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Metabolic Reprogramming of Triple-Negative Breast Cancer: The Role of miRNAs

Amal Qattan*

Abstract: MicroRNAs (miRNAs) are well known to influence the expression of the genes that regulate critical cellular functions. Various reports have suggested that they play critical roles in breast cancer metabolism through the regulation of various metabolic pathways, including the metabolism of glucose, lipids, glycolysis and the mitochondrial tricarboxylic acid cycle (TCA). miRNAs regulate the metabolic process by targeting key molecules (enzymes, kinases transporters) or by modifying the expression of key transcription molecules. In addition, miRNAs can indirectly regulate mRNA translation by targeting chromatin-remodeling enzymes. Furthermore, miRNAs influence the expression of both oncogenes and tumor suppressors and have a major impact on PI3K/AKT, HIF, and MYC signal transduction, which contributes to the metabolic phenotype in human cancer. Although human epidermal growth factor and endocrine therapies have been effective in treating breast cancer, for locally advanced and distant metastases mortality remains high. Drug resistance and recurrence remain major hurdles for advanced breast cancer therapy. Given the critical influence of metabolic reprogramming in the progression of neoplasm, tumorigenesis and metastasis, research should focus on novel targets of metabolic enzymes to reverse drug resistance and improve overall survival rates. Blocking the miRNAs that contribute to metabolic reprogramming or the use of exogenous miRNAs as antisense oligonucleotides, may be an effective way to treat aggressive, chemo-resistant cancers. This review summarizes current knowledge on the mechanism of action of miRNAs in altering the metabolism of cancer cells and presents possible therapeutic approaches to treating breast cancers that are resistant to current drugs.

Keywords: Metabolic reprogramming, triple-negative breast cancer, miRNAs (miRs), therapy

1 Background

MicroRNAs (miRNAs) are small, non-coding RNAs, 18-21 nucleotides in length, which modify gene expression post-transcriptionally and act as intracellular mediators in various biological processes. Because miRNA deregulation is a common feature of malignancies, they are arguably the most important species of RNA identified in cancer. Several studies have shown that, depending on the type of cancer, miRNAs can act as either oncogenes or tumor suppressors. Some miRNAs have been correlated with the prognosis of the disease or detected in serum/plasma for diagnostic purposes. Moreover, miRNA signatures are associated with tumor subtypes and clinical outcomes, suggesting that deregulated miRNAs may represent novel therapeutic targets [1, 2]. Various forms of miRNAs are differently expressed in subtypes of breast cancer [3, 4]. According to mRNA profiling, there are at least six distinct molecular subtypes of TNBC, and the miRNA expression among them may vary. These subtypes include luminal androgen receptor (LAR), basal-like ones (BL1 and BL2), a stem-like (MSL), a mesenchymal (M), and the immunomodulatory (IM) subtype [5].

Although comprehensive clinical data is scanty, progress has been made in our understanding of the complex molecular events that regulate TNBC metabolic phenotypes. miRNAs clearly play critical roles in the regulation of cancer cell metabolism through their actions on the expression of the genes involved in the processes. Just as miRNA profiles are altered in each subtype of breast cancer, specific metabolic profiles are observed in tumors to have differing expressions of ER and PgR. For example, TNBC shows a significantly higher level of glycolysis...
than other types of breast cancer do, and requires higher GLUT-1 expression. The Warburg effect is the best-known metabolic phenotype observed to date in tumors; this is the phenomenon by which cancer cells rely for energy on aerobic glycolysis. The dysregulation of miRNAs affects the rate of glycolysis [6], and miRNAs such as miR-129, which enhances GLUT-1 expression, therefore contributing to the Warburg effect [7]. Malignant transformation is also associated with excessive glucose uptake, de novo fatty acid synthesis, glutaminolysis, altered lipid metabolism, aerobic glycolysis, and an increase of reactive oxygen species (ROS) [8].

The finding that the benign tumors and stromal fibroblasts associated with cancer cells also use aerobic glycolysis is the basis for the reverse Warburg effect. It has been suggested that cancerous epithelial cells can trigger fibroblasts to undertake aerobic glycolysis and thus produce pyruvate and lactase. These metabolites of glycolysis feed the cancer cells, leading to increased proliferation [7]. Therefore, many papers highlight the link between ROS production, glucose metabolism and the epithelial mesenchymal transition (EMT), a feature associated with basal cell like breast cancer. Increased malignancy is also linked with increased glycolytic metabolism [9, 10]. The mechanism(s) remains to be established by which enhanced glycolytic activity and increased ROS are activated; the latter directly suppresses aerobic glycolysis by inhibiting pyruvate kinase (PKM2) and inducing NADPH production. This facilitates antioxidant synthesis and thus permits the accumulation of glycolytic intermediates [8]. How signaling pathways control energy metabolism is an important question, since studying metabolic pathways may yield new targets in treating drug-resistant and metastatic breast cancers [11, 12]. For example, phosphatidylinositol 3-kinase has a critical role in cancer metabolism. PI3K/Akt is thought to be involved in the modulation of FASN, a vital enzyme necessary for the de novo synthesis of fatty acids. Up-regulation of FASN has been found in premalignant lesions and most human cancers. Cancer cells depend on de novo fatty acid synthesis, which is suppressed in normal cells having low FASN expression [12, 13]. Interestingly, the expression of FASN is regulated negatively by miR-195 via a unique binding site in FASN 3'-UTR, which in consequence inhibits migration and invasion [13]. In addition, PI3K is targeted by miR-123a, miR-136, miR-320, miR-422, and miR-506, which taken together gives strong evidence for the role of miRNAs in regulating cell metabolism.

In order to produce the great amount of ATP necessary for cancer cell proliferation, it is necessary to have a metabolic shift to aerobic glycolysis (the Warburg effect). Research strongly suggests that miRNAs are involved in cancer cell proliferation, in part because miRNAs have been shown to affect carbohydrate metabolism as well as lipid metabolism. Whether restoring normal aerobic metabolism to a cancerous cell could halt the process of tumorigenesis and whether miRNAs are involved is still under investigation. To date, the evidence suggests that miRNAs act directly by interacting with metabolic transporters, metabolic enzymes and the vital signaling pathways involved in metabolism, and by controlling the expression of oncogenes and tumor suppressors. This review centers on the crucial roles of miRNAs in modifying metabolism in cancer cells.

2 miRNAs mediate metabolic reprogramming in breast cancer

Cancerous cells use metabolic reprogramming in order to enhance biosynthesis, growth, and survival [14]. The metabolic reprogramming of cancer cells is achieved through a complex interplay of regulatory networks involving phosphatidylinositol 3-kinase (PI3K), mTOR, protein kinase B (Akt), PTEN and 5' AMP-activated protein kinase (AMPK). While many miRNAs regulate glucose, lipid and the metabolism of amino acids by modulating gene transcription, the lack of information on major metabolic processes in the mammary glands has meant that metabolic reprogramming by miRNAs in breast cancer has not been studied in detail [8]. It is well known that cancer cells use mechanisms of aerobic energy production rather than oxidative phosphorylation [7, 15]; most fundamental metabolic processes such as increased rates of glucose uptake and glycolysis, suppressed gluconeogenesis, and lactic acid fermentation are altered. Abnormal glucose metabolism is associated with changes in miRNA expression and miRNAs have been shown to respond to the adaptation of cells to different glucose conditions. For example, in mesenchymal stem cells under hyperglycemic conditions, there is reduced miR-32-5p synthesis, leading to the promotion of the cell cycle by the targeting PTEN.

In malignant cells, the deregulation of glucose transporters (GLUTs) produces a high glucose requirement and increased glucose uptake, which accelerates cancer cell metabolism. GLUT expression also correlates positively with the pathological grade of breast cancer. In breast cancer cells, six types of glucose transporters can be found: GLUT1, GLUT2, GLUT3, GLUT4, GLUT5, and GLUT12. The GLUT1, GLUT2 and GLUT3 transcripts are elevated, in many cancers, but surprisingly mRNA for GLUT4 and GLUT5 has not been detected. TNBC tumor homogenates and also locally advanced breast cancers
show an increased expression of GLUTs. Much research has unequivocally demonstrated that GLUT1 and GLUT3 are much higher in many types of tumor [16, 17]. GLUT1 expression is higher in TNBC than in other subtypes of breast cancer [10, 18, 19]. In particular, GLUT1 is believed to be an important rate-limiting step in the control of glucose transport to cancer cells, but interestingly it is nearly undetectable in benign tumors and normal epithelial tissue [17]. miRNAs regulate the expression of several of the genes that facilitate glucose uptake. miR-129 targets GLUT1; miR-106a and miR-195-5p target GLUT3; and miR-93 targets GLUT4 [20]. The increased expression of miR-19a, miR-19b and miR-130b has been shown to down-regulate GLUT-1. miR-195-5p is a direct regulator of GLUT3, which is elevated in most malignant tissues. miR-195-5p inhibits cell growth and proliferation, and promotes apoptosis by suppressing GLUT3 expression, which suppresses proliferation (Figure 1) [6, 21]. This evidence suggests that exploiting or silencing miRNAs may open new horizons for cancer therapy.

**Figure 1:** miRNAs regulate breast cancer cell metabolism. Red bars indicate inhibition of the target. The change to aerobic glycolysis in cancer cells of the breast is facilitated by miRNAs, which also regulate the expression of GLUT transporters and the hexokinase 2 (HK2) enzyme involved in glycolysis.
2.1 Role of miRNAs in glycolysis

Apart from glucose transport, glycolysis also provides cancer cells with energy. A number of studies have suggested that miRNAs control glycolytic enzymes and thus the regulation of irreversible steps in glycolysis. The target enzymes include 6-phosphofructo-1-kinase (PFK1), hexokinase (HK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). It is known that the human genome encodes four HK isoforms, HK1, HK2, HK3, and HK4. HK2 is the key component of the Warburg effect, and a promising target for novel therapy. miR-143 inhibits glycolysis by down-regulating HK2, a glycolytic enzyme that converts glucose to glucose-6-phosphate. This mechanism was confirmed for breast cancer by Fang in 2012 [22] and in colon cancer by Gregersen in 2012 [23]. Many reports have shown that miR-143 targets HK2 and thus regulates the metabolism of glucose in cancer, indicating that it may become a novel target for cancer chemotherapy. Interestingly, miR-155 has been found to repress miR-143 by action on C/EBPβ, which is a transcriptional activator of miR-143, and to up-regulate HK2 expression at the post-transcriptional level. In addition, administration of cholesterol-modified agomir-143 (systemic delivery) significantly inhibited tumor growth in TNBC mice, suggesting a potential therapeutic regimen for TNBC [24]. miR-155 is known to control the expression of HK2 in breast cancer cells through a dual-switch mechanism. Initially, miR-155 represses miR-143, which negatively regulates HK2. Then, miR-155 stimulates HK2 transcription through the activation of STAT3, by down-regulating SOCS1, a STAT3 inhibitor in breast cancer cells [17, 25, 26]. Another important enzyme associated with breast cancer cell metastasis is phosphoglucone isomerase (PGI), which has been implicated with the metastasis and invasion. PGI, a cytosolic enzyme that catalyzes the interconversion of glucose-6-phosphate and fructose-6-phosphate, plays a fundamental role in gluconeogenesis and glycolysis. PGI regulates miR-200 family expression, which directly affects the EMT in breast cancer [8, 27].

In contrast to the intracellular level of miR-122, which does not significantly differ whether metastatic or non-metastatic, the level of exosomal (circulating) miR-122 is correlated with the metastatic capacity of breast cancer [28, 29]. Exosomal miR-122 inhibits glucose uptake by downregulating PKM2 leading to enhanced cancer cell proliferation [29, 30]. PKM2 allows the tumor to use phospho-metabolites, which are essential precursors involved in the synthesis of amino acids. During aerobic glycolysis, lactate dehydrogenase (LDH) is transported out of cells by the monocarboxylate transporter 1 (MCT1). In order to fulfill the high-energy demands of cancer cells, the solute carrier family 16 member 1 (SLC16A1) is targeted by miR-124, which directly regulates the formation of lactate by the overexpression of SLC16A1. In human breast cancer miR-210 also indirectly improves aerobic glycolysis by repressing mitochondrial respiration. The expression of miR-200a is reduced in breast cancer and the level of mitochondrial transcription factor A (TFAM) is increased. TFAM directly activates mitochondrial respiration and regulates both the production of ROS and mitochondrial oxidation [31].

2.2 Role of miRNA in TCA Cycle

Though cancer cells usually prefer aerobic glycolysis for the production of energy to the TCA cycle, the TCA cycle also provides cancer cells with energy and is targeted by miRNAs. A shift of glucose metabolism to aerobic glycolysis is common in cancer cells. Studies predict that miR-107 and miR-103 will up-regulate pantothenate kinase (PanK). PanK expression is essential to the coenzyme A pathway and it catalyzes the phosphorylation of pantothenate to 4-phosphopantothenate. Acetyl CoA and lipid levels are regulated by miR-103 and miR-107 [6, 32, 33]. In addition, miR-19a, miR-19b, miR-122a, miR-148a, miR-152, miR-299-5p, miR-421, and miR-494 regulate the genes that encode TCA enzymes [6]; the first miRNA linked to metabolic control was miR-122. miRNAs are also important players in the control of the TCA cycle in that modulate transcription factors in breast cancer such as the myelocytomatosis oncogene (MYC) and the hypoxia inducible factors HIF1-α and HIF1-β [6].

2.3 Role of miRNAs in homeostasis and metabolism of lipids

Cancer metabolism varies according to both the metastatic site and the expression of proteins such as the fatty acid synthase (FASN) which are associated with lipid metabolism. Research has revealed the overexpression of FASN in breast cancer cells, facilitating their development and survival. miR-142-3p, miR-195, miR-320, and miR-424 were found to inhibit FASN [39, 40]. The first miRNA found to be associated with lipid metabolism was miR-122 [34], which has been implicated in the increased synthesis of lipids and cholesterol-rich membranes in cancer cells [35]. The inhibition of miR-122 (a tumor suppressor) disrupts lipid storage and lipid metabolism in breast cancer cells [8, 32, 41]. In addition, glutathione S-transferase Pi 1
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For the treatment of drug-resistant breast cancer, targeted metabolic reprogramming by miRNAs may be a novel and promising anti-cancer therapy. To effectively treat breast cancer, drugs that target metabolic enzymes and their regulators (miRNAs) may help to overcome difficulties in treatment [47]. miRNAs have been shown to be therapeutically effective in managing cancer. One study showed that suppressing glucose and glycolysis by inhibiting HK and blocking GLUTS can improve breast cancer therapy [43]. Another study revealed that a tumor suppressor p53 pathway induces let-7, miR-34, miR-107, and miR-200, which regulate MYC, LDHA, and sirtuin 1 (SIRT1) [32]. Targeting LDHA, MYC, SIRT1, and other metabolic pathways with miR34 may be an effective and innovative therapy for breast cancer.

Moreover, it has been demonstrated that metformin plays a pivotal role in reprogramming cancer cell metabolism and directly modulating genes and miRNAs [48]. Metformin acts by affecting the progression and relapse of different types of cancer when combined with a chemotherapeutic agent. The anticancer metabolic actions of metformin involves miRNA modulation [8, 49]. Several studies have shown that the systematic administration of miRNAs helps to counter the growth of breast cancers and to reduce cell toxicity. miR-34 expression in breast cancer is decreased as it is in other types of cancer [43]. These miRNAs have important tumor suppression functions and negatively regulate oncogenes by targeting RAS and MYC [32].

### 2.4 Role of miRNAs in signaling pathways involved in cancer metabolism

Signaling pathways that have been deregulated, including PI3K/Akt/mTOR, AMPK, c-Myc and HIF, are hallmarks of cancer cells [32, 44]. The relationship between imbalanced signaling pathways and deregulated miRNAs is associated with the abnormal metabolism of cancer cells. The major pathways associated with the metabolic reprogramming of breast cancer cells include PI3K/AKT/mTOR and PTEN. In breast cancer, the expression of glycolytic enzymes and the translocation of glucose transporters cause an increase in glycolysis via the AKT pathway. In turn, activating the AKT pathway regulates metabolism via HIF-1α [8, 32, 44]. Studies have also shown that AKT isoforms regulate miRNAs, for example, the miR-200 family in breast epithelial cells, such that their expression is reduced with activated AKT2 [45]. The p53 pathway may inhibit the expression of GLUT1 and GLUT-4 thus affecting glycolysis, and can also activate hexokinase 2 (HK2) [6, 46]. To date, miR-25, miR-29, miR-30d, miR-34a, miR-125b, miR-142, miR-194, and miR-215 are known to control the activity and abundance of p53 [6]. Of these, miR-25, miR-30d, and miR-125b negatively regulate p53 by directly binding to the 3′-UTR of p53 mRNA, whereas the other molecules modify the regulators of p53, such as MDM2 [6].

### 3 miRNAs Therapeutics in breast cancer metabolism

For the treatment of drug-resistant breast cancer, targeted metabolic reprogramming by miRNAs may be a novel and promising anti-cancer therapy. To effectively treat breast cancer, drugs that target metabolic enzymes and their regulators (miRNAs) may help to overcome difficulties in treatment [47]. miRNAs have been shown to be therapeutically effective in managing cancer. One study showed that suppressing glucose and glycolysis by inhibiting HK and blocking GLUTS can improve breast cancer therapy [43]. Another study revealed that a tumor suppressor p53 pathway induces let-7, miR-34, miR-107, and miR-200, which regulate MYC, LDHA, and sirtuin 1 (SIRT1) [32]. Targeting LDHA, MYC, SIRT1, and other metabolic pathways with miR34 may be an effective and innovative therapy for breast cancer. Moreover, it has been demonstrated that metformin plays a pivotal role in reprogramming cancer cell metabolism and directly modulating genes and miRNAs [48]. Metformin acts by affecting the progression and relapse of different types of cancer when combined with a chemotherapeutic agent. The anticancer metabolic actions of metformin involves miRNA modulation [8, 49]. Several studies have shown that the systematic administration of miRNAs helps to counter the growth of breast cancers and to reduce cell toxicity. miR-34 expression in breast cancer is decreased as it is in other types of cancer [43]. These miRNAs have important tumor suppression functions and negatively regulate oncogenes by targeting RAS and MYC [32].

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### Table 1: MicroRNAs, target pathways, and gene regulation of breast cancer metabolism.

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Target genes</th>
<th>Metabolic activity/Pathway</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>miR-210</td>
<td>ISCU</td>
<td>Hypoxia, Krebs cycle, glycolysis</td>
<td>[34]</td>
</tr>
<tr>
<td></td>
<td>ISCU, COX10</td>
<td>Hypoxia, ROS, glycolysis</td>
<td>[35]</td>
</tr>
<tr>
<td>miR-155/miR-143</td>
<td>SDHD, NDUFA4</td>
<td>HIF-1α, ROS, TCA cycle, Hypoxia</td>
<td>[34]</td>
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<tr>
<td>miR-378</td>
<td>HK2</td>
<td>Aerobic glycolysis</td>
<td>[25]</td>
</tr>
<tr>
<td>miR-124</td>
<td>ESRRG, GABPA, ERR</td>
<td>Oxidative phosphorylation</td>
<td>[36]</td>
</tr>
<tr>
<td>miR-200</td>
<td>PGI</td>
<td>Glycolysis</td>
<td>[27]</td>
</tr>
<tr>
<td>miR-126</td>
<td>PI3K</td>
<td>Down-regulation</td>
<td>[38]</td>
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</tbody>
</table>

(GSTP1) is a potent regulator of lipid metabolism in TNBC. In prostate cancer, miRNAs have been found to regulate GSTP1 [42], suggesting evidence for the role of miRNAs in TNBC tumorigenesis. One study showed that inactivation of GSTP1 reduces tumorigenesis activity in TNBC [43]. Thus, targeting lipid metabolism via exogenous miRNAs may be a novel therapeutic strategy for combating TNBC.
4 Conclusion

Current research on miRNAs as major regulators of cancer metabolism provides a foundation for understanding the mechanisms responsible for the aberrant metabolism of cancer cells. Collectively, the data described in this mini-review support the hypothesis that miRNAs act to modulate the glucose metabolism by regulating vital factors including the transporters (GLUT) or key enzymes (HK2, PKM2). The addition of exogenous miRNAs or the silencing of specific miRNAs may be capable of halting metastasis. These findings provide future directions for the targeting of metabolic enzymes, which may be a promising strategy for overcoming drug-resistant cancers. It remains clinically challenging to control breast cancer in patients who are resistant to chemotherapy. More research is needed on whether the circulating and extracellular miRNAs are involved in cancer metabolism or in regulating the key glycolytic genes. It is also important to find which types of miRNA are key to the reprogramming of glucose metabolism. To treat breast cancer, future research should further explore the innovative delivery methods of the exogenous miRNAs that target metabolic pathways.

Abbreviations

miRNAs: MicroRNAs
TNBC: Triple-negative breast cancer
TCA: Tricarboxylic acid
GLUTs: Glucose transporters
ROS: Reactive oxygen species
PKM2: Pyruvate kinase isozyme M2
FASN: Fatty acid synthase
PFK1: 6-phosphofructo-1-kinase
HKs: Hexokinases
PGI: Phosphoglucone isomerase
SLC16A1: Solute carrier family 16 member 1
TFAM: Mitochondrial transcription factor A
GSTP1: Glutathione S-transferase Pi 1
SIRT1: Siruvin

Declarations

Ethical Approval and Consent to participate: NA

Consent for publication: NA

Competing interest: The author declares that they have no competing interest.

References


