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Metabolic genes in cancer: their roles in tumor progression and clinical implications

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Abstract

Re-programming of metabolic pathways is a hallmark of physiological changes in cancer cells. The expression of certain genes that directly control the rate of key metabolic pathways including glycolysis, lipogenesis and nucleotide synthesis are drastically altered at different stages of tumor progression. These alterations are generally considered as an adaptation of tumor cells; however, they also contribute to the progression of tumor cells to become more aggressive phenotypes. This review summarizes the recent information about the mechanistic link of these genes to oncogenesis and their potential utility as diagnostic markers as well as for therapeutic targets. We particularly focus on three groups of genes; GLUT1, G6PD, TKTL1 and PGI/AMF in glycolytic pathway, ACLY, ACC1 and FAS in lipogenesis and RRM1, RRM2 and TYMS for nucleotide synthesis. All these genes are highly up-regulated in a variety of tumor cells in cancer patients, and they play active roles in tumor progression rather than expressing merely as a consequence of phenotypic change of the cancer cells. Molecular dissection of their orchestrated networks and understanding the exact mechanism of their expression will provide a window of opportunity to target these genes for specific cancer therapy. We also reviewed existing database of gene microarray to validate the utility of these genes for cancer diagnosis.

Keywords

Metabolism; Oncogenesis; Diagnostic marker

1. Introduction

1.1. Metabolic changes in cancer

Energy homeostasis of a normal cell is balanced by at least three metabolic pathways including glycolysis, lipogenesis and tricarboxylic acid (TCA) cycle, and these pathways are also closely linked to amino acid as well as nucleotide biosynthesis. Although normal cells utilize a variety of energy sources such as glycogen, fatty acids and amino acid, glucose is considered as a key energy source for their growth. Glucose is taken up by the glucose transporter system and is converted to pyruvate through the glycolysis pathway (1). Pyruvate is then converted to acetyl-CoA and utilized as a substrate for the TCA cycle in mitochondria. While the TCA cycle

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generates ATP through its oxidative phosphorylation, an intermediary metabolite, citrate, is exported into the cytoplasm and is converted to acetyl-CoA which is used as an initial substrate for generating fatty acids through the lipogenesis pathway (2,3). Fatty acids serve not only as energy storage but also provide key components for membrane biosynthesis and also play important roles in cell signaling by modifying various signal proteins.

On the contrary to the normal cells, tumor cells exhibit quite abnormal behavior by re-programming these metabolic pathways. It has long been recognized that the cancer cells need to drive higher rate of energy metabolism because of their active proliferation rate and motile nature. With the same reasons, tumors become more hypoxic and therefore they need to rely on non-oxidative energy source such as glycolysis as originally reported by Warburg (4). On the other hand, higher rate of lipogenesis in cancer cells seems to be contributing both to building mass (cell membrane etc) and generating energy (beta-oxidation) (5). The rate of lipogenesis is also significantly accelerated in tumor cells in order to compensate for the higher rate of proliferation. This metabolic re-programming triggers a series of cascade of events in tumor cell physiology and often generates harmful byproducts such as ROS and sometimes even imbalance nucleotide pool for DNA replication that promote a mutation rate through the activation of mutators and/or the defect of DNA repair system. It was also shown that the germline mutations were significantly correlated with the incidence of various types of cancer in clinical setting (6-8). The major source of ROS in normal cells is oxidative phosphorylation; however, ROS is also generated by other pathways such as NADH oxidation. In fact, many cancer cells have high turn-over of glycolysis and promote the NADH oxidative pathway which consequently generates high amount of ROS. On the other hand, it is known that many cancer cells have malfunctioning mitochondria which results in lower oxidative phosphorylation. However, this is not always the case and recent evidence demonstrated that many cancer cells still have intact mitochondria with normal function of TCA cycle.

It is increasingly evident that many genes involved in metabolic pathways play direct roles in tumorigenesis and tumor progression. In the following sections, we review the most current information about these metabolic genes in regard to their physiological roles in tumorigenesis and underlining molecular mechanisms. We also discuss their possible utilities as diagnostic markers and potential therapeutic implications.

2. Glucose metabolism in cancer

2.1. Cancer cells depend on glycolysis for energy supply

In most cancer cells, the rate of glucose uptake is significantly elevated and oxidative phosphorylation in mitochondria is often decreased compared to normal cells. This effect was first noted by Otto Warburg in 1929 and is called as aerobic glycolysis or the Warburg effect (4). Rapidly growing cancer cells suffer from a lack of oxygen and nutrition due to the diffusion limits of blood supply, and therefore, persistent glucose metabolism and generation of lactate is thought to be an adaptation of tumor cells to hypoxia. Interestingly, however, cancer cells prefer to utilize glycolysis for their energy supply even under normoxic condition when they are grown in culture medium. Although glycolysis is far less efficient in generating ATP than oxidative TCA cycle, it is much faster than the oxidative pathway and is independent of mitochondrial function which is often dysfunctional in cancer cells. In fact, Ramanathan et al. showed that even when mitochondrial function was completely blocked, i.e., inhibition of oxidative ATP production, the level of ATP was not significantly altered in tumor cells (9). However, how cancer cells reprogram this metabolic alteration and whether it is essential for tumorigenesis is still not fully understood. One key factor which links glycolysis and tumorigenesis is the tumor suppressor, p53. This gene appears to block glycolytic pathway through its target TIGER (TP-53-induced glycolysis and apoptosis regulator) by decreasing the glycolytic metabolite fructose-2,6-bis-phosphate which stimulates glycolysis and inhibits

gluconeogenesis (10). p53 has also been shown to downregulate another glycolytic enzyme, phosphoglycerate mutase (PGM). Furthermore, EGFR which is highly expressed in many cancers has been shown to inhibit autophagic cell death by maintaining intracellular glucose level through stabilization of the sodium/glucose cotransporter 1 (SGLT1) (11). In addition, oncogenes such as Ras and Src have been reported to promote glycolysis by activating glucose transporter (GLUT) 1 which is a key gene of glucose uptake (12,13). Therefore, re-programming the glycolytic pathway is considered to play critical roles in tumorigenesis and tumor progression. Currently, there are at least four genes in the glycolytic pathway that are known to be directly involved in oncogenesis, namely GLUT1, PGI/AMF, G6PD and TKTL1 (Table 1). The product of GLUT1 is capable of transporting glucose across the hydrophobic cell membrane, which is the first rate-limiting step of glucose metabolism. The upregulation of GLUT1 and GLUT3 with increased glucose uptake has been shown in various tumors including oesophageal, gastric, breast and colon cancers (62-65). Phosphoglucose isomerase/autocrine motility factor (PGI/AMF) catalyzes the second glycolytic step, the isomerization of glucose-6-phosphate to fructose-6-phosphate (66). Recent studies revealed that PGI/AMF is a multifunctional moonlighting protein which is associated with not only glycolysis but also cancer cell migration, invasion, growth, survival, and angiogenesis. Glucose-6-phosphate dehydrogenase (G6PD) and transketolase-like-1 (TKTL1) are involved in an important branch of glycolysis, pentose phosphate pathway. Both G6PD and TKTL1 are key enzymes for ribose production, and therefore, they are considered to play roles in tumor cell proliferation (17, 67).

2.2. GLUT1 mediates glucose uptake in cancer cells

Glucose is a polar molecule and cannot penetrate endothelial cells or plasma membrane by simple diffusion, and therefore, uptake of glucose across cell membranes requires transporter proteins. GLUT 1, GLUT3, and GLUT4 are members of GLUT/SLC2 family and they are known to regulate glucose uptake (68,69). The GLUT family is a transmembrane protein which has 12 transmembrane domains with both amino and carboxy-terminal ends exposed to the cytoplasmic side of the plasma membrane. GLUT1 is found at variable levels in many tissues, while GLUT3 and GLUT4 are expressed in a tissue-specific manner (65,70). Increased expression of GLUT1 has been shown in various types of cancers including hepatic, pancreatic, breast, esophageal, brain, renal, lung, cutaneous, colorectal, endometrial, ovarian and cervical carcinoma. (71-80). Notably, high expression of GLUT1 is significantly correlated with decreased survival in breast cancer. The expression of GLUT1 and glucose uptake was also strongly increased in rat renal oncocyctic tubules when renal oncocytomas were induced by chemicals (81). These data suggest that GLUT1 acts as an oncogene in a variety of cancers. In fact, ectopic expression of GLUT1 in Chinese hamster ovary cells led to a higher rate of glucose and thymidine uptake when cells were exposed to glucose-deficient conditions, indicating that GLUT1 support tumor cell growth (82). Increased GLUT1 expression and glucose uptake enables rapidly growing cancer cells to acquire energy even under hypoxic condition by harnessing glycolysis. Of note, hypoxia-inducible factor (HIF) which is up-regulated in many cancers is known to enhance the expression of GLUT1 and other enzymes that are necessary for glycolysis (83,84). In addition, HIF-1 promotes angiogenesis through upregulation of VEGF, which facilitates intake of oxygen as well as glucose by tumor cells (85). Interestingly, HIF-1 is induced not only by hypoxia but also by Ras through PI3K/Akt, resulting in VEGF upregulation (86). Therefore, GLUT1 plays an important role in the proliferation of cancer cell by supplying energy source, and the expression of this gene is critically balanced by various oncogenes and tumor microenvironment.

The phenomenon of elevated glucose uptake has been clinically exploited to detect tumor cells by positron emission tomography (PET) scan using the glucose analogue tracer 2-fluorodeoxy-D-glucose (2-FDG) (87,88). Importantly, the dependence of cancer cells on glycolysis can also

be utilized to selectively inhibit cancer cells in chemotherapy. 2DG is a glucose analogue and is converted to 2DG-6-phosphate which inhibits glycolytic enzymes, phosphoglucose isomerase and hexokinase. Consequently, 2DG-treated cell cannot effectively use glucose as energy source, which results in the energy deprivation and the following growth arrest of tumor cell (89). These results suggest a potential utility of 2DG as anti-cancer drug, although 2-DG is known to cause hypoglycemic symptoms because it also reduces glucose in normal tissues, especially in the brain which heavily relies on glycolysis for energy supply (90,91). Recently performed phase I clinical trial (NCT00096707) for 2-DG-treatment on several types of cancer patients showed that 2-DG exhibited positive responses in patients who were treated orally with this compound. These data support our continuing hope that 2DG serves as a lead compound to develop a better drug to target glycolytic pathway for cancer therapy. In addition, the results of phase I/II clinical trials have shown that the combination of 2-DG and γ -radiation was well tolerated in cerebral glioma patients (65). Therefore, such combination therapies by taking advantage of the dependence of cancer cells on glycolysis may also be a promising approach for cancer treatment.

2.3. G6PD is a key enzyme of pentose phosphate pathway

G6PD is a key enzyme to produce ribose-5-phosphate via pentose phosphate pathway, which is essential for RNA and DNA synthesis in rapidly growing cells (17,67). Another crucial role of G6PD is to generate NADPH which is an essential factor for glycolysis, and the reducing power of NADPH is necessary to neutralize oxidative stress, e.g., to maintain the reduced form of glutathione which serves to detoxify free radicals and peroxides (92). Therefore, G6PD is thought to contribute to cancer growth and survival by producing ribose and NADPH through pentose phosphate pathway. In fact, elevated levels of expression and activity of G6PD are frequently observed in breast, colon, endometrial, cervical, prostatic, and lung cancers (93, 94). Interestingly, ectopic expression of G6PD in NIH 3T3 cells was shown to significantly increase intracellular levels of NADPH and glutathione and also to promote anchorage-independent cell growth (17,92). Furthermore, these cells were shown to be tumorigenic as well as angiogenic in nude mice, suggesting that G6PD acts as oncogene.

The higher rate of glycolysis in cancer cells generates increased number of metabolites such as hydrogen ions and lactate that cause acidification of the cells. Although acidosis can stimulate invasion, migration, mutagenesis, and radioresistance in cancer cells, it also causes apoptosis through the p53 pathway (1,95). Elevated glycolytic flux via HIF1 causes lactate production by upregulation of lactate dehydrogenase to increase pyruvate-to-lactate flux (96, 97) and also by pyruvate dehydrogenase kinase (PDK) to block pyruvate recruitment into the tricarboxylic acid cycle (98,99). To avoid resultant acidosis and maintain an intracellular pH, cancer cells need to pump out H^+ -ion by utilizing Na^+/H^+ antiporter or by monocarboxylate transporter which transports H^+ with lactate (100,101). Considering the fact that ectopic G6PD expression increases the level of NADPH and glutathione, G6PD may contribute to cancer cell survival by maintaining the intracellular pH and redox balance.

Due to the critical role of G6PD in tumorigenesis, this enzyme is considered to be an excellent therapeutic target. Buthionine S'R'-sulfoximine (BSO), a glutathione depletion agent, is known to inhibit G6PD, and this compound was shown to suppress colony formation of G6PD-expressing cells in soft agar (17). BSO is currently in phase I clinical trial (NCT00006027, NCT00002706, NCT01007305, NCT00002706, NCT00002730, NCT00005835 and NCT00661336). Another promising inhibitor of G6PD is 6-aminonicotinamide (6-AN) which has been used as a modulator of smooth muscle contraction (18). 6-AN is also capable of suppressing pentose phosphate pathway by inhibiting 6-phosphogluconate dehydrogenase which results in NADPH reduction, suggesting that this compound can be an effective inhibitor of pentose phosphate pathway. Furthermore, combination of 2-DG and 6-AN has been shown

to enhance the radiosensitivity in human glioma and squamous carcinoma cell lines (102). Therefore, a combination of inhibitors for G6PD and glucose transporter can be an effective approach to selectively suppress cancer cell growth.

2.4. Transketolase-like 1 supports tumor proliferation through pentose phosphate pathway

Thiamine (vitamin B1) - dependent transketolase is another key enzyme of pentose phosphate pathway. Transketolase regulates the nonoxidative pathway of pentose phosphate pathway, while G6PD is responsible for the oxidative pathway. Like G6PD, TKTL1 gene was also shown to be strongly expressed in various carcinomas including ovarian, nasopharyngeal, colon and urothelial carcinomas (103-105). Increased expression of TKTL1 is correlated with higher tumor stages, invasion, and poor prognosis (104,106). Importantly, inhibition of TKTL1 by RNAi in nasopharyngeal carcinoma cell line (CNE) dramatically down-regulated transketolase activity and significantly inhibited proliferation of the cells (19). In addition, knockdown of the expression of TKTL1 by siRNA in colon cancer cell line (LoVo) was accompanied with decreased proliferation and G0/G1 arrest (107). Furthermore, TKTL1-knockdown sensitized colon carcinoma cells (HCT116) to oxidative stress-induced apoptosis (108). On the other hand, induction of TKTL1 by thiamine promotes cell growth in Ehrlich's ascites tumor cells (103). Of note, thiamine is metabolized to thiamine pyrophosphate, a cofactor of transketolase, which is involved in ribose synthesis, and promotes cell replication. Therefore, TKTL1 contributes to tumor progression by promoting cell survival and also by providing ribose via pentose phosphate pathway for tumor cell growth. To date, a specific inhibitor for TKTL1 has not been identified (20); however, this enzyme is considered to be a rational target for cancer therapy.

2.5. PGI/AMF has dual roles as a glycolytic enzyme and a cytokine

PGI was originally isolated as "autocrine motility factor (AMF)" from the conditioned medium of human A2058 melanoma cells. As a tumor secreted cytokine, PGI/AMF has been shown to be involved in cell migration, invasion, proliferation, survival, and angiogenesis (109). Interestingly, PGI/AMF plays another important role in glycolysis and gluconeogenesis and this enzyme catalyzes the second glycolytic step, the isomerization of glucose-6-phosphate to fructose-6-phosphate (22). Cell surface receptor of PGI/AMF, gp78/AMFR, is overexpressed in various metastatic tumors along with PGI/AMF, and their presence in the serum and urine is correlated with a poor prognosis and tumor progression (21,110-117). Ectopic expression of PGI/AMF in murine fibroblasts and fibrosarcomas rendered the cells highly motile and transformed phenotype *in vitro* and tumorigenicity *in vivo* (118,119). Furthermore, orthotopic implantation of pancreatic tumor cells that ectopically expressed PGI/AMF produced local tumors and liver metastases (120). On the other hand, the suppression of PGI/AMF expression led to the inhibition of cell proliferation and tumorigenicity followed by mesenchymal-to-epithelial transition (121). Furthermore, down-regulation of PGI/AMF in mouse embryonic fibroblasts and human fibrosarcoma caused premature senescence which is regulated in part by tumor suppressor genes. (21,122). It has been shown that the tumor suppressor p53 down-regulated PGI/AMF and that cyclin-dependent kinase inhibitor p21 was increased in PGI/AMF knock-down cells, suggesting that inhibition of PGI/AMF may be an effective way to suppress tumor cells by inducing senescence (21). Erythrose 4-phosphate (E4P) and mannose 6-phosphate (carbohydrate phosphates) are known to specifically inhibit PGI/AMF and considered to induce senescence to tumor cells (22). Therefore, these compounds and their analogues may potentially serve as effective anti-cancer drugs. Interestingly, the level of PGI/AMF in urine has been shown to be increased in patients with transitional cell carcinoma of bladder, therefore urinary PGI/AMF may be a useful marker for diagnosis of bladder cell carcinoma (111).

3. Lipogenesis and cancer

3.1. Lipogenic pathway is activated in cancer cells

Re-programming of lipogenic pathway is one of the most significant alterations of tumor cell physiology and at least three genes in this pathway are known to play key roles in tumor progression, namely ACLY, ACC and FAS. Triacylglycerol is an esterified form of glycerol which consists of three fatty acids including palmitate, oleic acid and alpha-linolenic acid and it is stored mostly in hepatic and adipose cells to maintain energy homeostasis. As a first step of fatty acid synthesis, pyruvate needs to be converted to acetyl-CoA in the mitochondria. Acetyl-CoA is then incorporated into TCA cycle which produces citrate in the presence of sufficient amount of ATP. Accumulated citrate is exported to the cytoplasm where it is catalyzed by ATP citrate lyase (ACLY) to generate cytosolic acetyl-CoA which is a key precursor of fatty acids. Acetyl-CoA is then carboxylated by ACC to synthesize malonyl-CoA which is then converted to palmitate (16-carbon saturated fatty acid) as the first fatty acid in lipogenesis by the key rate limiting enzyme (123). ACC and FAS are both highly expressed in the embryonic cell and the functions of both enzymes are essential for development. In fact, mice deficient in these enzymes died at embryonic stage (124,125). On the other hand, it is well recognized that fatty acid synthesis pathway is significantly activated at a relatively early stage in various types of tumors, and the key genes involved in this pathway including ACLY, ACC and FAS are considered to play critical roles in tumorigenesis and cancer progression (Table 1). In this section, we will discuss the functional roles of these three genes in tumor progression and potential therapeutic as well as diagnostic implications.

3.2. ATP citrate lyase generates cytosolic acetyl-CoA for lipid synthesis

Citrate is generated by citrate synthase in the TCA cycle and is exported to the cytosol through mitochondrial citrate transporter. It is then converted by ACLY to cytosolic acetyl-CoA which serves as an essential component for fatty acid synthesis. While the expression of ACLY is low in normal cells, it is significantly up-regulated in various types of tumors (126-130). Of note, phosphorylated ACLY (active form of ACLY) was found to be positively correlated with clinical stages of lung cancer (23). Furthermore, ACLY inhibitors such as siRNA and SB-204990 block the production of acetyl-CoA and consequently suppress cell growth *in vitro* and *in vivo* (24,25). Blocking ACLY with siRNA causes the suppression of Akt signaling and thus results in the loss of tumorigenicity *in vitro*. These results indicate that ACLY plays a role in tumorigenesis and tumor cell survival and suggest a potential clinical utility of these compounds. Interestingly, (-)-hydroxycitric acid (HCA) which is a known competitive inhibitor of ACLY significantly reduced levels of cholesterol, LDL and triglycerides without apparent side effects in clinical studies. HCA is derived from a subtropical plant, *Garcinia gummi-gutta*, which has been consumed as food and traditional medicine in India, suggesting that HCA may be used as chemo-preventive food supplement.

3.3. Acetyl-CoA carboxylase is associated with tumor progression

ACC is an enzyme of ATP-dependent carboxylase and converts acetyl-CoA to malonyl-CoA which then serves as a substrate for FAS to generate fatty acids. There are two isozymes of ACC (alpha and beta) whose expressions are regulated by a variety of factors such as nutrition, hormones and other physiological responses (131). The function of ACC alpha appears to be essential for embryonic developmental as an ACC alpha deficient mouse is embryonic lethal. On the other hand, RNA and protein levels of ACC alpha have been reported to be significantly increased in tumor cells and they were also associated with the up-regulation of FAS expression (132). Interestingly, the amount of phosphorylated ACC (p-ACC) was found to be dramatically increased in lung cancer and other "high-energy-consuming" cells, even though phosphorylated ACC is an inactive form and its expression is related with better survival of cancer patients (26). It was also reported that inhibition of ACC alpha with a chemical reagent,

TOFA (5-(tetradecyloxy)-2-furancarboxylic acid), or shRNA resulted in cell cycle arrest and apoptosis of tumor cells and that this effect was reversed by addition of palmitate in culture medium (27,133). Palmitate is a structural component of cell membrane and also serves as an energy source; however it also acts as a signaling molecule, although the exact role of this fatty acid in tumorigenesis is yet unclear. Nevertheless, a small molecule which can specifically inhibit the ACC activity is expected to potentially work as an anti-cancer drug.

3.4. Fatty acid synthase is up-regulated at an early stage of cancer

FAS is a multifunctional enzyme which is composed of seven functional domains (KS; β -ketoacyl synthase, MAT; malonyl-CoA-/acetyl-CoA-ACP-transacylase, DH; dehydratase, ER; β -enoyl reductase, KR; β -ketoacyl reductase, ACP; acyl carrier protein, TE; thioesterase) (134-136). All these activities coordinately synthesize fatty acid using Acetyl-CoA and malonyl-CoA as a primer and a carbon donor, respectively. The FAS gene is abundantly expressed during embryonic development; however, the expression of this gene is restricted to liver, lactating breast and brain in adult tissues (137-139). On the other hand, FAS is significantly up-regulated in a variety of cancers at an early stage and its expression is positively correlated to poor survival of patients (29-31). In breast cancer, both FAS and HER2 are expressed at premalignant stage such as DCIS (Ductal Carcinoma *in Situ*) (140,141), and their expression tends to be higher in more malignant cells. Importantly, inhibition of FAS expression in tumor cells by siRNA or small chemicals induces cell growth arrest and concomitant apoptosis. Therefore, these results suggest that FAS is involved in the early stage of tumorigenesis, possibly by blocking apoptosis. Indeed, a transgenic mouse which is specifically expressing FAS in prostate has been recently shown to develop *in situ*, non-invasive tumor (32). Although how FAS induces cell transformation is yet to be elucidated, forced expression of FAS was found to stimulate cell growth and reduce sensitivity to tyrosine kinase inhibitors (HER2 inhibitor) *in vitro* (33), suggesting that FAS may exert its oncogenic property by up-regulating HER2. Furthermore, it is reported that tumor suppressor protein, PTEN, is capable of suppressing FAS and down-regulation of PTEN resulted in significantly higher expression of the FAS gene (29). Therefore, dysregulation of PTEN which is often observed in breast cancer patients and resultant up-regulation of FAS are likely contributing to breast tumorigenesis at an early stage by blocking apoptotic signaling. In this context, it should be noted that inhibition of FAS significantly augmented the expression of pro-apoptotic genes including BNIP3, DAPK2 and TRAIL (132).

FAS gene is regulated by several transcriptional factors including sterol regulatory element binding protein (SREBP) which binds to consensus sequence, SRE/E-box, on the FAS promoter (142,143). Interestingly, FBI-1 which is known as a proto-oncogene directly interacts with SREBP and synergistically enhances the expression of FAS gene (144), suggesting a link of another oncogene to FAS-induced tumor progression. The FAS gene is also regulated by environmental factors. For example, hypoxic condition was shown to cause up-regulation of the FAS gene through increase in ROS in cancer cell (143). Indeed, the overexpression of FAS is often observed in hypoxic region of tumors and this up-regulation may contribute to tumor progression by blocking apoptosis signaling and in turn enhancing tumor cell survival as well as promoting chemo-resistance.

FAS has been considered an ideal target for cancer treatment due to its specific expression in tumor cells. In fact, treatment of tumor cells with pharmacological inhibitors of FAS such as cerulenin, C75 and Orlistat leads to cell cycle arrest and apoptosis (29,30,34-42). However, the specificity of action of these inhibitors is still a concern. Cerulenin harbors a highly reactive epoxy group that may also interact with other proteins and may affect processes other than fatty acid synthesis. C75 was designed to be a less reactive (and therefore potentially safer) form of the classical FAS inhibitor, cerulenin (43). When given i.p., C75 rapidly caused stools

to become extremely loose or liquid, and this was accompanied by weight loss, decreased food intake, and inhibition of normal paper-shredding behavior of animal. On the other hand, Orlistat appears to be more specific; however, this drug needs to be administered orally and the effects of Orlistat are largely confined to the gastrointestinal tract, where it inactivates pancreatic lipase (44). This compound is also known as an anti-obesity drug and inhibits the thioesterase domain of FAS. Therefore, developing a more specific and less toxic drug to block the function of FAS is necessary and it is currently under intensive study.

One of the unique features of FAS enzyme is its secretory form. A highly-sensitive ELISA (FASgen, Inc.) system is indeed available, and it has been reported that the expression level of FAS in serum is strongly associated with tumor stage and survival of patients with a variety of cancers, suggesting the utility of the secreted form of FAS as a diagnostic and prognostic tool (45,46)

4. Mitochondrial enzymes in cancer

Dysregulation of mitochondrial function is a hallmark of cancer cell and several key genes are identified that are closely linked to tumor progression, namely SDH (succinate dehydrogenase), FH (fumarate hydratase) and IDH (isocitrate dehydrogenase). Mutations or loss of SDH and FH genes are known to be oncogenic and they are considered to be tumor-suppressors (Table 1, 58-60). There are four subunits of SDH (A, B, C, D) that are assembled as complex II in mitochondrial electron-transport chain. Although complex II generally converts succinate to fumarate, mutations of SDHB, SDHC and SDHD cause accumulation of succinate and inhibit PHD (prolyl hydroxylase) function which induces degradation of HIF1 α (145). On the other hand, mutated FH cannot convert fumarate to malate and consequently induces PHD inactivation. Therefore, dysfunction of SDH and FH enzymes cause the activation of HIF1 α which enhances tumorigenic-related signaling such as angiogenesis. However, the exact molecular mechanism of the tumor suppressive function of SDH and FH is yet to be defined.

The NADP⁺ dependent IDH gene, which converts isocitrate to α -ketoglutarate, is often mutated at amino acid 132 in glioblastomas (146), and protein level of IDH was found to be frequently increased in many metastatic ductal carcinoma compared to normal cells (147,148), suggesting an oncogenic role for this gene in the mitochondria. IDH is considered as one of the major producers of NADPH which is required for fatty acids and cholesterol biosynthesis; however the transgenic mice of IDH exhibited fatty liver, hyperlipidemia and obesity but not tumor (149). How IDH contributes to tumor progression is still not clearly defined; however, one attractive theory is that IDH contributes to defense system in cancer cell against ROS which often causes cell death via DNA break. In this context, it should be noted that cancer stem cell has a powerful ROS-scavenging system through up-regulation of GSH (glutathione) and its related genes such as IDH and Foxo1 which regulates antioxidant related genes (150). Therefore, IDH may contribute to the maintenance of stemness of tumor stem cell. However this hypothesis needs more rigorous testing.

5. Nucleotide synthesis in cancer

5.1. Nucleotide metabolism in cancer cells

Nucleotides are key components of DNA and RNA structures and they also serve as important sources of cofactors such as CoA and NAD in cellular signaling. Therefore, dysregulation of nucleotide biosynthesis has profound effects on normal cellular physiology which often result in neoplastic transformation of the cell. When cells become cancerous and highly proliferative, they require excess and balanced supply of nucleotides for their growth and survival. However, when this balance is perturbed, DNA gains considerable chances of further mutations, which leads to more malignant characteristics of the tumor cells. Therefore, nucleotide metabolism

plays an important role in tumorigenesis and tumor progression. There are a series of key enzymes that are involved in the nucleotide biosynthesis and modification including CTP synthetase, thymidylate synthase, dihydrofolate reductase, IMP dehydrogenase, ribonucleotide reductase, DNA polymerase, and DNA methyltransferase. These enzymes are indeed markedly up-regulated in many types of cancer, and therefore, they are considered to be valid target for cancer therapy (153-159). Among these enzymes, ribonucleotide reductase and thymidylate synthase are particularly attractive because the level of these enzymes is highly elevated in various cancers and they are shown to be directly involved in tumor initiation (Table 1). Therefore, this section focuses on these two genes and discusses their potential utility for therapeutic targets as well as diagnostic markers.

5.2. Ribonucleotide reductase is double-face protein as tumor suppressor and oncoprotein

Ribonucleotide reductase (RNR) which is a key enzyme of rate limiting step in dNDP biosynthesis has been shown to play a critical role in tumorigenesis and tumor progression (152,160). This enzyme reduces ribonucleoside diphosphates (NDPs) to deoxyribonucleoside diphosphates (dNDPs) by tyrosyl radical reaction with Fe(III) cluster. The enzyme is composed of two non-identical homo-dimeric subunits; RRM1 and RRM2 (47). The large R1 subunit with a molecular mass of 90 kDa has a catalytic domain and is encoded by the RRM1 gene whose protein level is constant throughout the cell cycle. On the other hand, the small R2 subunit with a molecular mass of 45 kDa has tyrosine residue as a free radical scavenger with diferric iron, which can reduce NDPs to dNDPs. p53R2 (RRM2b) is a homologous gene of the RRM2 with 80% sequence similarity and is originally identified as a target gene of the p53 tumor suppressor protein (161,162). The expression of RRM2 gene is usually maintained to be higher than that of RRM1 and it reaches a maximum during S phase. The gene is known to be regulated by cell cycle-associated transcription factors, such as NF-Y and E2F (163,164), and therefore, the cell-cycle dependent activity of RNR enzymes is controlled by the level of RRM2. When the expression level of RRM2 is reduced, p53R2 binds to RRM1 subunit to form active RNR complex which can supply dNDPs for repairing damaged DNA. Therefore, RNR has a critical role in DNA repair during cell-cycle and their expressions are stringently regulated.

RRM2 and p53R2 are found to be markedly up-regulated in many types of cancer cells in patients, indicating the direct roles of these genes in tumor progression (160). In addition, ectopic expression of the RRM2 gene was shown to increase membrane-associated Raf1 expression, MAPK2 and Rac-1 activation, which resulted in enhanced metastatic potential in a xenograft model, suggesting that RRM2 is also involved in tumor progression (48). In this context, it should be noted that over-expression of RRM2 was found to enhance cellular invasiveness through activation of NF-kB which increases MMP9 expression (165,166). To further gain insight into the role of RNR in tumorigenesis, transgenic mice of RRM1, RRM2 and p53R2 have been recently established (47). The mice over-expressing RRM2 and/or p53R2 in the lungs were found to generate tumors in around 40% of the animals, providing direct evidence to show that these genes indeed act as oncogenes.

On the contrary, RRM1 has a tumor suppressor activity, as shown by gene transfer experiments in both mouse and human cell lines (167). Ectopic expression of RRM1 in human and mouse lung cancer cell lines significantly up-regulated the PTEN gene, suppressed migration and invasion as well as metastasis formation in an animal model (164). In clinical studies, the median disease-free survival exceeded 120 months in the group of patients with tumors that had high expression of RRM1 compared to the patients who had low level of RRM1 (168). The molecular mechanism of these striking and contrasting differences between RRM1 and RRM2 in their pathogenic roles during tumor progression is not well understood; however,

they provide important tools to further investigate the pathological roles of dNDP biosynthesis in tumorigenesis.

Inhibition of nucleotide biosynthesis in tumor cells by anti-metabolites is one of the classic approaches for cancer treatments and this approach continues to be effective. RNR inhibitors are classified into several groups as translational, dimerization and catalytic inhibitors. The catalytic inhibitors are further divided into subgroups, inhibitors of sulfhydryl groups, allosteric inhibitors and substrate analogues. Several antisense oligonucleotides (ASOs) specific to RRM2 are in clinical trials. GTI-2040 (combination of capecitabine) has just completed a clinical trial and is already in clinical use for renal cancer. CALAA-01 is a mixture of RNAi and nanoparticle, and therefore, resistant to nuclease degradation. CALAA-01 is currently in phase I trial (NCT00689065).

5.3. Thymidylate synthase acts as an oncogene by altering nucleotide metabolism

Thymidylate synthase (TYMS) plays a key role in the biosynthesis of thymidine monophosphate (dTMP) which is an essential substrate of DNA synthesis. TYMS is a 74 kDa protein and forms a homodimer which catalyzes reductive methylation of deoxyuridine monophosphate (dUMP) to generate dTMP using a cofactor, CH_2H_4 -folate. Expression of TYMS is controlled by the transcription factor E2F which is linked to cell-cycle regulation and proliferation (169,170), and inhibition of this enzyme results in cell arrest. The results of microarray and immunohistochemical studies indicate that the expression of this enzyme is significantly up-regulated in various tumors including breast, bladder, cervical, kidney, lung and gastrointestinal cancers (171-176). The high expression of TYMS is also associated with poor clinical outcomes in these cancers, suggesting that TYMS acts as an oncogene. In fact, ectopic expression of TYMS has been shown to confer normal cell with transformed and tumorigenic phenotype in a xenograft model (54). Notably, the elevated level of TYMS expression was also shown to result in more invasive and metastatic abilities in these cells. Furthermore, recent study of TYMS transgenic mice revealed that over-expression of this gene caused pancreatic islet hyperplasia and islet cell tumors (55). Importantly, mutations at the active site of this enzyme diminished the ability of tumor formation in mice, suggesting that imbalance of nucleotide pools by increasing levels of TYMS enhances mutations and thereby causes oncogenic transformation.

TYMS has been recognized as an effective target for anti-cancer therapy, and several inhibitors of TYMS have been used clinically for over 30 years. Among these drugs, 5-fluorouracil (5-FU) has been widely used for many types of cancer; however, 5-FU is known to have unwanted side-effects due to its broad specificity. Recently, several analogs of folates have been developed as a new class of TYMS inhibitors, and some of them are currently in clinical trials. Raltitrexed (RTX, Tomudex or ZD1694) is in various phases of clinical trials with combination of several anti-cancer drugs for solid tumors, colon and rectal cancers and leukemia, and another antifolate drug, ZD9331, has completed phase II trial for the treatment of ovarian cancer (NCT00014690). Although these drugs appear to be effective, their potential long-term side effects are of some concern because they generally have broad specificity, and developing more specific small chemicals is needed to generate more effective therapeutic drugs.

6. Diagnostic value of metabolic genes

Due to the high level of expression at various stages of tumor tissues, the metabolic genes are considered to serve as diagnostic as well as prognostic markers to predict patient outcome. Immunohistochemistry is still the most reliable method to examine this possibility, but it is generally not quantitative or cost effective for testing a “signature” of multiple genes at clinical setting. However, recent availability of data base of microarray analyses allows us to easily assess the diagnostic value of any combination of genes or “signature” for various types of

cancer. Figure 2 shows such analyses for four different types of tumors including breast, prostate, lung and colon cancer, using GEO microarray database, and the analyses include at least five different data base for each cancer type. In breast cancer, GPI, ACLY, RRM2 and TYMS genes are highly up-regulated in at least four different cohorts out of seven independent studies. In prostate cancer, FASN and RRM2 were markedly upregulated in all data base, while TKTL1, GPI, ACLY and TYMS are also significantly expressed in at least 3 cohorts out of 5 studies. In lung cancer, four genes including GLUT1, ACLY, RRM2 and TYMS were significantly upregulated in at least 5 independent studies. Moreover three genes including GPI, ACLY and RRM2 genes were upregulated in colon cancer of all studies. These data indicate that a different combination of metabolic genes may serve as “signature” for each type of cancer. We then examined whether a signature of the metabolic genes could predict disease-free survival in four independent studies of breast cancer. Figure 3 indeed indicates that the signatures of GPI, ACLY, RRM2 and TYMS genes have a strong predictable value for breast cancer patient outcome, which is consistent with the notion that these metabolic genes act as oncogenes in breast cancer. Therefore, it is expected that further analysis of different combinations of metabolic genes may reveal a “signature” with more predictable values for each type of cancer.

7. Conclusions and Perspectives

During the course of tumor initiation and progression, cancer cells need to reprogram their metabolic pathways in order to respond to the demanding requirements for their own growth. This re-programming is accomplished by both genetic and epigenetic alterations of various metabolic genes, and the dysregulations of some of these genes are directly involved in the initial step of transformation while others contribute to maintenance and acceleration of malignant phenotypes. However it is still not clear how and when these changes occur in normal cells. For example, the dysregulation of the FAS gene is often observed at very early stages of cancer and in benign tumors, suggesting the direct role of this gene in tumor initiation. However, how and what causes this dysregulation remains unknown, and understanding the mechanism and identifying the factors contributing to these changes is of paramount interest. It is suspected that not only carcinogens, but also dietary factors, hormonal balance, inflammatory conditions and tumor microenvironment such as stroma and ECM are all likely to be involved in the re-programming process of metabolic pathways. In this context, it is worth noting that some metabolic abnormalities such as diabetes and even ageing are linked with higher incidence of cancers. However, whether these abnormalities are directly involved in tumorigenesis remains to be determined.

It is well established that HIF1, AMPK and LKB play central roles in keeping the balance of cell metabolism for survival and growth under various stressful environments such as hypoxic, acidic and low nutrient conditions. Furthermore, recent findings indicate that the sensitivity of tumor cells to dietary restriction is closely associated with activation of PI3K pathway, suggesting a key role of this pathway in balancing metabolic homeostasis. It is also noted that the PI3K/AKT pathway directly controls lipogenesis by up-regulating SREBP1 which is considered to be a master control gene of various lipogenic genes (177). Oncogenes that encode transcription factors are also actively involved in regulation of cellular metabolism. For example, Myc is known to regulate Glut1 and other genes in glycolysis. Myc can directly bind the promoters of these genes with other co-factors (eg. E2F1 for nucleotide metabolism and HIF1 for glucose metabolism) and significantly up-regulate the target genes. Therefore, metabolic changes in tumor cell are also modulated by activation of these oncogenes; however, dissecting the exact molecular mechanism of this process is critically important in order to identify a specific target for both preventive and therapeutic intervention.

Another key question is homeostasis and crosstalk of each pathway after the re-programming in cancer cells. For example, dysregulation of the Glut1 gene affects not only glycolytic pathway but also lipogenesis because glycolysis generates substrate for TCA cycle which ultimately provides a precursor for lipogenesis. In addition, glycolysis and lipogenesis are closely linked through the redox pathway. Despite ill-functioning mitochondria and the Warburg effect, the balance of these pathways is still well maintained in the cancer cells during their survival and aggressive growth, suggesting that an alternative balancing mechanism needs to be in place, although how this compensatory mechanism works is yet to be defined.

The higher glucose uptake is indeed observed in the majority of tumor cells as originally found by Otto Warburg. However, impairment of mitochondrial function in cancer cells is still a controversial issue. These conflicting observations are perhaps due to the different experimental approaches including different tumor cell lines, culture methods (mostly monolayers) and assay procedures of mitochondrial functions. Rodríguez-Enríquez et al. recently addressed this question using spheroid tumor model (178). The authors showed that mitochondrial activities markedly decreased in a late stage of spheroid, whereas activity of glycolysis significantly increased with concomitant over-expression of HIF1. Therefore, the level of mitochondrial function appears to be dependent on the stage of tumor growth and location of each cell in tumor mass. The central region of tumor mass is often necrotic and hence under hypoxic condition which profoundly affects the balance between mitochondrial function and glycolytic flux. It is also known that stabilization of HIF1 α is induced by oncogenes such as Ras, Src and Myc followed by stimulation of aerobic glycolysis. In addition, expression level of H⁺-ATP synthase, a key enzyme of oxidative phosphorylation, was shown to be significantly decreased in lung cancer cells, and blocking the enzymatic activity promoted glycolytic flux (179). Furthermore, the inhibition of the same enzyme enhanced cell survival by attenuating ROS which is known to control apoptosis (180), while augmentation of mitochondrial metabolism induced suppression of tumor growth (181). Fantin et al. also showed that enhancement of mitochondrial metabolism by inhibiting LDHA diminished tumorigenicity of cancer cells (182). These results suggest that mitochondrial function is likely to be intact in tumor cells; however, the level of their activities is heavily dependent on their microenvironment, mainly on availability of oxygen in the tumor mass, and that different tumor cells adapt different survival strategies and energy metabolism even in the same tumor mass by changing the balance between glycolysis and oxidative phosphorylation.

Re-programming of metabolic pathways in cancer stem cell is another important aspect of recent tumor biology and has critical therapeutic implication. Are the metabolic genes already mutated in cancer stem cells? If so, when and how does it occur? How do they affect the abilities of self-renewal and differentiation of the cancer stem cell? What are the roles of niche or microenvironment in the metabolism of the tumor stem cell at both primary and metastatic sites? Answers to these questions are virtually unknown at present; however, understanding the underlining mechanism of metabolic re-programming in cancer stem cell may provide important clues for novel therapeutic targets. It should be noted that cancer stem cell is likely to be responsible for chemo-resistance according to the recent stem cell theory. Therefore, elucidating these questions may also provide us with a tool to overcome the problem of chemoresistant cancers.

Identifying small chemicals to specifically intervene in metabolic pathways and inhibit the function of metabolic genes is considered to be a promising approach to develop a novel type of anti-cancer drug, and it is under active investigation. Some of these compounds, such as 2-DG for glycolysis and CALAA-01 for nucleotide metabolism, are already in clinical trials. For lipogenesis, FAS is particularly an attractive target because of its specific expression in various types of cancers and blocking this enzymatic function is known to induce tumor cell apoptosis. However, considering the balancing and compensatory mechanism of each pathway in cancer

cells, simultaneous blocking of multiple pathways instead of targeting a single gene is likely to be a more effective approach. Because the metabolic genes are mostly up-regulated at an early stage of cancer, they are also considered to be ideal targets for chemo-prevention which is by far the most cost effective way to fight cancer. Some of the metabolic genes and their products are also likely to serve as useful diagnostic tools, and FAS and PGI are promising examples. Perhaps more aggressive proteomics approach using serum and urine from a cohort of patients with various cancers may identify better diagnostic markers of metabolic pathways.

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Fig. 1. Metabolic genes in cancer

GLUT1; glucose transporter 1, G6PD; Glucose-6-phosphate dehydrogenase, TKTL1; transketolase-like-1, RRM2; ribonucleotide reductase subunit M2, p53R2; p53-inducible ribonucleotide reductase small subunit 2 homolog, TYMS; thymidylate synthase, FH; fumarate hydratase, SDH; succinate dehydrogenase, IDH; isocitrate dehydrogenase, ACLY; ATP citrate lyase, ACC; acetyl-CoA carboxylase, and FAS; fatty acid synthase

	GEO ID	GLUT1	G6PD	TKTL1	PGI	ACLY	ACC	FASN	RRM2	TYMS	Sample number Normal / Tumor
Breast	GSE5364										13 / 183
	GSE10797										10 / 56
	GSE7904										7 / 43
	GSE8977										15 / 7
	GSE5847										47 / 48
	GSE9574										15 / 14
Prostate	GSE3744										7 / 40
	GSE8919		N/A								17 / 90
	GSE3325										6 / 7
	GSE3868										4 / 26
	GSE8956										18 / 71
	GSE5377		N/A		N/A						3 / 17
Lung	GSE5364										12 / 17
	GSE10072										49 / 58
	GSE7670										27 / 27
	GSE12428										28 / 35
	GSE8589			N/A	N/A	N/A	N/A	N/A			6 / 69
	GSE6044		N/A		N/A						5 / 39
Colon	GSE4115										73 / 79
	GSE2514		N/A								10 / 10
	GSE10950										24 / 24
	GSE6988										25 / 27
	GSE5206										5 / 100
	GSE5364										9 / 9
	GSE4183										8 / 45

Fig. 2. The expression profile of metabolic genes in clinical samples

The expressions of nine metabolic genes in four different types of cancers (breast, prostate, lung and colon) were examined using the GEO microarray database, and identity of each cohort was shown by GEO ID number. A closed box indicates significantly positive expression ($p < 0.05$), and the number of normal and tumor samples in each cohort was also shown in the right column. N/A; Not Available.

Fig. 3. Kaplan-Meier analysis of metabolic genes for breast cancer patients

Microarray data of 663 breast cancer patients from four independent cohorts were normalized and examined by meta-analysis for PGI, ACLY, RRM2 and TYMS. P value was calculated by log-rank test.

Table 1

Function of Gene	Up-regulation in Cancer (IHC)	Cell growth (<i>in vitro</i>)			Xenograft Tumor	Tumor in TG mouse	Secretion	Inhibitor	Reference
		Ectopic expression	Inhibition						
Glucose metabolism									
GLUT1	Lung, Colon, Ovary, Bile Duct	Promote	—	Promote	—	—	2-DG	14_16	
G6PD	Glucose-6-phosphate dehydrogenase	Promote	—	Promote	—	—	6-AN, BSO	17_18	
TKTL1	Tran sketolase	Promote	Arrest	—	—	—	siRNA, Oxythiamine chloride	19_20	
PGI/AMF	Glucose phosphate isomerasa/ Autocrine motility factor	Promote	Arrest	Promote	Promote	Serum, Urine	siRNA, E4P, M6P	21_22	
Lipid metabolism									
ACLY	Lung	—	Arrest	Promote	—	—	siRNA, SB-204990	23_25	
ACC1	Lung, Breast	—	Arrest	—	—	—	siRNA, TOFA	26_28	
FAS	Fatty acid synthase	Promote	Arrest	Promote	Promote	Serum	Cerulananin, Orlistat, C75, C93	29_46	
Nucleotide metabolism									
RRM2	Lung	—	Arrest	Promote	Promote	—	siRNA, GTI-2040	28_47_50	
p53R2	Lung	Promote	Arrest	—	Promote	—	siRNA	47_51_53	
TYMS	Lung, Breast, Colon, Cervical	Promote	Arrest	Promote	Promote	—	siRNA, ZD1694, 5-FU	54_57	
Mitochondrial metabolism									
SDHB,C,D	Succinate dehydrogenase	—	Promote *	Promote *	—	—	siRNA	58_59	
Fh1	Fumarate hydratase	—	ND	ND	—	—	siRNA	60	
IDH1	Isocitrate dehydrogenase	Promote **	—	—	—	—	siRNA, Oxalomalate	61	

* Tumor growth was measured in athymic nude mice implanted 143B human osteosarcoma or 143B SdhB shRNA clone. Inhibition of SdhB promoted the tumor growth *in vitro* and *in vivo*.

** Ectopic expression of IDHR132H mutant in U-87 MG cells elevated HIF-1 alpha expression which promoted tumor growth.

ND; No difference.