

## Roles of microRNAs in metabolic reprogramming of breast cancer

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### Abstract

Breast cancer cells exhibit deregulated metabolism. They require increased glucose uptake and glycolysis-associated enzymes to produce adenosine triphosphate by aerobic glycolysis rather than oxidative phosphorylation. Glutamine metabolism and fatty acid synthesis are also enhanced to meet the rapid and sustained cell growth. Triple-negative breast cancer and human epidermal growth factor receptor-2-positive breast cancers demonstrate significant metabolic reprogramming with increased levels of glucose and glutamine metabolism. Increasing evidences also suggest that micro-ribonucleic acids play important roles in the regulation of metabolic enzymes of breast cancer cells in post-transcriptional manner. Human epidermal growth factor receptor-2 and oestrogen receptor signalling pathways could have crosstalk with micro-ribonucleic acids in metabolic regulation network. The current narrative review was planned to go through recent advances on the role of micro-ribonucleic acids on metabolic reprogramming in breast cancer cells.

**Keywords:** Breast cancer, Phenotype, miRNA, Metabolic reprogramming, Glycolysis, Glutamine.

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### Introduction

It has been long known that cancer cells exhibit deregulated metabolism,<sup>1</sup> and that altered energy metabolism plays an important role in cancer initiation and progression besides being essential for the transformation of normal cells to cancer cells. Malignant cells use aerobic glycolysis to produce adenosine triphosphate (ATP) rather than oxidative phosphorylation despite the presence of oxygen. Enhanced fatty acid synthesis and glutaminolysis are also fundamental alterations of cancer cells that support unlimited proliferation.<sup>2</sup>

Micro-ribonucleic acids (miRNAs) are endogenous ~22nt non-coding RNAs and they bind to the 3'-untranslated regions (3'-UTR) of target messenger-RNAs (mRNAs) and regulate the expression of target mRNAs post-

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transcriptionally. One miRNA can bind to multiple target mRNAs and multiple miRNAs may bind to the same target mRNAs. Given the role of miRNAs in cancer, it is becoming clear that the deregulation of miRNA expression profiles participates in the regulation of carcinogenesis and progression of various cancers. Increasing evidences also suggest that miRNAs predominantly regulate key metabolic genes associated with cancer cells, which facilitate metabolic reprogramming.

Breast cancer can be classified into different molecular phenotypes based on hormonal status: Luminal A, Luminal B, human epidermal growth factor receptor-2 (HER-2)-positive and basal-like. The complexity of hormonal signalling pathways adds a layer of complexity in the metabolic regulation network in breast cancer. Until now, the molecular mechanisms of metabolic reprogramming in breast cancer are largely unknown.

The current narrative review was planned to discuss the regulatory role of miRNAs on the metabolic reprogramming of breast cancer.

### Metabolic reprogramming in breast cancer Glucose metabolism

Early in 1920s, Warburg<sup>1</sup> first revealed the phenomenon that cancer cells demonstrate increased glycolysis to produce energy for proliferation compared to normal cells, which was called the Warburg effect, and it happened despite the existence of oxygen. Meanwhile, in order to satisfy the increased glycolysis requirements, cancer cells require increased glucose uptake and glycolysis-associated enzymes.

Glucose transporters (GLUTs) facilitate import of glucose through the plasma membrane and are first rate-limiting proteins for glucose metabolism. In breast cancer cells, researchers have detected elevated levels of GLUTs.<sup>3</sup> Based on the molecular subtypes of breast cancer, triple-negative breast cancer (TNBC) and HER-2-positive breast cancer cells have higher levels of GLUT expression and elevated glycolysis compared to other subtypes.<sup>3</sup>

Hexokinase 2 (HK2) is the first regulatory enzyme in glycolysis. A study<sup>4</sup> detected HK2 positive in breast cancer cases (44%) and its expression was related to clinical outcome and an increased risk of recurrence. Pyruvate

kinase M2 (PKM2), which converts phosphoenolpyruvate (PEP) to pyruvate, controls the final step of glycolysis. PKM2 is widely overexpressed in TNBC cells, and inhibition of PKM2 improves the efficacy of chemotherapy and reverses drug resistance in TNBC patients.<sup>5</sup> Pyruvate dehydrogenase (PDH) converts pyruvate to acetyl-coenzyme. Breast cancer stem cells showed decreased expression of PDH and increased PDH kinase 1 (PDK1), which acts in concert with activated glycolysis under hypoxic conditions.<sup>6</sup> Lactate dehydrogenase-A (LDHA) is a nicotinamide adenine dinucleotide + hydrogen (NADH)-dependent enzyme and controls the conversion of pyruvate to lactate. One study showed that TNBC exhibited highest LDHA expression compared to other subtypes, and tumour LDH level could be a predictor for TNBC brain metastasis.<sup>7</sup>

### Glutamine metabolism

Breast cancer cells are known to show "glutamine addiction", meaning that they cannot survive in the absence of glutamine.<sup>8</sup> Glutamine metabolism is thought to be a key source of energy besides glycolysis in breast cancer cells.<sup>8</sup> Breast cancer cells also utilise glutamine-derived alpha ( $\alpha$ )-ketoglutarate to synthesise acetyl coenzyme A (CoA), which is a substrate of lipid, protein and nucleotide synthesis. Similar to glucose metabolism, immunohistochemical (IHC) studies indicated that TNBC and HER-2-positive breast cancer had higher expression of glutamine metabolic enzymes compared to other subtypes.<sup>9</sup>

Glutamine catabolic activity could be expressed by glutamate-to-glutamine ratio. A clinical study reported that malignant breast cells possessed an increased glutamate-to-glutamine ratio compared to benign cells.<sup>10</sup> Another study also revealed that invasive breast cancer cells had higher glutamate-to-glutamine ratio than non-invasive breast cancer cells.<sup>11</sup>

Glutamate secreted into stroma by breast cancer cells could have an impact on the tumour micro-environment, and

increase tumour cell aggressiveness. MDA-MB-231 cells release glutamate to promote cell invasiveness through up-regulating Rab27-dependent recycling of the transmembrane membrane type 1-matrix metalloproteinase (MT1-MMP), whereas normal mammary epithelial cells do not have such effects.<sup>12</sup>

### Lipid metabolism

Normal breast cells acquire the majority of fatty acids through extracellular lipids under normal circumstance. On the contrary, tumour cells satisfy their fatty acids requirement mostly via de novo biosynthesis. Three key enzymes related to fatty acid synthesis have been shown to be indispensable for cancer cell proliferation. ATP citrate lyase (ACLY) converts citrate to acetyl-CoA, and is the first key enzyme of fatty acid synthesis. ACLY activity was found to be significantly increased in breast cancer cells by 16-fold compared to normal breast cell, and the silencing of ACLY by small interfering RNA (siRNA) in Michigan Cancer Foundation-7 (MCF-7) cells could induce cell apoptosis.<sup>13</sup>

Acetyl-CoA carboxylase (ACC) converts acetyl-CoA to malonyl-CoA, and this represents the irreversible and rate-limiting enzyme of fatty acid synthesis. A study<sup>14</sup> reported that ACC was highly expressed in breast carcinoma in situ, which indicated that the turning point of ACC expression occurred at cancer initiation. Inhibition of ACC suppresses breast cancer stem cells self-renewal and results in the reduction of fatty acid synthesis and induction of cell apoptosis.<sup>15</sup>

Fatty acid synthase (FASN) is responsible for the endogenous palmitate synthesis. Up-regulation of FASN may be related to tumour initiation, progression and prognosis.<sup>16</sup> HER-2 breast tumours have the highest FASN expression, while TNBC tumours have the lowest.<sup>17</sup>

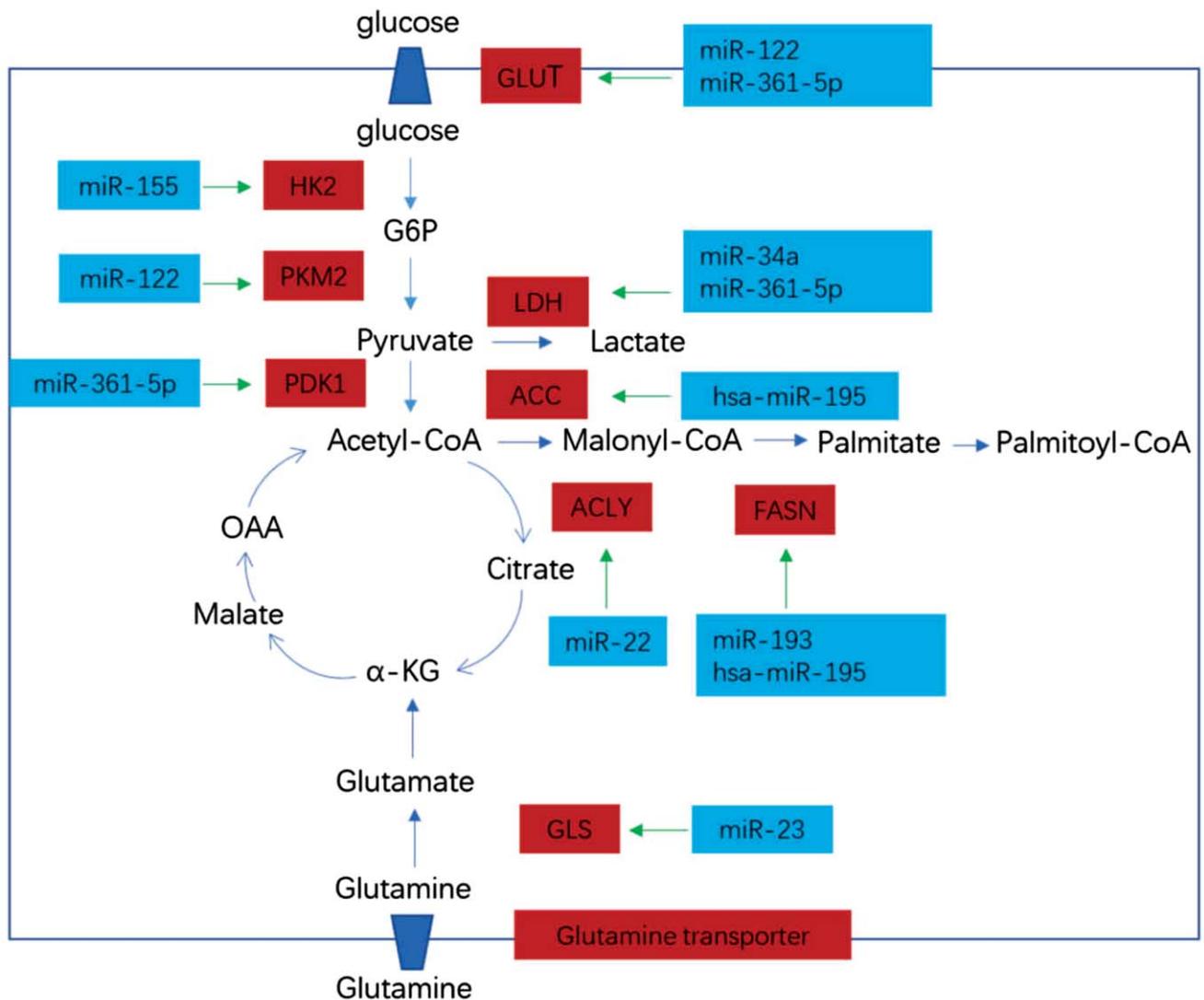
### miRNAs regulate glucose metabolism

Further, miRNAs mainly negatively regulate the

**Table-1:** List of micro-ribonucleic acids (miRNAs) involved in metabolic reprogramming of breast cancer cells.

Metabolism	Target gene	miRNA	miRNA functions	Reference
Glucose metabolism	GLUT1	miR-122, miR-361-5p	Decrease GLUT1 expression; Induce the translocation of GLUT1	18, 20
	PKM2	miR-122	Decrease PKM2 expression	18
	LDHA	miR-34a, miR-361-5p	Down-regulate the activity of LDHA	19, 20
	PDK1	miR-361-5p	Down-regulate the activity of PDK1	20
	HK2	miR-155	Up-regulate HK2 expression	21
Glutamine metabolism	glutaminase	miR-23	Decrease glutaminase expression	23
Lipid metabolism	ACLY	miR-22	Decrease ACLY expression	26,27
	ACC	hsa-miR-195	Decrease ACC expression	29
	FASN	miR-193, hsa-miR-195	Decrease FASN expression	28, 29

GLUT1: Glucose transporters-1, PKM2: Pyruvate kinase M2, LDHA: Lactate dehydrogenase-A, PDK1: Pyruvate dehydrogenase kinase 1, HK2: Hexokinase 2, ACLY: Adenosine triphosphate citrate lyase; ACC: Acetyl coenzyme A carboxylase, FASN: Fatty acid synthase.



**Figure-1:** The micro-ribonucleic acids (miRNAs) involved in metabolic reprogramming of breast cancer cells.

expression of their target genes in post-transcriptional fashion. The miR-122 secreted by breast cancer cells could suppress glucose consumption in pre-metastatic niche cells and promote metastasis through reducing expression of GLUT1 and PKM2. These effects could be reversed by treating recipient cells with anti-miR-122 (Table-1).<sup>18</sup> One study showed that LDHA was a direct target of miR-34a in MDA-MB-231 and MCF-7 breast cancer cells (Figure-1). The miR-34a could inhibit glucose uptake of breast cancer cells through targeting of LDHA and leading to decreased breast cancer cell proliferation (Table-1).<sup>19</sup>

Moreover, miR-361-5p also down-regulates the activity of PDK1 and LDHA in breast cancer cells (Figure-1). The overexpression of miR-361-5p in MDA-MB-231 cells could

induce the translocation of GLUT-1 from plasma membrane to intracellular compartments, thereby decreasing glucose uptake (Table-1) (Figure-1).<sup>20</sup> Also, miRNAs could exert promoting effect on expression of metabolic enzymes. A study<sup>21</sup> showed that miR-155 up-regulated HK2 expression through activation of signal transducer and activator of transcription3 (STAT3) (Table-1) (Figure-1).

### miRNAs regulate Glutamine metabolism

Onco-proteins c-Myc, p53 and hypoxia-inducible factor 1 $\alpha$  (HIF1 $\alpha$ ) are main regulators responsible for metabolic reprogramming in cancers. The miRNAs may also regulate glutamine metabolic enzymes in breast cancer cells.

Increased c-Myc expression was reported in 30-50% of

invasive breast tumours.<sup>22</sup> High expression of c-Myc in breast cancer cells indicates increased requirements of glutamine for proliferation. In a study of P-493 B cells, c-Myc enhanced glutamine metabolism through increased mitochondrial glutaminase expression via suppression of miR-23 (Table-1).<sup>23</sup> Inhibition of c-Myc decreases glutaminase activity, reduces uptake of both glucose and glutamine, and reduces cell growth.<sup>24</sup>

Over-expression of c-Myc could induce mammary cancer in transgenic mice. The miRNAs, including mir-20a, mir-20b, mir-9 and mir-222, are involved in c-Myc-induced mammary carcinogenesis.<sup>25</sup>

### miRNAs regulate Lipid metabolism

Tricarboxylic acid (TCA) cycle-derived citrate is the substrate for de novo lipid biosynthesis. Several miRNAs were reported to be involved in lipid biosynthesis in breast cancer cells. Koufaris et al.<sup>26</sup> reported that miR-22 had the strongest association with metabolic reprogramming, while miR-22 was significantly down-regulated in breast tumours, and may be associated with disease outcomes in breast cancer patients (Table-1). Ectopic expression of miRNA-22 in MCF-7 cells could repress the invasiveness of breast cancer cells through decreasing ACLY expression (Figure-1). Over-expression of miRNA-22 also suppressed the activity of ACLY which caused a consistent reduction in lipogenic acetyl-CoA and palmitate biosynthesis, leading to reduced fatty acid biosynthesis. Suppression of ACLY with siRNA had similar effects on lipid biosynthesis to that of miR-22, implying that miRNA-22 mediates its effect via modulating ACLY activity (Table-1) (Figure-1).<sup>27</sup>

Anti-diabetic drug metformin increased miR-193 while decreasing FASN expression in TNBC. Suppression of miR-193 abolished the effect of metformin on FASN, suggesting that FASN is a target of miR-193 (Table-1) (Figure-1).<sup>28</sup> Ectopic expression of hsa-miR-195 in MDA-MB-231 and MCF-7 cells directly suppressed the expression of ACC and FASN, resulting in reduced proliferation, invasion and migration of breast cancer cells (Table-1) (Figure-1).<sup>29</sup>

### miRNA and hormonal receptors in breast cancer cells

Breast cancer can be divided into four molecular subtypes with specific expression of oestrogen receptor (ER), progesterone receptor (PR) and HER-2. HER-2 and ER signalling pathways could have crosstalk with metabolic regulations. Thus, the expression pattern of miRNA in breast cancer should have relationship to ER, HER-2 and intrinsic subtypes.

Luminal type of breast cancer demonstrates a specific expression profile of miRNA, such as increased Let-7 and miR-21 expression compared to non-luminal type of breast cancer.<sup>30</sup> Also, miRNA may regulate ER expression through an ER-miRNA regulatory loop. A study showed that miR-206 could inhibit ER translation, while activation of ER could also block miR-206 expression.<sup>31</sup> Oestrogen could inhibit miR-21 expression by ER activation in MCF-7 cells.<sup>32</sup> This effect of the suppression of miR-21 and miR-26 by oestrogen may be mediated through c-Myc activation.

Lowery et al.<sup>33</sup> identified predictive miRNAs signatures related to ER (miR-135b, miR-190, miR-217, miR-218, miR-299, miR-342), PR (miR-377, miR-520f-520c, miR-527-518a, miR-520g) and HER-2 (miR-181c, miR-302c, miR-30e, miR-376b, miR-520d) statuses by a stepwise artificial neural networks (ANN) analysis. Similarly, a study<sup>30</sup> showed specific miRNAs related to ER/PR status (miR-25, miR-200a, miR-142-5p and miR-205) and specific to HER-2 status (miR-154, miR-195, miR-107, miR-126, let-7g, let-7f, miR-10b). One network analysis in breast cancer indicated key regulation of miRNAs on tumour metabolism under trastuzumab treatment. Among the leading effective miRNAs members, hsa-miR-216b played an important role, in particular miRNA-mediated regulatory mechanisms.<sup>34</sup> Studies in trastuzumab-resistant HER2-positive breast cancer cells demonstrated that miR-375 was down-regulated and predicted to target insulin-like growth factor (IGFR). Thus, miR-375 may restore the sensitivity of breast cancer cells to trastuzumab, whereas miR-375 inhibition may confer the trastuzumab resistance.<sup>35</sup>

### Conclusion

Metabolic reprogramming may be regulated by miRNAs via three distinct mechanisms. First, miRNAs could affect the expression or activity of metabolic enzymes through regulation of transcription factors. Second, miRNAs could directly regulate the expression of metabolic enzymes. Third, miRNAs may affect other miRNAs mutually to form a regulation network through chromatin remodelling. It is important to identify the key miRNAs and elucidate the complex regulatory network driving metabolic reprogramming of breast tumour cells. With increasing knowledge about the miRNA-mediated regulation of metabolic reprogramming, there is hope that novel therapeutic miRNA targets would be identified that will overcome the difficulties in the treatment of drug-resistant breast cancer.

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