Review

Regulation of Glucose Metabolism in Hepatocarcinogenesis by MicroRNAs

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In the past decade, considerable effort has been made in elucidating the mechanism underlying the high level of aerobic glycolysis in cancer cells. While some recent studies have attempted to address this issue, the potential role of microRNAs in this process has not been explored until recently. These studies have demonstrated involvement of just five deregulated miRNAs in glucose metabolism in hepatocarcinogenesis. This review discusses the metabolic significance of these miRNAs in hepatoceullular carcinoma, their targets in glycolysis, gluconeogenesis, and pentose phosphate pathways, and provides an insight into the therapeutic potential of targeting specific miRNAs.

Key words: Hepatocellular carcinoma; Glucose metabolism; MicroRNAs

INTRODUCTION

A hallmark of cancer cells is that they metabolize glucose primarily by aerobic glycolysis, whereas most normal cells catabolize glucose by oxidative phosphorylation in the mitochondria. The proliferating cancer cells can metabolize as much as 10-fold more glucose to lactic acid than corresponding normal tissues under aerobic conditions (1). While glycolysis and subsequent oxidative phosphorylation produce 36 ATP molecules/mole of glucose, lactate production by glycolysis generates just 2 ATP molecules, making this process energetically inefficient. This shift in glucose metabolism accompanied by enhanced uptake of glucose and conversion of pyruvate to lactate, known as the Warburg effect, is beneficial to cancer cells because it facilitates production of macromolecules required for cell proliferation (2,3).

Insufficient knowledge of the exact role of drastic alteration in energy metabolism in cancer phenotype and a dearth of information concerning the potential relationship between metabolic deregulation and oncogene activation or tumor-suppressor inactivation probably contributed to the lack of interest in this unique glucose metabolism characteristic of many types of cancer, particularly liver tumors. Interestingly, the augmented uptake and metabolism of glucose generally correlate with poor prognosis of many tumor types (4–6). After almost six decades since Warburg observed the higher glycolytic rate in cancer cells, there has been a renewed interest in understanding the significance of this process in the development of cancer.

Although considerable effort has been made in understanding the functional significance of higher rates of glycolysis and production of lactate in malignant cells (7), the possibility of altered gluconeogenesis that could potentially facilitate the glycolytic pathway was not explored until recently. A mouse model where liver tumors can be induced by feeding a choline-deficient and amino acid-defined (CDAA) diet, in the absence of any exogenous chemicals or virus (8), was used to understand this process at different stages of tumor development (9). A distinct advantage of using this model system was that only the liver contains a full complement of all the enzymes essential for gluconeogenesis. Further, tumorigenesis in this model involves steatosis, inflammation, fibrosis, and insulin resistance that are the hallmarks of human hepatocellular carcinoma (HCC) (10,11). Using this model system, we have shown that the expression of all the critical enzymes and a transcription factor involved in gluconeogenesis are suppressed in the liver tumors. Inhibition of glucose production by

gluconeogenesis could facilitate persistent glycolysis. Further, the excess of glucose-6-phosphate accumulated due to inhibition of gluconeogenesis could be diverted to the pentose phosphate pathway (PPP) for the production of nucleotides required for cell proliferation.

While some recent studies have focused on the mechanisms underlying the altered glucose metabolism in cancer cells, the potential role of microRNAs in this process is only now being explored. These studies have revealed a few microRNAs, notably miR-1, miR-23a, miR-34a, miR-155, and miR-199a, that target specific enzymes involved in glycolysis, gluconeogenesis, and the PPP. miR-1, miR-34a, and miR-199a are significantly down-regulated in HCC, whereas miR-23a and miR-155 are upregulated. These miRNAs can, therefore, function as oncogenes or tumor suppressors. This review has made an effort to discuss the specific targets of these miRNAs in the metabolic pathways and the mechanism(s) by which they modulate these pathways to facilitate initiation, progression, and maintenance of cancer cells, particularly in hepatocarcinogesis. We have also provided an insight into the therapeutic potential of targeting these specific microRNAs.

miRNA-23a

We have demonstrated that miR-23a is significantly upregulated in diet-induced rodent models of HCC (9, 12), as well as in primary human HCC (9). Interestingly, another study identified a correlation between higher miR-23a expression and liver cirrhosis and intrahepatic metastasis (13). Although some studies have reported diminished hepatic glucose production (gluconeogenesis) induced by IL-6-mediated activation of STAT3 (14, 15), it was not until recently that the mechanism underlying this inhibition was shown to be at least partially due to the upregulation of miR-23a. Hepatic gluconeogenesis is a vital metabolic pathway functioning as the primary source of the body's glucose during fasting (16). The rate of gluconeogenesis in the liver is controlled by three crucial enzymes: glucose-6-phosphatase (G6PC), phosphoenolpyruvate carboxykinase (PEPCK), and fructose-1,6-biphosphatase (Fbp1) (16). Our study showed that IL-6–STAT3 signaling activates the expression of miR-23a, which directly targets the key gluconeogenic enzyme G6PC and its transcription coactivator peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α). In addition, an inverse correlation between miR-23a expression and mRNA levels of PEPCK, another key enzyme linked to gluconeogenesis, was observed in primary human HCC samples. Importantly, the expressions of all three enzymes were significantly suppressed in primary human HCC tissues (9). Further, G6PC protein level and enzymatic activity were also significantly

reduced in primary human HCC samples (9). This is highly significant because all three reactions associated with these enzymes are irreversible (16). STAT3 is known to suppress hepatic gluconeogenesis through the downregulation of PEPCK and G6PC independent of PGC-1α (15). While the PGC-1α-independent suppression of G6PC by STAT3 can be explained by direct targeting of this key gluconeogenic enzyme by miR-23a (9), the mechanism underlying PGC-1α-independent regulation of PEPCK remains to be explored.

These observations collectively prove that drastic reduction in glucose production in HCC is, at least in part, caused by miR-23a overexpression. Inhibition of gluconeogenesis in HCC may lead to the accumulation of glucose-6-phosphate (G6P), a key metabolic intermediate, within the cell. G6P can then be utilized in a variety of metabolic pathways including glycolysis for energy production and the PPP for NADPH regeneration and nucleotide synthesis. Increased G6P flux through these pathways may aid tumor cells in maintaining high rates of proliferation.

In addition to its role in hepatic gluconeogenesis, PGC-1α plays a regulatory role in mitochondrial respiration and biogenesis (17,18). Through the upregulation and coactivation of nuclear respiratory factors (NRFs) 1 and 2, transcription factors for key mitochondrial regulators including mitochondrial transcription factor A (TFAM), PGC1α promotes mitochondrial biogenesis and consequently enhances cellular oxygen consumption (17). Although this mitochondrial function of PGC-1α has been extensively studied in adaptive thermogenic tissues (muscle and brown adipose tissue) (18), such a function has yet to be thoroughly explored in HCC. It is possible that miR-23a-mediated suppression of PGC-1α can facilitate a metabolic switch from oxidative phosphorylation to anaerobic glycolysis in HCC.

Besides miR-23a, IL6-STAT3 signaling can also upregulate the expression of oncogenic transcription factor cMyc (19). Conversely, cMyc can repress miR-23a expression in prostate cancer and lymphoma cells (20). In these cells, miR-23a has also been implicated in the suppression of glutamine metabolism by directly targeting glutaminase 1 (GLS1) (the kidney isoform of GLS) (20). cMyc-mediated suppression of miR-23a in lymphoma plays an important role in glutamine metabolism that leads to the production of energy and anabolic precursors (21). In contrast, both miR-23a and glutaminase activity are drastically higher in primary HCC tissue compared to normal liver tissue (9,22). This concurrent enhancement of both miR-23a expression and glutamine metabolism could be a result of enhanced activity of glutaminase 2 (GLS2) (the liver isoform of GLS) since coexpression of GLS1 and GLS2 has been reported in

multiple cancer cell lines and human cancers (23). In this context, a recent study examining in vivo metabolism in oncogene-induced tumors has demonstrated that GLS2 is the only form of GLS expressed in the normal liver, as anticipated, but cMyc-induced liver tumors replace this GLS2 expression with GLS1. In contrast, MET-induced liver tumors exhibited an attenuation of GLS2 in the absence of associated upregulation of GLS1 (24). It is likely that the appearance of GLS1 in the myc-induced tumors, but not in met-induced tumors, is due to suppression of miR-23a by cMyc. It is important to explore the complex interaction of IL-6–STAT3 signaling and cMyc with miR-23a and the role of miR-23a in glutamine metabolism in HCC.

miRNA-1

We have demonstrated the tumor-suppressor characteristics of miR-1, which is silenced by promoter methylation in primary human HCC (25). miR-1 suppresses tumor cell proliferation by directly targeting mRNA coding for the oncogenic proteins MET, FOXP1 (25,26), and HDAC4 (27). Reduced miR-1 levels in the serum appear to correlate with shorter overall survival of HCC patients independent of age, sex, tumor stage, and previous treatments (28). A noteworthy observation is that miR-1 can also augment HBV replication (29), a risk factor for cirrhosis and liver cancer (30,31).

Another study from our laboratory has also identified a tumor-suppressive role for miR-1 in lung cancer where it targets oncogenic proteins FOXP1, MET, PIM1, and HDAC4 (26). A recent investigation has shown that miR-1 acts as a tumor suppressor in lung cancer cells by targeting multiple enzymes within the PPP: glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), and transketolase (TKT) (32). Glucose flux through the PPP helps cancer cells to meet the high demand for nucleotide synthesis through the production of ribose-5-phosphate and reduces oxidative damage via elevated levels of NADPH (33). In this context, it should be emphasized that PPP also provides glycolytic intermediates, fructose-6-phosphate, and glyceraldehydes-3-phosphate, which can be metabolized through glycolysis to generate ATP and anabolic precursors (16). Overall, the shift in the primary glucose metabolism caused by reduced levels of miR-1 facilitates glycolysis, nucleotide synthesis, and regeneration of NADPH. It will be interesting to determine whether a similar regulation of PPP and glycolysis by miR-1 occurs in the liver.

The direct targeting of G6PD by miR-1 is of significance to HCC because this miRNA is epigenetically suppressed in HCC (25). Further, G6PD is the first and rate-limiting enzyme in the PPP that is also significantly upregulated in HCC (34,35). Therapeutic delivery of miR-1 is likely to reduce G6PD level/activity, thus impairing NADPH production, macromolecule synthesis, and subsequently tumor proliferation. A reduction in NADPH production and the consequent increase in ROS also sensitize cells to radiation-induced cell damage (36). However, it will be important for such a delivery of miR-1 to be targeted to tumor cells to prevent systemic oxidative damage. Because the metabolic functions of G6PD play an important role in survival, proliferation, and anticancer drug resistance (36), it is emerging as an important diagnostic and therapeutic target for many types of cancer. Therefore, it will be critical to investigate the use of high-throughput analysis of G6PD expression and activity in HCC specimens as a potential diagnostic marker (36) and the possibility of targeted miR-1 delivery as a therapeutic option for HCC. In this context, we have used DODMA-based cationic lipid nanoparticles (LNP-DP1) for systemic delivery of miR-122, a liver-specific miRNA with tumor-suppressor functions (12,37), to the liver and HCC (38). A key observation was that delivery of miR-122 to the normal liver and HCC in vivo caused significant inhibition of expression of miR-122 target genes and suppressed HCC xenograft growth in a mouse model of HCC (38). This approach could be extended to miR-1 and other downregulated miRNAs relevant to glucose metabolic pathways in HCC to determine their potential to arrest tumor growth.

miRNA-34a

miR-34a that is transcriptionally activated by p53 (39) is significantly downregulated in metastatic HCC tissues (40). It is considered a tumor suppressor due to its ability to induce apoptosis and inhibit cell growth by directly targeting MYCN (41), and its capability to directly target MET causing inhibition of cell migration and invasion of HCC-derived cells (42,43). It also inhibits cellular glycolysis by directly targeting hexokinase 1 (HK1), hexokinase 2 (HK2), and glucose-6-phosphate isomerase (GPI) (44). HK1 and HK2 are responsible for the first reaction in glycolysis where glucose is phosphorylated to G6P. This phosphorylation locks glucose in the cell by inhibiting its transport mediated by glucose transporters. The conversion to G6P also acts to maintain a low concentration of glucose in the cell to facilitate the uptake of extracellular glucose via glucose transporters (16). These data suggest that augmentation of miR-34a expression can lead to a decreased rate of glycolysis, resulting in suppression of tumor growth (44).

miR-34a appears to be a universal tumor suppressor, its expression being lost in multiple solid tumors and hematopoietic malignancies. Importantly, it antagonizes several oncogenic processes including cell proliferation, apoptosis, stemness, and metastasis. Further, a miR-34a

mimic has shown promise in the therapy of a variety of cancers, both in vitro and in vivo in mouse models (45). More recently, the observation that knockdown of miR-34a targets, sirtuin 1 (SIRT1) and BCL2, enhances susceptibility of HCC cells to IL-24-induced cell death has resulted in development of an oncolytic adenovirus vector that coexpresses miR-34a and IL-24 selectively in HCC cells. Remarkably, treatment of xenograft HCC tumors with this adenovirus resulted in complete tumor remission with no recurrence (46). Based on these encouraging results, miR-34a is the first miRNA mimic developed for human cancer therapy and is undergoing phase I clinical trial for primary and metastatic HCC (47,48).

miR-34a may potentially play a large role in cellular metabolism through the targeting of SIRT1, a key NAD-dependent deacetylase that regulates a wide range of metabolic processes including lipid metabolism, glucose metabolism, and expression of other metabolic regulators (49,50). Being negative regulators of each other, both miR-34a and SIRT1 positively control their own expression by attenuating the other (49). Interestingly, miR-34a is one of the most upregulated miRNAs expressed in human nonalcoholic steatohepatitis (NASH), a form of nonalcoholic fatty liver disease (NAFLD) (51), and its expression positively correlates with the severity of NAFLD (52). The attenuation of SIRT1 by miR-34a is thought to contribute to the NAFLD phenotype by regulating liver cell apoptosis and cholesterol metabolism (52). Additionally, SIRT1 deacetylates PGC-1α, which increases its ability to coactivate HNF4α to upregulate gluconeogenic enzymes. Interestingly, SIRT1-mediated deacetylation of PGC-1α had no significant effect on the upregulation of mitochondrial genes targeted by PGC-1α (53). By targeting SIRT1, miR-34a expression may lead to decreased cellular glucose production through the reduced activity of PGC-1α. The inhibition of gluconeogenesis can reduce glucose secretion of NAFLD cells and allow for increased fatty acid production. This is of significance because, although rare, NAFLD can lead to hepatocarcinogenesis (54). It is possible that upon development of HCC, miR-34a that was initially upregulated at the NAFLD stage will be significantly inhibited, while miR-23a expression will be upregulated to facilitate increased cellular proliferation while maintaining low levels of glucose secretion.

miRNA-155

We have shown that miR-155 is significantly upregulated in primary mouse and human HCC compared to its expression in pair-matched normal liver tissues (55). miR-155 is considered an oncomiR due to its ability to directly suppress expression of the tumor suppressor CCAAT/enhancer-binding protein β (C/EBPβ) (55) and promote tumorigenesis by activating Wnt signaling in the liver

(56). miR-155 is also significantly upregulated in other cancers including lymphoma and breast cancer (57).

Although miR-155 promotes tumorigenesis by a variety of mechanisms, a recent study in breast cancer cells has demonstrated that its overexpression increases the rate of glucose consumption and lactate production through regulation of numerous enzymes involved in glucose transport, glycolysis, and lactate production. The enzymes include hexokinase 2 (HK2), glucose transporter 1 (GLUT1), phosphofructokinase 2 (PFK2), pyruvate dehydrogenase isoform M2 (PKM2), and lactate dehydrogenase isoform A (LDHA). However, only the mechanism of miR-155-induced upregulation of HK2 has been elucidated. Here miR-155 upregulates HK2 through activation of STAT3 and suppression of miR-143 that can directly target HK2 (58). The activation of STAT3 by miR-155-mediated targeting of suppressor of cytokine signaling 1 (SOCS1), a negative feedback regulator of JAK/STAT signaling (59), has not been demonstrated in HCC. However, it may be a relevant mechanism of HK2 regulation, as miR-155 is upregulated (55) and STAT3 is activated (60) in HCC (60). Further, the miR-143 level is higher in human HCC tissues compared to its level in pair-matched normal liver tissues (61). Interestingly, knockdown of HK2 can also reduce cell proliferation and cell survival in breast cancer cells and suppress tumor growth in xenograft mice (58). Thus, miR-155 may be a key metabolic regulator that leads to increased rates of glucose consumption through aerobic glycolysis and lactate production. Further investigation is needed to elucidate the mechanisms underlying these metabolic regulations and their potential to modulate metabolism in the liver.

miRNA-199a

miR-199a-3p and miR-199a-5p are both downregulated in primary human HCC compared to the adjacent normal liver tissues (62,63) and exhibit tumor-suppressor functions in HCC (64). The ability of miR-199ab-5p to directly target hypoxia-inducible factor 1α (HIF-1α) (65), a subunit of the heterodimeric transcription factor HIF-1 that mediates the cellular response to hypoxia (66), in cardiac myocytes has important metabolic implications. HIF-1 activity is associated with alterations in cellular metabolism through transcriptional activation of numerous genes that result in increased cellular glucose uptake, glycolysis, lactate production, lactate secretion, and mitochondrial autophagy (66). Interestingly, HIF-1 inhibition has been linked to the therapeutic effects of antioxidants in preventing tumor growth and development (67). miR-199a suppression may play a vital role in HCC pathogenesis by augmenting HIF-1α expression and HIF-1 activity. This regulation of HIF-1 activity by miR-199a-5p in HCC, however, requires further investigation. Future therapeutic strategies should aim to deliver
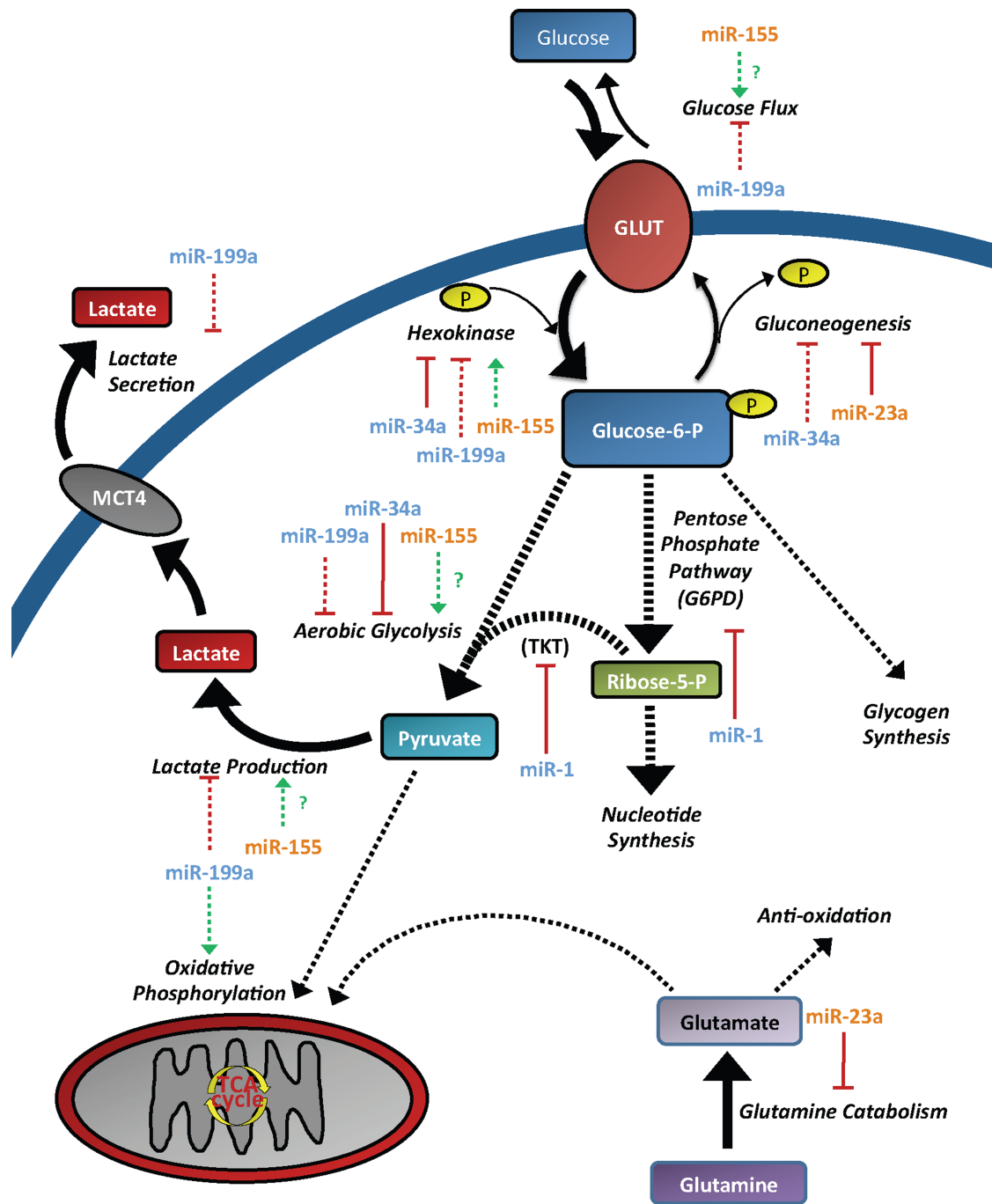
Figure 1. MicroRNA-mediated metabolism. The figure shows aspects of microRNA-mediated glucose and glutamine metabolism in cancer. Black arrows show the catabolic and anabolic processing of metabolites. The larger black arrows represent pathways that are upregulated in HCC, and the broken arrows signify that metabolic intermediates are not shown. MicroRNAs in blue are downregulated in HCC, and those in orange are upregulated. MicroRNA-mediated regulations are shown in red for inhibition and green for activation. The broken arrows represent mechanisms in which the microRNA does not directly target an enzyme within the specified metabolic pathway. The question marks next to some of the miR-155 regulations indicate mechanisms that are yet to be elucidated. GLUT, glucose transporter; MCT4, monocarboxylate transporter 4; G6PD, glucose-6-phosphate dehydrogenase; TKT, transketolase.

miR-199a directly to HCC tumor cells to block hypoxia-induced metabolism by inhibiting HIF-1α expression.

CONCLUDING REMARKS

Metabolic Shift in Cancer

From the discussion of the roles of different miRNAs in metabolic processes (see Fig. 1), it is evident that the metabolic adaptations seen in cancer are, at least in part, mediated by miRNAs. The expression of some miRNAs may mediate large shifts in glucose metabolism; however, other miRNAs may only act as a fine-tuning mechanism to make more minor changes. No single miRNA can be accountable for the full shift observed in cancer metabolism. Conversely, the synergy of multiple miRNAs may lead to a tumor-specific metabolic profile.

Benefits of a Metabolic Shift

As tumors grow, it becomes increasingly difficult for the innermost cells to obtain oxygen from the blood, creating a hypoxic environment. Hypoxia within tumors leads to a necessary shift away from oxidative phosphorylation toward glycolysis as the primary source of ATP generation (3). The high rate of glycolysis may help maintain a low concentration of intracellular glucose, creating a gradient that favors the flux of glucose into the cell. Similarly, it may be highly beneficial for HCC tumors to suppress gluconeogenesis to maintain this influx of glucose. Indeed, our studies demonstrating miR-23a-mediated suppression of G6PC suggest cellular accumulation of G6P (9). G6P is a major metabolic intermediate that can be funneled down a variety of pathways including glycolysis, oxidative phosphorylation, glycogen synthesis, and the PPP (see Fig. 1).

The PPP utilizes G6P to generate ribose-5-phosphate and in the process restores NADPH levels. NADPH can be used as a cellular reducing agent to regenerate glutathione (GSH) in order to reduce ROS levels. NADPH is also utilized in other anabolic pathways including lipid and cholesterol synthesis. Ribose-5-phosphate is utilized for the production of purine nucleotides that are necessary for tumors to achieve high rates of proliferation. Any excess ribose-5-phophate can also continue through the PPP to produce the glycolytic intermediates fructose-6-phosphate and glyceraldehydes-3-phosphate. In turn, these intermediates can be utilized in aerobic glycolysis to generate pyruvate and ATP. Thus, by facilitating high rates of proliferation and glycolysis while regenerating NADPH to combat harmful ROS, a shift in metabolism of glucose toward the PPP could be highly beneficial for continued proliferation and maintenance of tumor cells.

Application of Metabolomics

Although significant efforts have been made in determining the regulatory mechanisms of miRNAs in metabolism, a metabolomic approach would be ideal to obtain a global perspective on such regulations. Stable isotope-resolved metabolomics (SIRM) is emerging as a powerful technology that when combined with miRNA manipulation (overexpression or knockdown) will allow researchers to gain a more comprehensive understanding of how the expression of specific miRNAs can affect the metabolic profile of cancer cells. In SIRM, cells are allowed to grow with a heavy isotope-labeled metabolite that is structurally similar to an endogenous metabolite. The metabolic breakdown of the labeled metabolite is characterized via nuclear magnetic resonance (NMR) and gas mass spectroscopy (MS), wherein isotopically labeled atoms in metabolic intermediates can be detected independently of typical atoms within other metabolites. Importantly, this metabolic profiling can also be performed in an in vivo setting, enabling physiologic studies in whole animals/humans [for a detailed discussion of SIRM, see (68)]. This analysis will ultimately provide a detailed insight into the role of miRNAs in regulating the levels of specific intermediates in glucose metabolism and thus sustaining the growth of tumor cells. Further, utilization of this sensitive and highly reproducible technique is likely to identify potential alternate targets for cancer therapy.

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