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A Metabolic Hypothesis of Cell Growth and Death in Pancreatic Cancer

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Introduction: Tumor cells, just as other living cells, possess the potential for proliferation, differentiation, cell cycle arrest, and apoptosis. There is a specific metabolic phenotype associated with each of these conditions, characterized by the production of both energy and special substrates necessary for the cells to function in that particular state. Unlike that of normal living cells, the metabolic phenotype of tumor cells supports the proliferative state.

Aim: To present the metabolic hypothesis that (1) cell transformation and tumor growth are associated with the activation of metabolic enzymes that increase glucose carbon utilization for nucleic acid synthesis, while enzymes of the lipid and amino acid synthesis pathways are activated in tumor growth inhibition, and (2) phosphorylation and allosteric and transcriptional regulation of intermediary metabolic enzymes and their substrate availability together mediate and sustain cell transformation from one condition to another.

Conclusion: Evidence is presented that demonstrates opposite changes in metabolic phenotypes induced by TGF-β, a cell-transforming agent, and tumor growth-inhibiting phytochemicals such as genistein and Avemar, or novel synthetic antileukemic drugs such as STI571 (Gleevec). Intermediary metabolic enzymes that mediate the growth signaling pathways and promote malignant cell transformation may serve as high-efficacy nongenetic novel targets for cancer therapies.

Key Words: Growth signaling pathways—Glucose intermediary metabolism—Pentose cycle-ribose synthesis—Cancer.

Decades of intensive research have identified many possible mechanisms for the development of common human malignancies including tumor-inducing genes, environmental factors, and signal transduction pathways. The genetic alterations reported to date in pancreatic cancer include frequent mutations of the K-*ras*, *p53*, *p16*, and *Smad4* genes, which were reported to be associated with accelerated disease progression and poor prognosis (1,2). Genetic abnormalities that influence cellular responses to hormonal growth regulators and their signaling pathways have been reported in connection with other major tumor types as well (3–8). The general understanding of malignant cell behavior currently emphasizes strong genetic regulation of human cell functions. It has been well established through molecular genetic studies that there is a great variance in the expression of human genes in response to environmental changes, nutrition, lifestyle, and age. The continued genetic characterization of human cancer reveals an ever more complicated architecture of potential cancer promoter and suppressor genes that show great variability and are extremely difficult to modulate. The diverse mechanisms of tumor induction have in common their resultant influence on metabolism-altering normal potential for differentiation, cell cycle arrest, and apoptosis (Fig. 1). In spite of their great genetic potential to express different phenotypes corresponding to the different degree of differentiation, tumor cells are uniformly characterized by a specific high glucose-utilizing metabolic phenotype and poor differentiation (9–11). Tumor cells use glucose primarily for intracellular anabolic processes, mainly the synthesis of nucleotides, whereas other anabolic reactions such as lipid and protein synthesis pathways are depleted of glucose carbons (12). It is our overall hy-

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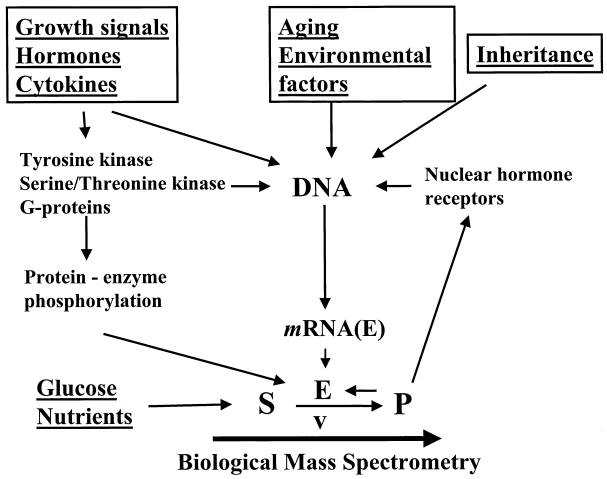

FIG. 1. The regulatory effect of cell growth-controlling signals follows two possible routes: they regulate metabolic enzymes through protein phosphorylation or through the transcription of genes. Glucose and nutrients, conversely, affect cell metabolism by altering substrate availability for various metabolic pathways. Glucose-P and nutrient components directly influence metabolism as allosteric regulators of metabolic enzymes (E). Metabolic activity is also influenced by substrate/product ratios and the regulatory effect of intracellular substrates (S) and products (P) on nuclear receptors. The overall metabolic effect of all regulatory pathways can be determined by biologic mass spectrometry after measuring the flux of glucose or other nutrient carbons through various metabolic pathways. The flow of carbons toward the synthesis of different macromolecules determines the metabolic phenotype of tumor cells. According to the availability of substrates and the activity of metabolic enzymes, tumor cells may undergo proliferation, differentiation, cell cycle arrest, and apoptosis as described in Table 2.

pothesis that the effects of growth signals and genetic factors that transform human cells from the resting state to the proliferating one are mediated by metabolic phenotype changes aimed toward supplying the rapidly dividing cells with an optimal source of carbons at the expense of cell differentiation.

In this article, we review the evidence for the hypothesis, delineating the conversion of growth signals into changes in metabolic pathway activity during the growth and death of pancreatic, lung, and epithelial malignancies.

CHARACTERIZATION OF METABOLIC PHENOTYPES IN TUMOR CELLS

A well-known characteristic of malignant cells is their ability to compete for glucose about 10 to 50 times more intensely than the surrounding normal tissue (9–11). This metabolic characteristic of tumors is used when the radioactive analog glucose tracer ^{18}fluoro-deoxy-glucose is detected by positron emission tomography (FDG-PET) for the diagnosis and classification of human malignancies. It has been demonstrated using PET that the increased rate of glucose accumulation in cancer cells strongly correlates with increased malignancy and invasiveness (13–16). Proliferating cells are highly dependent on de novo synthesis of purines and pyrimidines as well as ribose for nucleic acid synthesis. Glucose is a particularly important substrate from which tumor cells produce ribose, the backbone of RNA and DNA molecules. Before the advent of stable isotope and mass spectrometry, the use of glucose in tumor cells had been studied using enzymology methods. Simple studies of glucose uptake and oxidation can also be done using radioactive tracers.

The advantage of the stable isotope and mass spectrometry technique is in its ability to determine the quantity and position of label incorporation into biomolecules that are synthesized during cell cycle progression and proliferation by normal and tumor cells. Such capability was first demonstrated in the study of the glycogen synthesis pathway using uniformly labeled U^{13}C-glucose in experimental animals (17). In consequent studies, the analysis of label incorporation into glutamate for the purpose of sampling α-keto-glutarate pools provided the basis for the study of pyruvate dehydrogenase (PDH) and pyruvate carboxylase activity as well as the anaplerotic flux of the TCA cycle (18). Anaplerosis refers to the reactions that allow the entry of carbon into the TCA cycle intermediate pools other than via citrate synthase. Any carbon that enters the cycle as acetyl-CoA is oxidized to carbon dioxide and water; any carbon that enters the citric acid cycle via an anaplerotic pathway is not oxidized, but must be disposed of by some other route. Glutamate dehydrogenase is one possible route providing equilibrium between alpha-ketoglutarate and glutamate; some other reactions include pyruvate carboxylation, transamination reactions, and propionate carboxylation. Taking advantage of the knowledge of mass isotopomer distribution of a biomolecule, fatty acid synthesis can directly be studied using 1, 2–^{13}C-acetate or deuterated water (19). We recently have developed a method of using [1, 2-$^{13}C_2$]glucose for metabolic phenotyping studies that provides unique information on the carbon flow through the oxidative and nonoxidative branches of the pentose cycle by its labeling pattern in ribose. [1, 2-$^{13}C_2$]glucose symmetrically labels oxaloacetate when it is carboxylated via pyruvate carboxylase and carbon 2 and 3 of α-keto-glutarate of the TCA cycle. When it is converted to acetyl-CoA by pyruvate dehydrogenase, it labels carbon position 4 and 5 of α-keto-glutarate for the study of glucose oxidation and generates a mass isotopomer distribution pattern in fatty acid for the determination of the new fraction of lipid synthesis as

well as the ^{13}C enrichment of acetate in pancreatic adenocarcinoma cells (20).

Future studies using labeled fatty acids, glycerol, or ribose can be developed to further characterize the role of these substrates in different metabolic phenotypes. Such studies can reveal metabolic activity and the identification of structural and regulatory macromolecules that are synthesized using primarily glucose carbons in human cells (21,22). Extensive reviews and methodologic reports published in specialty journals have demonstrated the usefulness of the stable isotope method in combination with biologic mass spectrometry in mammalian cell metabolic studies to reveal glucose-derived specific synthesis pathways of nucleotides, lipids, and amino acids associated with tumor proliferation, differentiation, or apoptosis (23–25).

The stable isotope approach in combination with biologic mass spectrometry complements molecular genetic approaches, which measure the expression, transcription, and activation of metabolic enzymes. Although molecular genetic studies provide information on the regulatory action of genes, hormones, and signal transduction pathways, nutrient and substrate availability ultimately influence substrate distribution in tumor cells and determine cell functions (Table 1). The most measurable significance of directly characterizing substrate distribution in tumor cells is the ability to determine the metabolic phenotype characteristic of tumor cell proliferation and to characterize the overall metabolic effect of tumor growth-regulating agents.

METABOLIC PHENOTYPE CHANGES IN RESPONSE TO TUMOR GROWTH-PROMOTING AND INHIBITING SIGNALS

Many potential growth-modulating factors have been identified and characterized through their signal transduction pathways. They all fall into two major signal types: one, such as steroid hormones, that acts on intracellular receptors and influences gene expression and the other, such as transforming growth factors, that acts on cell surface receptors and influences multiple enzyme activities by protein phosphorylation. We have previously characterized the overall metabolic effect of transforming growth factor (TGF-β) on lung epithelial carcinoma cells, which uses the tyrosine kinase signaling mechanism through the cell surface TGF-β receptor family and promotes the invasive transformation of various human cells (26). The accumulation of glucose carbons in nucleic acid ribose demonstrated a significant, dose-dependent increase in response to this growth factor (Fig. 2). Concomitant metabolic changes in response to TGF-β treatment included decreased glucose oxidation in the pentose and TCA cycles, indicating that invasive cell transformation is accompanied by nonoxidative metabolic changes and increased glucose utilization toward anabolic metabolic reactions of nucleotides (27). This increase in the nonoxidative metabolism of glucose in the pentose cycle provided an explanation at the molecular level for the principal metabolic disturbance observed in human tumors: increased glucose uptake with increased glucose utilization for nucleic acid synthesis and decreased glucose oxidation. These metabolic changes also explain how tumor cells are capable of dividing rapidly in the hypoxic environment.

Recent evidence has indicated that genistein, the natural tumor growth-regulating agent found in soy bean, decreases glucose uptake and glucose carbon incorporation into nucleic acid ribose in MIA pancreatic adenocarcinoma cells (Fig. 3) (20). Genistein has marked tyrosine kinase- and protein kinase-inhibiting properties (28,29), resulting in cell cycle arrest (30) and limiting angiogenesis (31) in several tumor models. The opposite

TABLE 1. *Comparison of research methods generally used for the characterization of human cell function*

Investigative method	Research techniques	Target molecules	Specific information provided
Molecular biology	PCR hybridization sequencing	DNA, RNA	Genetic abnormalities, expression of genes, gene sequences
Proteomics	LC/MS	Peptides and proteins	Composition and structure of structural and enzyme proteins
Enzymology	Enzyme activity measurements	Enzyme proteins	Enzyme activities, substrate utilization and production, metabolic control characteristics of enzymes
Biological mass spectrometry	Stable isotope labeling	RNA, DNA, amino acids, lipids, all intermediates of metabolism	Substrate flux through metabolic pathways, identification and contribution of substrates to macromolecule synthesis

LC, liquid chromatography; MS, mass spectrometry.

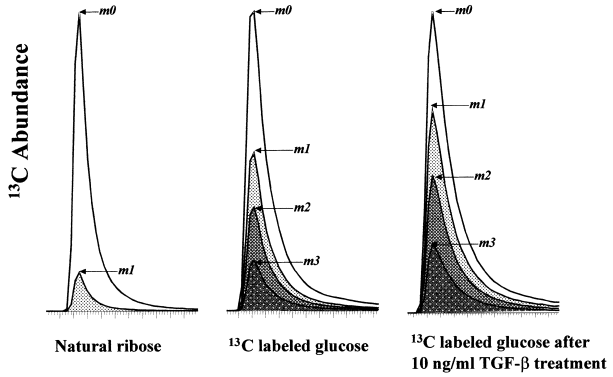

FIG. 2. Ribose mass spectral changes after TGF-β treatment (10 ng/mL) in cultures of H441 lung epithelial carcinoma cells. The chemical ionization mass spectral analysis reveals a significant increase in ^{13}C-carbon deposition into RNA from glucose after TGF-β treatment as shown by the increase in the shaded areas on the spectrum. Ribose mass spectra after chemical derivatization are detected at m/z 256 using biologic mass spectrometry, and mass isotopomers shown as m0, m1, m2, and m3 represent ribose molecules in RNA with 0, 1, 2, or 3 ^{13}C substitutions, respectively. Further details of the experimental procedures are described in reference 27.

changes in glucose carbon deposition into nucleic acid ribose, lactate, glutamate, and fatty acids after treating tumor cells with growth-promoting (27) or growth-inhibiting agents (20) indicate that glucose carbon redistribution between major metabolic pathways plays a critical role in the cell proliferation process.

METABOLIC HYPOTHESIS OF TUMOR GROWTH

Tumor cells, like other living cells, possess the potential for proliferation, differentiation, cell cycle arrest, and apoptosis. Associated with each of these conditions is a specific distribution of substrates between macromolecule synthesis pathways according to the metabolic needs of the given cell. Macromolecule synthesis pathways directly determine proliferating, differentiating, cell cycle arrest, and apoptotic phenotypes, which are characterized by different needs for the production of energy and substrates necessary for cells to function under different pathophysiologic conditions. It is clear from previous work on pancreatic, lung, and epithelial malignancies that invasive transformation is associated with characteristic metabolic changes. The typical metabolic phenotypes related to tumor cell formation and death are summarized in Table 2. Cell transforming agents, such as TGF-β and organophosphate pesticides, induce a severe imbalance in glucose carbon redistribution between cell proliferation-related and cell differentiation-related structural and regulatory macromolecules. This phenotype is characterized by increased glucose utilization specifically for nucleic acid synthesis through the nonoxidative branch of the pentose cycle with a concomitant decrease in glucose oxidation and the synthesis of glutamate, palmitate, and stearate directly from glucose. Conversely, agents that inhibit tumor growth such as genistein and the fermented wheat germ extract Avemar alter glucose utilization from a pattern typical of the proliferative phenotype to one of differentiated or apoptotic cells. Such evidence suggests the hypothesis that (1) cell transformation and tumor growth are associated with the activation of metabolic enzymes that increase glucose carbon utilization toward nucleic acid synthesis, whereas lipid and amino acid synthesis pathway enzymes are activated during tumor growth inhibition. (2) phosphorylation, allosteric regulation, and transcriptional regulation of intermediary metabolic enzymes and their substrates together mediate and sustain cell transformation from one condition to another. The effects of pathophysiologic and genetic factors that transform tumor cells from one functional state to another are mediated by metabolic phenotype changes aimed toward supplying the dividing cell with an optimal source of carbons at the expense of cell differentiation.

Figure 1 demonstrates the relation between extrinsic tumor growth-influencing factors and metabolic enzymes that are targets of growth-regulating signals. Tumor growth-modulating factors in the form of cell surface hormones bind cell-transforming factors that activate specific signal transducer pathways, resulting in activity changes of metabolic enzymes through either protein phosphorylation or genetic regulation. Superim-

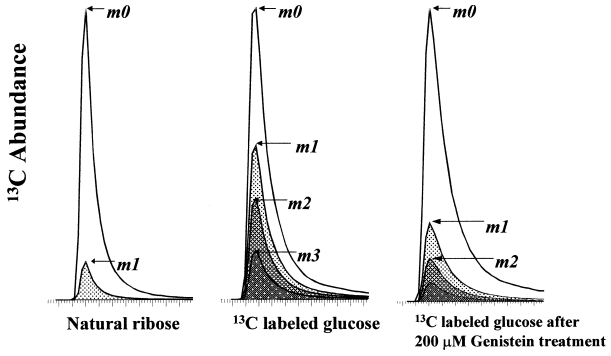

FIG. 3. Ribose mass spectral changes after genistein treatment (200 μM) in cultures of MIA pancreatic adenocarcinoma cells. The chemical ionization mass spectral analysis reveals a significant decrease in ^{13}C-carbon deposition into RNA from glucose after genistein treatment as shown by the decrease in the shaded areas on the spectrum. Ribose mass spectra after chemical derivatization are detected at m/z 256 using biologic mass spectrometry, and mass isotopomers shown as m0, m1, m2, and m3 represent ribose molecules in RNA with 0, 1, 2, or 3 ^{13}C substitutions, respectively. Further details of the experimental procedures are described in reference 22.

TABLE 2. *Metabolic phenotypes of tumor cells and their biologic characteristics*

Biologic phenotype and dominant cell cycle	Metabolic phenotype	Metabolic pathway and enzyme	Cell type; induction method; signaling pathway	Reference
Proliferating ("S" phase cycle dominance)	Distribution and continuous flow of glucose carbons through the nonoxidative steps of the PC for de novo DNA and RNA synthesis	Nonoxidative PC transketolase	H441 lung adenocarcinoma, K562 myeloid blasts; TGF-β or isofenphos pesticide; tyrosine/protein kinase activation	27
Differentiating ("G_0-G_1" phase cycle dominance)	Shift of glucose carbons to direct oxidation through G6PD and recycling of ribose carbons back into glycolysis; increased lipid and amino acid synthesis from the carbons of glucose	Oxidative pentose cycle, G6PD glycolysis, pyruvate kinase	MIA pancreatic adenocarcinoma; genistein, avemar, STI571; tyrosine/protein kinase inhibitors	20, 42
Cell cycle arrest ("G_0" or "G_2-M" phase cycle dominance)	Limited carbon flow through both the oxidative and the nonoxidative branches of the PC: limited RNA/DNA synthesis, limited NADPH production	Nonoxidative pentose cycle, transketolase; oxidative pentose cycle, G6PD	MIA pancreatic carcinomac, Ehrlich's ascites carcinoma in mice; oxythiamine, DHEA-S; no signal transducer pathways needed	39, 40
Apoptotic "G_0" cycle arrest	Limited glucose availability or direct inhibition of glycolytic, PC, or TCA cycle enzymes	Glycolysis, PC and TCA cycle	Fibroblasts, lymphoblasts, lung carcinoma; DOG or TNF-α; no signal transducer pathways needed	33, 34

PC, pentose cycle; DOG, 2-deoxy-D-glucose; TCA, tricarboxilic acid; TNF, tumor necrosis factor.

posed on this background, glucose, intracellular glucose metabolites, and various nutrients directly affect cell function in providing the substrates for intracellular synthetic reactions and energy production and as key allosteric regulators of metabolic enzymes. The metabolic characteristics of four distinctive phenotypes, namely proliferation, differentiation, cell cycle arrest, and apoptosis, that have been described in tumor cells in response to tumor growth-modulating treatments are summarized in Table 2. Tumor cells adapt to a high rate of glucose utilization and macromolecule synthesis. These processes, in turn, become highly dependent on the availability of glucose carbons and increased activity of glycolytic, pentose, and TCA cycle enzymes. As a feedback mechanism, the production of intermediary metabolites also regulates gene expression through intracellular nuclear receptors. Our proposed model is based on the direct determination of substrate flow through various metabolic pathways that control the oncogenic process under the influence of various signaling and growth regulatory events.

In support of our model, there are strong interactions between newly discovered signal transduction pathways and fundamental metabolic pathways such as glycolysis and the pentose cycle. Glucose deprivation is capable of inducing apoptosis of tumor cells on its own, even when other nutrients are plentiful (32,33). The c-*myc* oncogene directly regulates glucose transporter 1 and glycolytic gene expression in several tumor cells (34). Our hypothesis emphasizes that there is an apparent functional hierarchy within growth signaling, the translation of genes into proteins and enzymes that regulate various key metabolic pathways for macromolecule synthesis and energy production. The flow of information from the exterior of cells using specific signal transducer pathways and the flow of substrates to sustain these signaling events are key elements in the regulation of metabolism. These events also determine the optimum level of metabolic enzymes, which ultimately represent key regulatory check posts of carbon redistribution between major metabolic pathways. Metabolic enzymes control substrate flow according to the physiologic needs of a given cell as determined by its metabolic phenotype. The direct and indirect interactions between signal transducer pathways, substrates, and their intracellular target enzymes allow a fine regulatory mechanism in a functional hierarchy of genes and proteins, which together control cell events such as cell cycle progression, transformation, proliferation, hormone or enzyme secretion, differentiation, apoptosis, and growth. Accordingly, multiple genetic alterations and signaling pathways that cause tumor development directly affect glycolysis (35), the cellular

response to hypoxia (27), and the ability of tumor cells to recruit new blood vessels (36).

PRACTICAL IMPLICATIONS: THE HYPOTHESIS AT WORK

One of the consequences predicted by our hypothesis is that alteration of the proliferative phenotype is associated with specific metabolic changes as shown in Figure 4. It is well documented in the medical literature that the E7 oncoprotein, encoded by the oncogenic human papillomavirus (HPV) type 16, binds to the glycolytic enzyme type M2 pyruvate kinase (M2-PK). Pyruvate kinase exhibits a tetrameric form with a high affinity to its substrate phosphoenolpyruvate in normal cells and a dimeric form with a low affinity to phosphoenolpyruvate in tumor cells. As a result, tumor cells accumulate high levels of phosphorylated glycolytic metabolites to support nucleic acid synthesis at the expense of the carbon pools for lipid and amino acid syntheses. Investigations of HPV-16 E7 mutants and the nononcogenic HPV-11 subtype suggest that the interaction of HPV-16 E7 with M2-PK is linked to the transforming potential of the viral oncoprotein through metabolic adaptive changes (37). Another example involves the c-Myc oncogenic transcription factor, which regulates lactate dehydrogenase A and induces lactate overproduction. c-Myc, however, also controls other genes regulating glucose metabolism. In Rat1a fibroblasts and murine livers overexpressing c-Myc, the mRNA levels of the glucose transporter GLUT1, phosphoglucose isomerase, phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, and enolase were elevated. c-Myc directly transactivated genes encoding GLUT1, phosphofructokinase, and enolase and increased glucose uptake (38). These metabolites are necessary for nonoxidative nucleic acid synthesis (39) that can be precisely characterized using stable isotopes and GC/MS. Such an approach is an excellent screening method for studying the direct metabolic effects of new anti-tumor drugs or phytochemicals.

Another consequence of our hypothesis is that it is possible to alter the malignant phenotype by altering the metabolic functional capabilities of cancer cells. This principle can be applied to the development of new cancer treatment protocols and chemoprevention strategies for the ultimate benefit of patients. Cellular metabolism adapts to different cell events by specific glucose metabolic reactions. When this process that allows tumor cells to grow in an unlimited fashion is reversed by the inhibition of specific glucose metabolic enzymes, the malignant phenotype of the cells is also altered. One specific target site where such approach could bring new remedies for the treatment of cancer has been established within the nonoxidative reactions of the pentose cycle. Transketolase has been identified as the key enzyme in

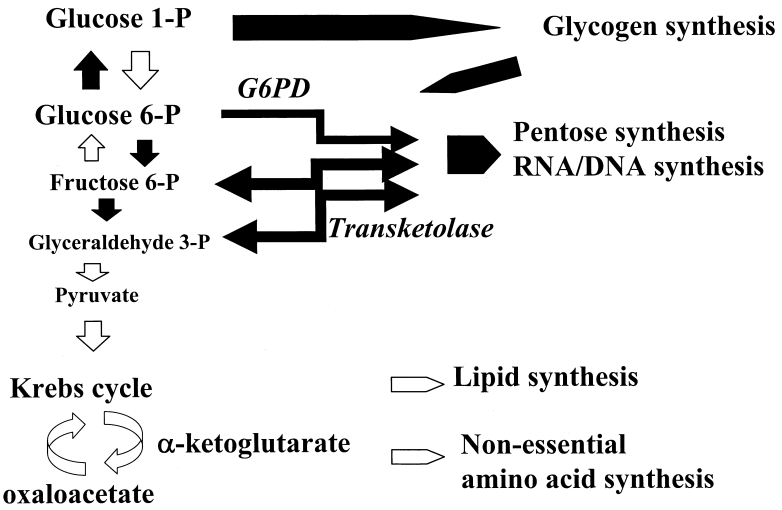

FIG. 4. The proliferating metabolic phenotype of tumor cells. This phenotype is characterized by a severe imbalance in glucose carbon redistribution between nucleotide, amino acid, and lipid synthesis pathways. Black-filled arrows represent increased flow of glucose carbons through pentose cycle reactions for the synthesis of nucleotides, and empty arrows represent a limited flow of glucose carbons for amino acid and lipid synthesis as well as oxidation. Undifferentiated cells are characterized by high rates of nucleic acid synthesis through the nonoxidative steps of the pentose cycle and the consequent decreased recycling of ribose carbons back into glycolysis. Continuous synthesis of cell membrane lipids and receptor proteins requires an optimal distribution of carbons through the TCA cycle and lipid synthesis pathways, which are inhibited by oncogenic metabolic changes. Inhibition of transketolase or G6PD results in cell cycle arrest, and the subsequent limited availability of glucose substrates for nucleic acid synthesis results in tumor cell apoptosis. Transketolase and G6PD of the pentose cycle represent prominent target sites for new anticancer strategies as demonstrated by Gleevec (46).

the regulation of glucose carbon recruitment for the de novo synthesis of nucleic acid ribose (40), and it also has an exceptionally high growth control coefficient in in vivo tumor proliferation. Because the blood plasma of mammalians contains only a very limited supply of five carbon sugars, it is inevitable that glucose recruitment for nucleic acid synthesis be one of the key metabolic regulatory steps where effective tumor growth control can be achieved. In previous studies, we successfully applied the chemically modified transketolase co-factor, oxythiamine, for the treatment of experimental cancer in animals (41). Oxythiamine treatment induced a dose-dependent arrest in the progression of the cell cycle in Ehrlich's tumor-hosting animals (42). Recent studies using novel tumor growth-inhibiting agents, such as the wheat germ extract Avemar for the treatment of human colorectal malignancies with advanced liver metastases (43–45) or STI571 (Gleevec) for the treatment of chronic myeloid leukemias (46), reveal strong inhibitory action on glucose use for nucleic acid synthesis as the central mechanism of anti-proliferative action.

CONCLUSIONS

Tumor cells assume their unique characteristics according to their diverse genetic aberrations. Their invasive and proliferative characteristics, however, are limited by the availability of substrates, nutrients, and metabolic pathway enzyme activities. Based on these factors, tumor cells exhibit distinct metabolic phenotypes determining the rate of proliferation, apoptosis, cell cycle arrest, and differentiation. Hormones, signaling pathways, environmental factors, and nutritional habits have a strong influence on these metabolic phenotypes. Understanding of adaptive metabolic changes in glycolysis and anabolic reactions in response to tumor growth-modulating agents is fundamental to the understanding of tumor pathophysiology in the pancreas. The proposed metabolic hypothesis of tumor cell growth and death permits a wide range of basic and clinical studies in developing new strategies to revert tumor-specific metabolic changes. Complex metabolic networks of key regulatory metabolic enzymes offer a large number of targets for direct intervention. This new class of metabolic regulators offers a potentially effective alternative to current gene-therapeuticals, chemotherapeuticals, and signal pathway regulators to achieve the same common endpoint effect of reducing cell proliferation through limiting glucose carbon use for nucleic acid synthesis.

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