Glucose Metabolism in Breast Cancer and Its Implication in Cancer Therapy

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ABSTRACT

It is well known that malignant cells have accelerated glucose uptake and metabolism in order to maintain their fast proliferation rates. With the increased influx of glucose into cancer cells, glycolysis is facilitated through a coordinated regulation of metabolic enzymes and pyruvate consumption. Shifting from mitochondrial oxidative phosphorylation to glycolysis and other pathways such as pentose phosphate pathway (PPP) and de novo fatty acid synthesis in the breast tumor provides not only energy but also the materials needed for cell proliferation. Glucose augmentation in tumor cells can be due to the elevated level of glucose transporter (GLUT) proteins, such as the over-expression of GLUT1 and expression of GLUT5 in breast cancers. Moreover, other factors such as hypoxia-inducible factor-1 (HIF-1), estrogen and growth factors are important modulators of glucose metabolism in the progression of breast carcinomas. Therapies targeting at the glycolytic pathway, fatty acid synthesis and GLUTs expression are currently being investigated. Restoring tumor cells to its normal glucose metabolic state would endow tumor specific and accessible treatment that targets glucose metabolism.

Keywords: Breast Cancer, Glucose Transport, Glycolysis, Fatty Acid Synthesis, Mitochondrial Metabolism

1. Introduction

Rapid growth and cellular proliferation are hallmarks of tumor. In order to survive, tumor cells have to adopt all possible mechanisms to meet their energy needs. The increased demand for energy also requires a higher metabolic rate of nutrients. In non-malignant cells, this is usually achieved through elevating mitochondrial oxidative phosphorylation (OXPHOS), which is the major source of cellular ATP. By contrast, in cancer cells, observations by Otto Warburg suggested that tumor cells might utilize the augmented glycolysis rather than mitochondrial phosphorylation of glucose as their main energy supply, and apply this less efficient pathway even under aerobic conditions [1]. This metabolic alternative is also closely associated with the aggressiveness of the tumor [2]. The universal ‘aerobic glycolysis’ of tumors suggests a ‘metabolic reprogramming’ of nutrients in those cells, which is characterized by facilitated glycolytic reactions, impaired OXPHOS and elevated de novo nucleotide and fatty acid biosynthesis. Since the capability of tumor cells to support their enhanced nutrient metabolism is obligatory for their growth and phenotype, mechanisms contributing to these changes may provide a biochemical basis for therapeutic strategies.

2. Glucose Metabolism in Cancer

Cells are highly organized and a constant supply of energy is required to create and maintain the biological orders that keep them alive. This energy is derived from the chemical bond stored in food molecules, which serves as fuel for cells. Glucose is a particularly important fuel molecule and dominates energy production in most animal cells. Generally, glucose metabolism provides chemical energy in the form of ATP and NADH through control of three-step continuous reactions, including glycolysis, the tricarboxylic acid (TCA) cycle and OXPHOS. The breakdown of each molecule of glucose into two smaller molecules of pyruvate is carried out through ‘glycolysis’, which involves a sequence of ten separate reactions, each of which produces different intermediates and is catalyzed by different enzymes. During glycolysis, energy is produced as activated carrier molecules (ATP and NADH) and at the end of glycolysis, there is a net
gain of two molecules of ATP for each glucose molecule that is metabolized. For many anaerobic organisms, glycolysis is the principal source of cellular ATP. By contrast, in most aerobic tissues, the pyruvate derived from glycolysis directly enters mitochondria, in which each molecule of pyruvate is converted to acetyl-CoA and CO₂. The complete oxidation of the acetyl groups in acetyl-CoA to CO₂ occurs in the TCA cycle, starting from citrate formation by the joining of acetyl-CoA to oxaloacetate (OAA). Moreover, this cycle accounts for two-thirds of total oxidation of carbon compounds in most cells and generates high-energy electrons carried by NADH and FADH₂. These high-energy electrons are then passed to an electron transport chain embedded in the inner mitochondrial membrane, which eventually combine with O₂ to form H₂O. With the movement of electrons along the respiratory chain, energy from NADH and FADH₂ is stored as an electrochemical proton gradient across the inner membrane of mitochondrial, which exerts a proton-motive force, driving the conversion of ADP and phosphate to ATP. Compared to glycolysis, mitochondrial OXPHOS produces 36 molecules of ATP per one molecule of glucose. Efficient OXPHOS relies on functional enzyme complexes of the mitochondrial respiratory chain, which are encoded by both nuclear and mitochondrial genes. Therefore, any deletions and mutations of mitochondrial DNA (mtDNA) will result in a defect of mtDNA-encoded polypeptides, which can cause a large spectrum of diseases featuring impaired mitochondrial metabolism such as cancer.

Cancer cells, characterized by uncontrolled proliferation in the hypoxic and poorly vascularized environment, require a significant amount of glucose and accelerated glucose metabolism to meet their need for energy and biosynthetic elements. However, a metabolic alteration observed by Otto Warburg (Warburg effect) in 1924, suggested that in contrast to non-malignant cells, which utilize mitochondrial OXPHOS to produce ATP in the presence of adequate oxygen and anaerobic glycolysis when adequate oxygen supply is not available, malignant cells preferentially depend on converting glucose into lactate rather than mitochondrial glucose metabolism even under high oxygen environments [1]. Though the exact molecular mechanisms underlying the ‘Warburg effect’ are unknown, the ‘aerobic glycolysis’, which presents elevated glucose transport, increased glycolytic activity and reduced mitochondrial glucose metabolism, has been one of the important tumor metabolic phenotypes. The advantage of this shifted energy conversion is likely to provide tumor cells adequate microenvironment for multiple bioenergetic and biosynthetic pathways [3].

2.1. Facilitated Glycolysis

Glycolytic enzymes in malignant cells were distinctive from their counterparts in normal cells [4-6]. For instance, phosphoglycerate mutase (PGM) and enolase, catalyzing glucose to pyruvate—the end-product of glycolysis, were phosphorylated at the tyrosine residues in the cells transformed with Rous sarcoma virus or stimulated by growth factors [7]. The same was observed with lactate dehydrogenase (LDH)-A, which is responsible for the conversion of pyruvate to lactate—a main product of anaerobic pathway. The phosphorylation level of these three enzymes correlated with the increased rate of glycolysis [7]. Typically in malignant human breast tissue, the overexpression of LDH-A was associated with tumor invasiveness. Accordingly, LDH-A has been proposed as a marker of tumor progression [8].

In addition to the phosphorylation of glycolytic enzymes, the enzyme activities were significantly increased in the malignant breast tissues compared to the normal and benign breast tissues, such as hexokinase (HK), phosphofructokinase (PFK), aldolase and pyruvate kinase (PK) (see Glycolysis in the Figure 1) [5,9]. For example, HK activation was related to the suppression of apoptosis since the promoter region of the HK gene contains the DNA responsible elements of both p53 and hypoxia-inducible factor (HIF)-1, and both mutated p53 and HIF-1 increased the HK expression [10]. HIF-1 is considered as an oxygen sensor and is stabilized under hypoxic stress. It transcriptionally modulates a number of genes as a metabolic adaptation in cancer cells [11,12]. HIF-1-induced activation of HK triggers the transloca-tion of HK from the cytoplasm to mitochondrial membranes with which it interacts and suppresses several key components of mitochondria-dependent apoptotic proteins [13]. Moreover, the activity of PK, which irreversibly catalyzes the last step of pyruvate formation, has been shown to have a strong connection with the malignancy of breast carcinomas [14].

The glycolytic end-product pyruvate is consumed differently in tumors. Pyruvate metabolism stands at the crossroad between oxidative and glycolytic pathways. Transportation of pyruvate into mitochondria results in its oxidation, whereas its maintenance in cytosol leads to the reduction of pyruvate. The inhibition of pyruvate oxidation in mitochondria is considered as a metabolic deviation that could explain Warburg effect of tumor cells [15]: down-regulation of pyruvate oxidation by phosphorylated pyruvate dehydrogenase (PDH) complex would redirect pyruvate into the cytoplasm, which will then be metabolized into lactate. Moreover, the mitochondrial metabolic flux of pyruvate through the left-hand side of the TCA cycle (from α-ketoglutarate to OAA) is much greater than that through the right-hand side (from acetyl-CoA to α-ketoglutarate), which may be due to the higher rate of glutaminolysis in tumor [16].
Glutaminolysis is the degradation of amino acid glutamine to glutamate, pyruvate and then lactate. Therefore, the consequence of accelerated glutaminolysis in tumor cells is an increased conversion of pyruvate into lactate. In supporting this information, several studies reported that there were higher utilization of both glucose and glutamine to generate lactate in tumor cells [17,18]. As glutaminolysis is an energy producing pathway, the application of glutaminolysis in tumor cells could be an adaptive metabolic switch in order to satisfy the ATP requirement [18]. Additionally, the reduced mitochondrial pyruvate oxidation was accompanied by an increased activity of cytosolic pyruvate enzymes in tumor cells, which would reduce the amount of this substrate transported to and metabolized in the mitochondria [19, 20].

The key molecule that drives glycolysis in cancer cells is HIF-1. HIF-1 is more stabilized in conditions such as nutrient depletion and reduced oxygen or hypoxic conditions. HIF-1 has been shown to induce the expression of several glucose transporters as well as most enzymes required for glycolysis [21]. Besides increasing the expression of HK in anaerobic metabolism, HIF-1 can also suppress PDH in mitochondria by inducing pyruvate dehydrogenase kinase (PDK)-1, which inhibits PDH activity by phosphorylation [22,23]. Since PDH catalyzes the mitochondrial oxidation of pyruvate to produce NADH and acetyl-CoA for ATP production, the inhibition of PDH by HIF-1 will result in a restricted entry of pyruvate into the mitochondria, a decrease of oxygen consumption, and consequently the transmission of metabolic flux to the lactate pathway [24]. Moreover, in malignant cells, there was a higher level of fructose-2, 6-bisphosphate

Figure 1. A brief outline from glucose uptake to mitochondrial metabolism followed by de novo nucleotide and fatty acid biosynthesis involved in this review.
(F-2, 6-BP), which is a powerful allosteric activator of glycolytic enzyme PFK-1. This increase could be due to a phosphorylative induction by the PFK-2 portion of the bifunctional enzyme PFK-2/fructose-2, 6-bisphosphatase in a HIF-1-dependent manner [25]. Furthermore, HIF-1 plays a transcriptional role in promoting LDH-A expression [26], which would attenuate tumor cell respiration and viability [27].

Diverting metabolism away from mitochondria towards cytoplasm provides malignant cells resistant to apoptosis. When cells switch from OXPHOS towards higher glycolysis, the consequent formation and accumulation of lactate can lead to cellular acidification, which would eventually facilitate tumor growth through acid-mediated extracellular matrix degradation and favour tumor invasion and metastasis (see Acidification in the Figure 1) [28]. Consequently, the persistence of this adaptive metabolic mechanism grants tumor cells a request of growth advantages. In this context, glycolytic enzymes serve as anti-apoptotic factors through inhibiting several components of mitochondria-dependent apoptosis [9, 29]. Additionally, the genetic theory of carcinogenesis regards glycolytic transformation as a consequence of activation of various oncogenes or tumor suppressor genes, including Akt/PTEN, p53 and c-myc [30]. Up-regulated Akt can be found in many cancers and could induce a glycolytic metabolic profile through augmenting HK expression and activity [31,32]. The PTEN (the antagonist of Akt) appears to be mutated in several cancers, which fails to control the activity of Akt. Gene p53 can also influence the metabolic balance in cells between glycolysis and OXPHOS. The p53 activity can favour OXPHOS through a transcriptional regulation of F-2, 6-BP by TIGAR (a p53-induced glycolysis and apoptosis regulator). TIGAR negatively regulates glycolysis by degrading F-2, 6-BP, which activates glycolytic enzyme PFK1 [33]. Moreover, p53 contributes to the synthesis of cytochrome c oxidase (COX)—a subunit of mitochondrial electron transport chain complex IV [30,33,34]. Restoring impaired COX is able to promote the conversion of ATP production from glycolysis to OXPHOS [34]. Accordingly, p53 mutation would result in the switch of ATP production from OXPHOS to glycolysis. Therefore, the metabolic phenotype is essential for the pathogenesis of cancer but not a ‘by-product’ of carcinogenesis.

2.2. Altered Mitochondrial Machinery

The original Warburg hypothesis was that it could be the damage of mitochondrial respiration that contributes to the enhanced glycolysis in tumor cells [1]. Mitochondria have been suggested to play an important role as an oxygen sensor to initiate the HIF-1 pathway under hypoxia [35]. Once hypoxia is initiated, reactive oxygen species (ROS) will accumulate on the site of complex III Q0 of mitochondrial respiratory chain and will stabilize rather than degrade the regulatory subunit of HIF-1 [35,36]. This results in the augmentation of HIF-1-induced gene expression and suppression of mitochondrial metabolism [22,23]. The role of mitochondria-derived ROS has also been implicated in a proposed vicious cycle linking mtDNA mutation in tumors. On the one hand, a burst of ROS production from mitochondria would favour oxidative dependent mitochondrial mutagenesis and hence promote tumorigenesis [37]; While on the other hand, mutation of mtDNA in the respiratory chain components could commence a cascade of events leading to an increase in electron leakage and ROS over-generation in the process of malignant transformation and tumor progression [38,39]. Several mtDNA mutations have been found in breast tumors [40], and the presence of mtDNA mutations in breast cancer cells is consistent with the intrinsic susceptibility of mtDNA to damage and persistent oxidative stress [41]. In terms of the oncogenesis of mtDNA mutation, critical evaluation and interpretation of the literature revealed that mtDNA mutation could enhance the specificity of diagnosis, detection and prediction of breast cancer growth and/or patients’ outcomes. Therefore, the mtDNA mutation may be used as a new molecular bio-marker for breast cancer [42].

The suppressed OXPHOS may also be interpreted as a transient adaptation in cancer cells, which disconnects glycolysis from dysfunctional OXPHOS, leaving glycolysis as the predominant ATP source, and then followed by an OXPHOS remodeling according to the growth demand of the cancer. The preference for glycolytic production of ATP in tumor cells could be due to 1) glycolysis generates ATP more rapidly although it is less efficient in long term than OXPHOS [43]; and 2) part of the carbon intermediates from glycolysis could be used for various biosynthetic pathways to keep a constant tumor growth [44]. However, deficiencies in mitochondrial respiratory chain components such as mtDNA impairment cannot explain tumorgenesis for different kinds of tumors because in some cases the over-expression of respiratory complexes was observed [45].

2.3. Glycolysis and Mitochondrial TCA Cycle

Provide Precursors for Tumor Biosynthesis

The persistent shift of malignant cells from mitochondrial OXPHOS to glycolysis for ATP production implicates that alternative pathways may be involved in maintaining cell growth. Despite of the energy production of glucose, catabolism also produces building blocks for tumor cells to divide and proliferate. Glycolytic intermediates formed in central catabolic reaction pathways can
be siphoned off by other enzymes that use them as biosynthetic precursors to produce amino acids, nucleotides and lipids for cell needs. Thus, the supply of materials for biosynthesis is another primary metabolic alteration associated with proliferating tumor cells from the view of glycolysis and TCA cycle [46]. The majority of macromolecules required for proliferation are generated de novo from glucose, which is controlled through chemical modification of these large molecules in response to changes in the cellular immediate environment [47]. The presence of different glucose metabolic pathways in the cell requires a precise regulation. For long-term adaptation, glucose metabolism in tumor cells requires a balance between elimination of some particular reaction pathways and elevation of the others to meet the minimum requirements. Hence, branching pathways of glycolysis and mitochondrial TCA cycle in malignant cells are likely to function as a distinctive way for their uncontrolled growth. These are of particular interest in understanding of the pentose phosphate pathway (PPP, also known as hexose monophosphate shunt) for de novo nucleotide biosynthesis and the de novo fatty acid synthesis in cancer cells.

2.3.1. Pentose Phosphate Pathway
Carbon derived from glycolytic flux to the PPP can be utilized to generate ribose-5-phosphate for de novo nucleotide biosynthesis. The large cellular nucleotide pool as well as the level and activity of different rate-limiting enzymes involved nucleotide synthetic pathways are the critical elements of maximal cell proliferative capacity. Compared with normal cells, tumor cells display an altered nucleotide metabolism, such as the larger nucleotide pool, higher enzymatic activity in nucleotide anabolic pathway as well as lower enzymatic activity in nucleotide catabolic pathway [48]. Understanding of the nucleotide biosynthesis in malignant cells offers possibilities to design selective therapies for human cancer. Besides the generation of ribose-5-phosphate from PPP to support nucleic acid synthesis, the reduced nicotinamide adenine dinucleotide phosphate (NADPH) was formed during PPP to provide reducing equivalents for not only lipid synthesis but also detoxification of reactive oxygen species [49]. Activities of enzymes involved in this pathway such as glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase have been reported to be increased in malignant breast tissues [50] as well as carcinomas and cancer cell lines from other origin [4,51,52].

The remaining pyruvate from aerobic glycolysis that is not converted to lactate enters the mitochondria and is then extruded from the TCA cycle at various steps for biosynthetic pathways to satisfy the increased demand of cell proliferation. Pyruvate could be converted to acetyl-CoA, which will join with OAA to form citrate in the presence of citrate synthase. After translocation of citrate from mitochondria to cytosol, cleavage of citrate back to acetyl-CoA provides the precursor for the synthesis of fatty acid, which may function as essential building materials of cell plasma membrane for rapid cancer growth (see PPP and De novo FA Synthesis in the Figure 1).

2.3.2. Fatty Acid Synthesis
De novo fatty acid synthesis involves the conversion of glucose to pyruvate and then to acetyl-CoA, which will work together with OAA to generate citrate. Besides glucose metabolism, metabolites from glutaminolysis provide another carbon source to facilitate de novo fatty acid synthesis. During glutaminolysis, glutamate is produced through deamination of glutamine and can be converted to α-ketoglutarate, which is then metabolized through TCA cycle to generate citrate [53]. The citrate formed from both glucose and glutamine metabolism in mitochondria will be transported into the cytosol and broken down to acetyl-CoA and OAA by ATP citrate lyase (ACL). The acetyl-CoA generated by ACL was then carboxylated to malonyl-CoA by a rate-limiting enzyme acetyl-CoA carboxylase (ACC). In addition, malic enzyme and the PPP produce the reducing equivalence NADPH for a preparation of fatty acid synthesis. Fatty acid synthase (FAS) is the major enzyme of lipogenesis. With the provision of NADPH, FAS catalyzes the condensation of acetyl-CoA and malonyl-CoA for the de novo synthesis of 16-carbon saturated free fatty acid palmitate (see De novo FA Synthesis in Figure 1) [54].

Fatty acids are the source of membrane components such as phospholipids and glycolipids. It also provides precursors of critical signaling molecules for proliferation and differentiation [55]. Fatty acids are generally acquired from diet and high level of fat in the Western food has been implicated in the development of human malignancies including carcinomas of colon, breast and ovarian [56-60]. In fact, except for some organs such as the liver, adipose tissue and lactating mammary gland, other normal cells rarely utilize de novo lipid synthesis pathway [61,62]; in contrast, the de novo fatty acid synthesis was significantly elevated in malignant tumors as reported by Medes et al. [63]. Consistently, the expression of enzyme FAS is highly up-regulated in a variety of human malignancies, but FAS is hardly detected in normal tissues [54]. Through immunohistochemical and metabolic studies, FAS over-expression has been detected in cancer tissues, its surrounding non-cancer tissues and in the blood of patients with cancer [64-71]. Evidence from breast cancer, prostate cancer, ovarian cancer and thyroid cancer studies suggested an association of FAS expres-
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2.4. Augmented Glucose Uptake

Although glucose is the primary energy source for most cells, it is hydrophilic and does not pass through plasma membranes by diffusion; therefore, cellular utilization of glucose needs specific carrier proteins. In mammalian cells, there are two pathways for glucose uptake, namely the Na⁺/glucose co-transporters [84] found primarily in small intestine and kidney, and GLUTs or solute carrier family 2A (SLC2A) expressed in all cells [85]. Thirteen members of GLUTs have been identified and classified into three groups; with well-characterized GLUT1-GLUT4 belonging to Class I, fructose carrier protein GLUT5 and newly identified GLUT7, GLUT9 and GLUT11 falling into Class II, and GLUT6, GLUT8, GLUT10, GLUT12 and the H⁺/myo-inositol transporter HMIT grouped into Class III, respectively [86]. Each of GLUTs presents specific tissue distribution, function and developmental regulation [87,88]. For example, GLUT1 expresses at variable levels in the early development of many tissues [89], and is believed to be responsible for basal glucose uptake [90,91]; GLUT2, regarded as glucose sensor, expresses in the liver, pancreatic islets, kidney and small intestine [92]; GLUT3 is abundant in the brain [93]; GLUT4 is restricted to adipose and skeletal tissues [94]; and GLUT5, having a high affinity to fructose instead of glucose, usually presents in small intestine and sperm cells [95,96].

The higher rate of glycolysis in tumor cells makes tumor cells access to glucose easier if necessary. To compete with normal cells for substrate supply, tumor cells employ this high glucose transport strategy to make them adapted in relatively poor vascularized zones. In fact, it has been shown that the efficiency of glucose entry into malignant cells was at least 20- to 30-fold higher than that in non-malignant cells [97]. The increase of glucose entry to tumor cells is initially achieved through up-regulation of specific glucose transporters in the plasma membrane [98,99].

Association between the malignancy of cells and increased glucose transportation and/or augmented GLUTs expression has been extensively investigated. Rat fibroblasts transformed by ras and src oncogenes have an increased rate of glucose uptake, which is due to activated GLUT1 expression through enhancer elements within GLUT1 promoter [100-102]. With positron emission tomography (PET) scan, clinical observations of tumor through labeled glucose analog, 18F-labeled 2-fluoro-2-deoxy-D-glucose (FDG) indentified an increase in GLUT1-mediated glucose uptake in 57 cervical carcinomas [103], 55 breast carcinomas [104], 34 non-small cell lung carcinomas [105] and 17 ovarian carcinomas [106]. Increased glucose uptakes as well as the over-expression of GLUT family members have been found involved in tumor aggressiveness [107,108], therefore, GLUT family members are considered as a potential therapeutic target [86,109]. In addition, oncogenic transformation itself may be an inducer of GLUT1 [100,101,110]. In lung and ovarian tumor, comparative increase in GLUT1 expression was observed in cancer patients with higher grade of malignancy, higher proliferative index and lower degree of differentiation [111,112]. Consistently, GLUT1 expression level reciprocally correlates with survival rate of cancer patients [112-114]. Since the expression and regulation of GLUTs are tissue and often cell-specific, expression of some isoforms may only be observed in tumor cells but absent in normal cells. For example, GLUT3, which is detected in lung, ovarian and gastric cancer tissues, was not found in adjacent non-tumor tissues [115]. Fructose transporter GLUT5 could be only found in the malignant breast tissue, but not in its...
counterpart normal tissues [116]. Identification in the breast tumor by reverse transcriptase-polymerase chain reaction (RT-PCR), immunohistochemistry and immunocytochemistry, variable expression patterns of GLUTs were observed in both malignant cell lines and tumor tissues. GLUT1 is widely considered responsible for glucose transport in breast tissue [117-120]. Existence of GLUT2 in breast was controversial and no apparent difference was observed between normal and malignant tissues [117,118,121]. GLUT3, which is more likely expressed in the brain, was not detected in either normal or tumor breast tissues [118], except of breast cancer cell line ZR-75 [122]. The expression of GLUT4 was found in various human malignant breast tissues, but not in human breast cancer cell lines - MCF-7, MDA-MB-435 and MDA-MB-23 [117,118,121]. The high-affinity fructose transporter GLUT5 was selectively expressed in human breast cancer tissues, indicating that fructose may be a good energy substrate in tumor cells [116,119]. A novel glucose transporter protein GLUT12 has been identified in breast cancer cell line MCF-7 [123, 124]. The perinuclear sub-cellular localization of GLUT 12 suggests that GLUT12 may traffic to plasma membrane as GLUT4 does and translocation of GLUT12 may contribute to hexose supply to breast cancer cells [86,123, 124].

HIF-1 has been suggested to be one of the factors responsible for GLUT1 up-regulation in tumor cells, due to either the prevention of proteosomal degradation or the enhanced synthesis of GLUT1. HIF-1 is a transcriptional factor that can bind to the GLUT1 promoter to facilitate GLUT1 transcription [125]. Gatenby et al. demonstrated that up-regulation of GLUT1 is an adaptive process to hypoxia in breast cancer [126]. Specific disruption of the HIF-1 pathway in human breast tumor MCF-7 cells with short hairpin RNA significantly attenuated GLUT1 expression [127]. In contrast, Burgman et al. reported a divergence between increased hypoxia-induced FDG uptake and the induction of GLUT1/ GLUT3 protein levels. This observation suggested that hypoxia-induced reduction of cysteine residues in the GLUT may be the mechanism underlying the hypoxia-induced increase in FDG uptake [128]. Moreover, in mouse mammary tumor cells, the insulin receptor substrate (IRS), which was shown to be involved in breast cancer invasion, survival and metastasis, could promote tumor invasion through GLUT1-mediated sustainment of aerobic glycolysis [129]. Furthermore, cellular oncogene c-myc, protein kinase Akt, and ovarian hormone estrogen are also known to induce GLUT1 expression [130-133]. In addition, it has been demonstrated that GLUT3 expression and glucose uptake can be up-regulated through secondary messenger cAMP pathway in breast cancer cell line ZR-75 [122].

Estrogen is a key element in the development and progression of breast cancer; therefore, anti-estrogens, such as tamoxifen, significantly improved the outcome of breast cancer patients. In human breast cancer cells, 17β-estradiol (E2) was able to induce higher rate of glucose consumption through glycolysis and TCA cycle, which lead to an increased glucose metabolism [134]. Rivenzon-Segal et al. observed that tamoxifen could suppress both estrogen-stimulated tumor growth and glycolysis. These changes were consistent with the observed down-regulation of GLUT1 expression. Thus, it is proposed that in vivo monitoring of glycolysis may function as a tool to reveal hormonal regulation of GLUT1 expression in breast cancer tumors, as well as to assess tumor response to hormonal therapy [133]. In addition to estrogen, GLUT1s expression correlates well with estrogen receptors (ER) expression level, especially ER-alpha [135]. E2 could elevate the protein levels of GLUT12 after 24 h treatment in ER positive, but not ER negative breast cancer cells, suggesting an ER-dependent hormonal regulation of these GLUTs [86,135]. Epithelial growth factor (EGF) is another important factor that regulates the expression of GLUTs. It has been demonstrated that EGF could enhance not only cell proliferation but also GLUT12 protein in MCF-7 human breast cells. This observation indicates that GLUT12 may contribute to the increased glucose uptake in breast cancer tissues [86,136].

3. Glucose Metabolism and Its Implication in Cancer Treatment

3.1. Blockage of Glycolysis

Due to the remarkable heterogeneity and adaptability of cancer cells, an effective pharmacological treatment plan is challenging. The molecular characteristics of cancers with identical histology are often dissimilar and molecular heterogeneity can even be observed within a single tumor [24]. Therefore, it is of critical importance to develop an anti-cancer drugs with a defined target and integrated molecular mechanisms participated by distinct cancers [24]. Though not all tumor cells use glycolysis as their main energy source and mitochondrial metabolism is completely functional in these cells [137,138], they do exhibit a higher sensitivity to OXPHOS-inhibiting drugs than their normal counterparts [139]. Therefore, metabolic alterations appear to be a unique signature of diverse molecular abnormalities in tumors especially those with suppressed mitochondrial function [24]. This observation makes ‘metabolic therapy’ a promising anti-cancer approach [138]. Metabolic therapy may have two advantages, namely ‘specificity’ and ‘generality’. Specificity is referred to the reason why tumor cells are more sensitive to metabolic inhibitors than their normal coun-
terparts [138,139]. Generality stands for its complete independence of specific signaling and/or epigenetic dysfunction [140]. Glycolytic suppressors have been demonstrated to be successful in experimental tumor models exhibiting that the inhibition of glycolysis and/or OXPHOS results in the delayed tumor growth and/or the impaired tumor cell viability in xenograft in nude mice and cell cultures in vitro [139,141]. As shown in the glycolysis pathway of the Figure 1, HK, PFK and PK are rate-limiting enzymes that catalyze three irreversible reactions in glycolytic pathway. Thus, identifying glycolytic inhibitors should first focus on these steps. In fact, some chemical drugs that target these reactions have been in clinical trials.

2-deoxyglucose (2-dg) is one of these drugs that is a glucose derivative where 2-hydroxyl group is replaced by hydrogen [142]. 2-deoxyglucose enters cells through the glucose transporter, therefore higher expressions of glucose transporter in tumor cells, the higher uptake of 2-deoxyglucose in those cells. Once 2-deoxyglucose enters the cells, it can be used as substrate of HK but will not go further into glycolysis like glucose [141]. Therefore, an augmented uptake of 2-deoxyglucose is likely to be used as a strategy for metabolic therapy of cancer. It has been documented that modification of 2-deoxyglucose improved its uptake in cells such as addition of hydroxyl group - esters, ethers and phosphoesters etc [143, 144], and/or replacement of hydroxyl group with fluorine, iodine, thiol or thioalkyl [145-151]. Moreover, any modification of glucose by either removal of the hydroxyl group or replacement of fluorine and/or methyl group at C-1 or C-6 positions would escape the control of HK and PFK. Furthermore, because of complexity of metabolic interactions, it will be better for a drug that can target one or more glycolytic steps and/or the potential paralleled pathways than just disrupt the rate-limiting step such as HK, PFK and PK. For example, modifications of glucose at C-3 and C-4 could block the cleavage of F-1,6-BP by adolase and lead to collapsed glycolysis.

Oxamate is another type of glycolytic blocker that prevents conversion of pyruvate to lactate through its inhibition of LDH [152]. LDH functions at the downstream of HK, PFK and PK and only becomes very active in anaerobic environment (see Acidification of the Figure 1). Thus, oxamate blocks glycolysis at a different level compared to 2-deoxyglucose, and is selective to kill anaerobic cells in in vitro cell cultures [150,152]. Because the conversion of pyruvate to lactate only occurs in reduced oxygen conditions, tumor cells with aerobic metabolism are not affected by this drug. From this point of view, oxamate can be considered as a selective inhibitor of glycolysis compared to 2-deoxyglucose, which inhibits both aerobic and anaerobic cells.

Dichloroacetate (DCA) is another anti-cancer agent, which targets glycolysis by inhibiting activity of PDK [24], which can negatively regulate PDH activity in the mitochondrion. As shown in Figure 1 (TCA cycle), PDH converts pyruvate to acetyl Co-A and, therefore, controls the flow of pyruvate to the citric acid cycle to generate ATP in the mitochondria [153]. It has been shown that DCA could reverse the glycolytic phenotype in a number of tumor cells through increasing the flux of pyruvate into the mitochondria and promoting glucose oxidation over glycolysis. This reverses the suppressed mitochondrial apoptosis in cancer and results in suppression of tumour growth in vitro and in vivo [24,30,154]. Therefore, it is a promising anti-breast cancer strategy to reverse glycolytic phenotype via inhibition of PDH with DCA.

For those cancers that have normal mitochondria glucose metabolism [137], glycolysis inhibition may not be efficient enough to arrest tumor cell growth while it may be more appropriate in this case to employ OXPHOS–inhibiting drugs such as rotenone, rhodamine (ro) and oligomycin [138]. A novel strategy for the treatment of tumor cells with aerobic metabolism is to use both OXPHOS inhibitors and glycolytic inhibitors. It has been demonstrated that when human breast carcinoma cells (MCF-7) were treated with rho 123 and 2-dg, hundred percent inhibition was observed as colony formation whereas rho 123 alone only achieved 50% inhibition and 2-dg alone did not inhibit colony formation, respectively [155]. Similar results were observed in xenografts of human breast cancer cells in nude mice. Combination of 2-dg and rho 123 abolished the tumor development while only partial or no inhibition was obtained with either drug alone respectively [156]. This result provides evidence that inhibition of both OXPHOS and glycolysis would significantly enhance therapeutic efficacy compared to each alone. In addition, inhibition of OXPHOS would convert tumor cells from aerobic to anaerobic glycolysis and hypersensitize these cells to glycolytic inhibitors [157,158]. Therefore, therapeutic approaches designed to inhibit both glycolysis and OXPHOS are considered a novel therapeutic strategy to selectively kill cancer cells [159,160].

Cells within the inner core and periphery of a tumor have different accessibilities to oxygen and nutrients. At the inner core, cancer cells are in a poorly oxygenated environment and rely heavily on anaerobic metabolism. The glycolytic inhibitors mentioned above are particularly effective against cancer cells with mitochondrial defects or under hypoxia condition, which are frequently associated with cellular resistance to conventional chemotherapy and radiation therapy [141,161]. Thus, it will be innovative to develop an anti-cancer protocol that
employs standard chemotherapy, radiation therapy and inhibition of glucose metabolism to increase the efficacy of cancer treatment.

3.2. Targeting de novo FA Synthesis

Because cancer cells rely greatly on fatty acid for their survival (de novo FA synthesis in the Figure 1), it is plausible that cancer treatment can utilize the suppression of FAS activity. Chemical blockage of fatty acid generation with a FAS inhibitor (cerulenin) exhibited cytotoxicity to a large range of human cancer cell lines [54]. Moreover, inhibition of cancer cell proliferation by tea polyphenol(-)-epigallocatechin 3 gallate (EGCG) and theaflavin (TF) was also associated with suppression of FAS expression through inhibiting PI3K-Akt-Sp1 signal pathway [162]. Diosgenin is a steroid sapogenin from plant and was shown to suppress FAS in HER-2-over-expressing breast cancer cells through modulating Akt, mTOR and JNK phosphorylation [163]. Furthermore, it was documented that amentoflavone isolated from S. tamariscina induced breast cancer cell apoptosis through suppressing the biosynthesis of FA [164].

In human breast cancers, increased expression of FAS is observed in infiltrating breast cancer tissue, and was always associated with malignant phenotypes and poor prognosis [165]. Ample evidence is available that supports FAS as an ideal target of anti-breast cancer therapy. For example, in estrogen dependent human breast cancer cells, inhibition of cancer lipogenesis by a FAS inhibitor significantly reduced ER protein and mRNA expression, leading to increased apoptosis [166]. Moreover, pharmacological inhibition of FAS activity in breast cancer cell lines SK-Br3, MCF-7 and MDA-MB-231 using cerulenin enhanced anti-cancer efficacy of Fluorouracil (5-FU) no matter of whether cerulenin and 5-FU were applied at the same time or one prior to the other. This finding indicates that FAS-catalyzed de novo fatty acid biogenesis plays a key role in regulation of breast cancer cells in response to other anti-cancer drugs [167]. Furthermore, up-regulation of FAS rendered human breast cancer cells (MCF-7 and MDA-MB-468 cells) to resist drug-induced apoptosis while reduction of FAS expression was able to efficiently reverse resistance to drug-induced apoptosis [168]. Therefore, FAS over-expression in cancer cells is a new mechanism of drug resistance and may be served as an ideal target for chemo-sensitization in breast cancer chemotheray [168].

There are two mechanisms that may explain how FA synthesis regulates malignant breast phenotype: one is through direct activation of pro-apoptotic factors and/or inactivation of anti-apoptotic proteins and the other is through alterations of cellular lipid metabolism. For the induction of apoptosis, it has been documented that inhibition of FAS with small interfering RNA (siRNA) significantly up-regulated pro-apoptotic factors such as Bcl-2/adenovirus E1B 19 kDa interacting protein-3 (BNIP-3), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), and death-associated protein kinase-2 (DAPK-2) [169]. Moreover, application of BNIP-3 siRNA in this setting significantly counteracted the effect of FAS siRNA induced apoptosis [170]. Furthermore, there was a significant inverse correlation between abundances of FAS and BNIP-3 in clinical samples of human breast cancer [169]. In addition, cerulenin-induced apoptosis in MDA-MB-468 breast cancer cells could be enhanced by down-regulation of anti-apoptotic factors such as XIAP, cIAP-1 and Akt, and up-regulation of apoptotic factor such as Bak in mitochondria [171]. For the alternation of cellular lipid metabolism, silencing ACC alpha (a rate limiting enzyme in FA biosynthesis) or FAS by siRNA technique revealed a significant decrease in palmitic acid synthesis, which led to induction of apoptosis concomitantly with reactive oxygen species (ROS) formation and mitochondrial impairment. Further study indicated that ACC was critical to breast tumor survival because over-expression of ACC alpha mutant that was resistant to ACC siRNA rescued the siRNA induced apoptosis. However, the same strategy did not work in the case of FAS mutant. Interestingly, the addition of palmitate or Vitamin E completely prevented the apoptosis induced by both ACC and FAS siRNA, suggesting an interaction among lipid metabolism, ROS formation and mitochondrial function [172]. Furthermore, besides lipogenesis, breakdown of fatty acids can also regulate fatty acid metabolism through carnitine palmitoyltransferase-1 (CPT-1). It has been observed that inhibition of CPT-1 with etomoxir resulted in cerulenine-like effects with down-regulation of FA synthesis [173]. Therefore, there is an increasing interest in identifying and developing new anti-tumor compounds that modulate FA metabolism.

3.3. GLUTs as a Target of Metabolic Therapy

It has been suggested that malignant transformation involves stimulation of glucose transporters and consequently elevation of glucose metabolism and glycolysis [174], therefore, understanding GLUTs participation in cancer could be helpful for the diagnosis and treatment of tumor tissue. Because GLUT1 was exclusively found in human breast cancer biopsies and its expression was strongly associated with tumor grade, proliferation as well as invasiveness [111,120,121], GLUT1 has been proposed to be a new marker for diagnosis and prognosis of human breast cancers, an indication of tumor aggressiveness, and a possible therapeutic target for patients with breast cancer [114,175]. It has been demonstrated that drug mediated inhibition of glucose transport in-
creased the effectiveness of several anti-tumor drugs and effectively induced growth arrest and apoptosis in lung, breast cancer and leukemia cell lines [175,176]. For example, combination of GLUT1 inhibitor with chemotherapeutic drug daunorubicin preferentially sensitized anti-cancer activity of daunorubicin and overcame drug resistance of cancer cells in hypoxia environment [176]. Since GLUT5 is specifically expressed in human breast cancer tissue rather than its normal counterpart, GLUT5 may also service as a novel diagnostic marker and therapeutic target for human breast cancer [177].

3.4. Summary and Potential in the Application of Herbal Research

The proposal of the “Warburg effect” and recent observations of 18F-labeled FDG uptake in tumor through PET opened a new era for cancer treatment by modulating glucose metabolism in tumor cells. Since glucose metabolism appears differently in tumor and normal cells, such as aerobic glycolysis in normal cells and anaerobic glycolysis in tumor cells, inhibition of anaerobic glycolysis in cancer cells provides a promising therapeutic approach to arrest cancer proliferation. The therapeutic approach can be achieved by the following strategies: the first is to inhibit glucose transport into the cells by targeting GLUT1 or GLUT5 gene expression. This can be fulfilled by employing short interfering RNA technology or drugs that inhibit GLUT function. The second is to inhibit glucose metabolism in cancer cells by employing drugs that target anaerobic glycolysis directly. The third is to inhibit both aerobic and anaerobic glycolysis in cancer cells. In this case, cancer cells that still utilize aerobic glycolysis will turn to utilize anaerobic glycolysis, and anaerobic glycolysis inhibitors will then significantly inhibit cancer cell proliferation. The fourth is to inhibit de novo fatty acid synthesis by inhibition of FAS activity. This can be achieved by direct inhibition of either enzymes involved in FA synthesis such as FAS and ACC or in combination of those with anaerobic glycolysis.

One of the strategies has been in the phase I and phase II clinical trials at present time [178], which employs the anti-diabetic drug metformin for the treatment of human breast carcinoma [179]. In addition, this strategy of inhibiting cancer through nutrient metabolism may provide opportunities to further understand anti-cancer mechanism of natural herbs. Accordingly, herbs that have hypoglycemic and/or hypolipidemic properties may be of use in cancer treatment. For example, berberine (BBR) is an isoquinoline alkaloid isolated from many botanical materials such as Cortex Phellodendri and Rhizoma Curcuma. Though the anti-tumor mechanism of BBR is not fully revealed, it has been demonstrated that BBR could induce cell cycle arrest in human breast cancer cells [180]. Moreover, it has been reported that BBR has comparable hypoglycemic and potent hypolipidemic activities in patients with type 2 diabetes [181]. Therefore, it is plausible that BBR regulation of glucose-lipid metabolism may contribute to its tumor suppression. Another example is that several herbs such as Panax Ginseng, Ganoderma Lucidum, Astragalus and Lycium Barbarum have been used in the most populated fighting cancer formulation [182-185], and their polysaccharides extract did have the strong capability in improving metabolic abnormalities in in vivo studies [186-189]. Furthermore, a saponin extract from Gynostemma Pentaphyllum (GP) - gypenoside has been demonstrated to inhibit tumors through inducing mitochondria-dependent apoptosis and cell cycle arrest at G0/G1 [190]. It has been demonstrated that GP is able to modulate blood glucose levels and reduce hyperlipidemia in the Zucker fatty rat [191]. In addition, the polyphenolic compound such as curcumin from Curcuma Longa, has been utilized for cancer therapy through different mechanisms such as cell cycle arrest, apoptosis, mitochondrial dysfunction and protein kinase dysfunctions [192,193]. Curcumin is also good anti-oxidants, which ameliorate high-glucose induced mitochondrial oxidative stress [194]. Therefore, understanding of herbal regulation of glucose metabolism in cancer cells will significantly contribute our knowledge how herbs can be effective in the treatment of cancer, and which will also provide fundamental evidence for clinical application of traditional herbs in the field of cancer.

Transportation of glucose/fructose into cells is mediated through glucose transporters - GLUTs. Once glucose enters the cell, it is phosphorylated by HK to produce G-6-P, which is further metabolized to pyruvate by glycolytic enzymes such as PFK, aldolase, PGM, enolase and PK. A part of carbon derived from glycolysis may flux into PPP, participating in the generation of ribose-5-phosphate to support nucleic acid biosynthesis. Standing at the crossroad between oxidative and glycolytic pathways, pyruvate can be converted to either lactate by LDH-A in cytosol or to acetyl-CoA by PDH in mitochondria. Accumulation of lactate would lead to cellular acidification. In the presence of citrate synthase in mitochondrial TCA cycle, the formed acetyl-CoA would be condensed with OAA to generate citrate, which is then transported to cytoplasm and broken down back to acetyl-CoA and OAA by ACL. Meanwhile, acetyl-CoA is carboxylated to malonyl-CoA by ACC. FAS functions to condense acetyl-CoA with malonyl-CoA to complete de novo FA synthesis, which relies on the provision of NADPH by PPP. The increased presence of malonyl-CoA could inhibit the oxidation of FA through suppressing CPT-1, which is a suppressor of FA biosynthesis.
Warburg phenomenon originally hypothesized an impairment of mitochondrial OXPHOS in tumor cells. In order to transiently adapt to increased demands of nutrients and oxygen, tumor cells boost glycolysis rather than OXPHOS as their main cellular source of ATP through increasing activities of glucose transporters and glycolytic enzymes. Augmented cellular acidification by lactate formation would facilitate tumor growth through acid-mediated extracellular matrix degradation, which then favors tumor invasion and metastasis. The persistency of this adaptive mechanism suggests that alternative pathways are necessary to support the constant supply of building-materials for cell growth. Glycolytic and TCA cycle intermediates can be siphoned off by enzymes that use them as biosynthetic precursors. Promoted PPP and FA synthesis pathways are two strategies that tumor cells use for de novo biosynthesis.

Bold arrows marked in this figure indicate preferential pathways in tumor cells; slashed arrow stands for metabolic pathway in non-malignant cells.

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REFERENCES


Glucose Metabolism in Breast Cancer and Its Implication in Cancer Therapy

doi:10.1016/S0968-0004(08)01344-9


Glucose Metabolism in Breast Cancer and Its Implication in Cancer Therapy

do:10.1101/gad.1756509


[71] M. S. Shurbaji, J. H. Kalbfleisch and T. S. Thurmond, “Immunohistochemical Detection of a Fatty Acid Syn-
Glucose Metabolism in Breast Cancer and Its Implication in Cancer Therapy

thase (Oa-519) as a Predictor of Progression of Prostate Cancer,” Human Pathology, Vol. 27, No. 9, 1996, pp. 917-921. doi:10.1016/S0046-8177(96)0218-X


Glucose Metabolism in Breast Cancer and Its Implication in Cancer Therapy

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[100] M. J. Birnbaum, H. C. Haspel and O. M. Rosen, “Trans- 


5740, 1979, pp. 729-731. doi:10.1038/282729a0


[169] S. Bandyopadhyay, et al., “Mechanism of Apoptosis In-


[192] D. Lamoral-Theys, et al., “Natural Polyphenols That Display Anticancer Properties through Inhibition of

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