary, and bind to, the mature miRNAs in order to prevent its interaction with the miRISC complex.<sup>165</sup> Corsten et al. (2007) transfected GBM cell lines with an anti-miR-21, implanted them into mice intracranially and monitored their growth over 6 days.<sup>95</sup> The knockdown of miR-21 resulted in a remarkable reduction in tumour volume.<sup>95</sup> Moreover, anti-miR-21 can also increase the chemo-sensitivity of GBM cells as shown by Wong et al. (2012).<sup>166</sup> They developed TMZ-resistant GBM sub-clones and treated them either with anti-miR-21 and TMZ or with TMZ alone. The inhibition of miR-21 suppressed the growth of the TMZ-resistant cells and, in the presence of TMZ treatment, cells treated with anti-miR-21 showed a further increase in their apoptotic rate compared to those that were not treated with anti-miR-21.<sup>166</sup> Another *in vitro* study also showed that cells transfected with anti-miR-21 prior to TMZ treatment had an increased TMZ-induced cell death compared to cells treated with TMZ alone.<sup>167</sup> Similarly, miR-21 inhibition has been shown to increase sensitivity of GBM cells to paclitaxel (taxol, an anti-microtubule agent)<sup>168</sup>, teniposide (VM-26, a topoisomerase II inhibitor)<sup>169</sup>, and 5-fluorouracil (a pyrimidine analogue)<sup>170</sup>, three chemotherapeutic agents which are being investigated for the treatment of GBM.<sup>171-173</sup>

The alternative concept in miRNA-based therapy, miRNA replacement, aims to restore the expression of downregulated miRNA via introducing vectors expressing these miRNAs.<sup>174</sup> For instance, systemic administration of miR-7-expressing vectors to orthotopic GBM xenografts resulted in decreased tumour growth.<sup>175</sup> Moreover, miRNA re-expression in GBM cells was shown to enhance the effectiveness of targeted-therapeutics. miR-451, for example, was reported to cooperatively suppress GBM neurosphere formation when administered in combination with imatinib.<sup>176</sup>

Furthermore, combinatorial approaches of miRNA-based therapeutics have been proposed using *in vivo* systemic administration of both anti-miR21 and miR34a mimetics. The treatment with anti-miR21 and miR34a combination significantly increased apoptosis and senescence compared to treatment with either anti-miR21 or miR34a alone.<sup>177</sup>

Since the above mentioned miRNAs are involved in the regulation of glycolytic metabolism in GBM, one can consider miRNA-based therapy as a new way forward to target glycolysis and disrupt the metabolic homeostasis in GBM cells.

## **5. Conclusion**

Aerobic glycolysis is a hallmark of GBM tumours. To date, great advances have been made to understand the role of miRNAs in the regulation of glycolytic metabolism in GBM. miRNAs regulate glycolytic metabolism by regulating the expression of glycolytic genes and the signalling proteins, in the *PI3K/Akt* pathway, that regulate glycolysis. Several miRNAs regulating the *PI3K/Akt* pathway in GBM have also been shown to directly regulate components of the glycolytic pathway in other cancers. Moreover, other differentially expressed miRNAs in GBM, which have not yet been link to GBM glycolytic metabolism, play metabolic regulatory roles in other tumours. Although the differential expression of these miRNAs in GBM could suggest a similar metabolic regulatory role in GBM, functional validation studies would be necessary before such links can be established.

In GBM, and in multiple types of cancer, miRNAs that function to suppress or promote glycolytic metabolism are found to be down- or upregulated, respectively. Emerging evidence in GBM suggest that inhibition of upregulated or the replacement of downregulated miRNAs could be a promising therapeutic strategy to target glycolytic metabolism in GBM. Moreover, the combination of miRNA-based therapy with molecular targeted therapy or conventional chemotherapy has been demonstrated to exert additive or synergistic effects. Nevertheless, measures should be employed to ensure the stability of miRNA-based therapeutics, improve targeted delivery systems and understand and control of off-target effects of miRNA therapeutics before they can widely be used in clinic.

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