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BRIEF REVIEW

Role of Thiamin (Vitamin B-1) and Transketolase in Tumor Cell Proliferation

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Abstract: *Metabolic control analysis predicts that stimulators of transketolase enzyme synthesis such as thiamin (vitamin B-1) support a high rate of nucleic acid ribose synthesis necessary for tumor cell survival, chemotherapy resistance, and proliferation. Metabolic control analysis also predicts that transketolase inhibitor drugs will have the opposite effect on tumor cells. This may have important implications in the nutrition and future treatment of patients with cancer.*

Introduction

Tumor cells have been shown to heavily utilize the nonoxidative transketolase pathway for ribose synthesis to build nucleic acid, in addition to the oxidative glucose-6-phosphate dehydrogenase (G-6-PD) pathway, the main ribose-producing reaction in the classical model of the pentose cycle (1–4). Metabolic substrate flux control coefficients of enzymes can be used to identify target sites that have maximal influence on the metabolic pathway flux, thereby assisting in the development of drugs that will have the highest possible efficacy (5). Transketolase not only predominates in the nucleic acid ribose synthesis process in mammalian cells but also has a remarkably high substrate flux control coefficient in liver cells and erythrocytes (approx 0.5) (6–7). It has recently been suggested that transketolase and its cofactor thiamin have a very high growth control coefficient in the Ehrlich tumor model (8). Therefore, modulation of transketolase has the potential to control substrate flow through the nonoxidative branch of the pentose cycle and control cell growth, a mechanism not yet targeted by anticancer drugs.

Metabolic control analysis predicts that stimulators of transketolase enzyme synthesis such as dietary thiamin support tumor cell survival and proliferation. Unfortunately, thiamin, a regulator of transketolase mRNA synthesis in the transketolase pathway and a cofactor of transketolase, is a common supplement in a wide variety of foods and is also given in high amounts to cancer patients as a prophylactic against nutritional deficiencies (9). Because of the crucial role played by thiamin in enabling a high rate of ribose synthesis in tumor cells, supplementation of the cancer patient's diet with thiamin may adversely affect cancer treatment.

Role of Pentose Cycle Reactions in Tumor Cell Nucleic Acid Ribose Synthesis—the Standing Model in Light of New Discoveries

The classical role of the pentose cycle in mammalian cells is the production of NADPH through carbon flow from C_6 sugars (hexoses) to C_5 sugars (pentoses). Pentoses are subsequently recycled back into glycolysis through carbon interchange reactions within the nonoxidative branch of the pentose cycle by transaldolase, transketolase, aldolase, and isomerases. The classic model of hexose-pentose-hexose cycling allows the oxidative reactions to produce only a limited amount of ribose in mammalian cells (1), and the contribution of the nonoxidative pathway has been difficult to determine. It is widely accepted that carbon interchange reactions are responsible for the labeling pattern of ribose isolated from nucleic acid, which nevertheless strongly indicates the involvement of transketolase in ribose synthesis of tumors by its labeling pattern on C-1 and C-5 with ^{13}C- or ^{14}C-labeled glucose as the tracer (2–4). The underlying assumption used by Katz and Rognstad (1) for modeling the pentose cycle in a comprehensive study of 1967 was that ribose production through the irreversible oxidative pathway

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significantly exceeds the amount of ribose deposited into nucleic acid on the basis of the RNA and DNA content of tumor cells. Following this assumption, on the basis of what was known in 1967, it was further reasonable to conclude that transketolase is not involved in net ribose synthesis in tumor cells, but in carbon exchange, which is responsible for the heavy labeling of C-5 of ribose in nucleic acid.

With the later discovery of continuously synthesized homologous nuclear RNA and nuclear ribosomal RNA precursors (half time 25 min) (10), it is clear that mammalian cell nucleic acid synthesis and turnover rates cannot accurately be estimated by the nucleic acid content of cells, as was assumed by Katz and Rognstad (1). It is now known that intron sequences are continuously cut out from RNA, then rapidly degrade during posttranslational processing, which reduces the actual RNA amount by ~100-fold in the measured RNA content in the cytoplasm. Therefore, it cannot be assumed that continuous RNA synthesis, processing, and turnover with continuous DNA repair observed in mammalian cells are totally accounted for by the oxidative reactions of the pentose cycle. It has been recently shown that the nonoxidative reactions of the pentose cycle play a major role in cell proliferation by producing 5-phosphoribosyl-1-pyrophosphate directly from glycolytic metabolites in eight different tumor cell lines (11). The recent report that poor prognosis was found in nasopharyngeal cancer patients with low G-6-PD enzyme activity in their tumors clearly indicates that transketolase has a major role in tumor cell physiology as well as in determining cellular response to tumor therapy (12). Oxythiamin, the chemically modified form of thiamin and a noncompetitive inhibitor of transketolase, decreases *in vitro* and *in vivo* tumor cell proliferation and ribose synthesis directly through the nonoxidative steps of the pentose cycle (3). Oxythiamin induces a prominent cell cycle arrest in the G_0-G_1 phase, as demonstrated in tumor-bearing mice (13). Despite the new facts about RNA/DNA synthesis and reports demonstrating the importance of transketolase in the ribose synthesis process of tumors that compromise many of the critical assumptions used by Katz and Rognstad (1), the original model of the pentose cycle has not been revisited to address the potential underestimation of ribose synthesis as well as the contribution of transketolase and its cofactor thiamin pyrophosphate (thiamin-PP) to net ribose synthesis.

Metabolic Control Analysis and Its Application to Tumor Cell Nucleic Acid Ribose Synthesis Pathways

Metabolic control analysis is a relatively new field in biochemistry in which the contribution of enzymes to the control of a pathway substrate flux is determined and expressed quantitatively by means of substrate flux control coefficients. Essentially, substrate flow control through linear metabolic pathways is distributed among enzymes; therefore, linearly connected metabolic reactions of the pentose cycle individually regulate hexose, pentose, and triose substrate flow. For each participating enzyme in the pathway, the contribution to substrate flow regulation can be measured and described in a control coefficient with a value between 0 and 1. Key enzymes with a strong regulatory effect (formerly rate-limiting steps) have flux control coefficients approaching 1; enzymes with weak regulatory potential have lower flux control coefficients (5). The effects of regulatory drugs on substrate flow are likely increased when they target enzymes with high flux control coefficients within the metabolic pathway.

Pentose cycle reactions have been characterized in terms of their flux control coefficients in liver tissue, erythrocytes, certain bacteria, and, very recently, in Ehrlich ascites tumor cells. In rat liver, it is determined that the enzymes that strongly regulate the oxidative path of the pentose cycle are glucokinase (EC 2.7.1.2) and G-6-PD (EC 1.1.1.49), with flux control coefficients achieving 0.6 and 0.42, respectively (6). In the same tissue, the nonoxidative pathway substrate flux control is dominated by transketolase (EC 2.2.1.1), with a flux control coefficient of 0.58. On the other hand, ribose phosphate isomerase (0.23), epimerase (0.14), transaldolase (0.08), and glucose phosphate isomerase (0.04) have limited strength in the regulation of carbon flow through the cycle. A similar substrate flux control role of transketolase in the nonoxidative pentose cycle (0.74) has been described in human erythrocytes (7). The growth control coefficient of transketolase in the Ehrlich tumor model was recently reported to be very high (14,15). The strong tumor growth control properties of transketolase and thiamin-PP make nonoxidative nucleic acid ribose synthesis not only a promising new target for cancer treatment but also a tumor growth-promoting site with a known mechanism of action.

A rational approach to inhibit pentose synthesis in tumor cells is to target key enzymes with high flow control coefficients within the pentose cycle. Accordingly, the oxidative pathway is best controlled through G-6-PD and the nonoxidative pathway through transketolase. Although G-6-PD effectively regulates the oxidative pathway, its role in ribose production is limited in tumor cells, and therefore G-6-PD inhibitors such as dehydroepiandrosterone or its derivatives do not accomplish their mission in experimental tumor therapy (3).

Transketolase function is strictly dependent on the presence of its coenzyme thiamin (vitamin B-1), which strongly binds to the enzyme protein. Thiamin has been described as a regulator of transketolase enzyme protein synthesis in normal and tumor cells (16,17). Consequently, excess thiamin has a major disadvantage in the nutrition and treatment of patients with cancer (9). The model of tumor cell ribose synthesis and the application of metabolic control analysis to its process indicate that excess thiamin, which promotes the synthesis of the transketolase enzyme protein and increases its activity, should be avoided. For example, if transketolase activity increases by 100% (doubled) because of increased thiamin intake, the rate of ribose synthesis and tumor cell growth will also increase by 58% (100% × 0.58, where 0.58 is the growth control coefficient of transketolase in human liver cells). If relative thiamin deficiency is present in tumor

cells with excess transketolase apoenzyme protein waiting for thiamin to arrive through the bloodstream, the regulating effect of thiamin on nucleic acid synthesis as the critical cofactor for the synthesis of ribose is even more dramatic. Thiamin deficiency has been described in experimental tumors (18) as well as in patients undergoing chemotherapy (19), and it is also commonly observed in patients with early stages of breast and bronchial carcinoma (20). Therefore, significant modifications are necessary in the treatment of cancer with greater emphasis on more rational and discriminating use of thiamin supplementation, while new transketolase inhibitor drugs need to be evaluated to limit transketolase activity and glucose use of tumor cells for nucleic acid synthesis and cell proliferation.

Tumor Cell Nucleic Acid Ribose Synthesis Pathways—Data Behind the Theory

Studies utilizing isotopically labeled glucose carbons recovered from *in vivo* hosted Yoshida tumors or *in vitro* cultured HeLa, Mia, H9, and Hep G2 cells unequivocally demonstrated that the nonoxidative part of the pentose cycle plays a significant role in tumor cell nucleic acid synthesis (1–4). More than 70% of nucleic acid ribose in these tumor cells was derived through transketolase, transaldolase, and triose phosphate isomerase reactions and only 10–15% was derived directly through the oxidative steps. In a recent experiment carried out in our laboratory, lung epithelial carcinoma (H411) cells demonstrated nucleic acid ribose synthesis almost entirely (99%) via the transketolase pathway, which indicates that there may be human tumors with poor prognosis that completely depend on transketolase and thiamin for *de novo* nucleic acid synthesis (20a).

Effect of Thiamin vs. Transketolase Inhibitors on Tumor Cell Nucleic Acid Synthesis and Proliferation

Although circumstantial links have been suspected between increased glucose utilization, pentose cycle activity, ribose synthesis, and tumor cell growth, few studies have directly addressed the mechanistic role of the oxidative and nonoxidative reactions in nucleic acid synthesis, cell transformation, and differentiation. Inasmuch as tumor growth is significantly stimulated during periods of nutrition support (21), the role of dietary supplements and the ramifications of thiamin supplementation to cancer patients are clinically relevant topics to address for scientists, dietitians, and care physicians who study, plan, and apply supportive treatments to cancer patients. Data in the medical literature lend wide support to the theory that thiamin-PP promotes mammalian cell nucleic acid synthesis and proliferation. Early reports from the late 1970s indicated that the reprogramming of the pentose cycle toward an increase in the nonoxidative path flux occurs in liver tumor cells (22). Liver tumor cells, but not regenerating or developing normal liver cells, showed a severalfold increase in 5-phospho-α-D-ribose-1-diphosphate synthase activity, which demonstrates the strong dependence of tumor cells on ribose phosphate production and nucleic acid synthesis (23). The intensive utilization of ribose and the salvage pathways for purine nucleotide synthesis have been demonstrated in leukemia cells of all four types (ALL, CLL, AML, and CML), which were rapidly saturated with low concentrations of 5-phosphoribosyl-1-pyrophosphate and less inhibited by the physiological feedback inhibitor adenosine 5′-monophosphate in culture (24). These findings were recently confirmed in human colon tumors and in chemically induced colon tumors in rats, where a severalfold increase in transketolase enzyme activity was demonstrated (25,26).

Oxythiamin, a thiamin derivative and noncompetitive inhibitor of transketolase, decreases DNA/RNA levels and proliferation of *in vivo* hosted Ehrlich tumor cells. Oxythiamin also reduced the ribose fraction in nucleic acid that arrived directly through the transketolase pathway in MIA pancreatic adenocarcinoma cells in culture. These changes in the nucleic acid ribose moiety were accompanied by a significant decrease in tumor cell proliferation in cultures of MIA cells as well as oxythiamin-treated mice hosting Ehrlich tumors (3). Decreased tumor growth was the result of a prominent G_1 cycle arrest without signs of direct tumor cell or host toxicity after oxythiamin treatment (13). On the other hand, thiamin increases the proliferation of human endothelial cells (27) and was shown to be trophic in neuroblastoma cell cultures (28). It has also been shown that substrate flux through the nonoxidative pentose cycle is rapid and large in cultured liver tumor cells compared with the oxidative reactions and glucose intake of the cell (29).

High-affinity thiamin transport deficiency in cultured fibroblasts results in the cessation of cell proliferation followed by increased cell death. Excess thiamin promptly rescues these cells and reestablishes normal cell proliferation in culture (30). High-affinity thiamin transporter protein deficiency clinically manifests with thiamin-responsive megaloblastic anemia, which points to DNA synthesis disturbances in proliferating bone marrow cells. Although the link between thiamin and DNA synthesis in erythropoietic cells has been known since 1969 (31), no studies in the medical literature have attempted to elucidate the mechanism by which thiamin directly affects DNA synthesis and cell proliferation. The fact that thiamin transporter protein-deficient cultured cells did not proliferate in the presence of glucose, which bypasses the thiamin-dependent steps in nucleic acid synthesis, demonstrates the limited contribution of G-6-PD to nucleic acid ribose synthesis and proliferation. It is apparent from these results that thiamin and ribose are equally important and required for rapid cell proliferation and that the metabolic control on this process is exerted by transketolase and thiamin-PP together. On the other hand, the limited synthesis of ribose phosphate accomplished through G-6-PD in normal cells makes future nonoxidative ribose synthesis inhibitors both selective and effective primarily on tumor cells.

Conclusions

This review highlights the crucial role of transketolase and its cofactor thiamin-PP in nonoxidative ribose synthesis, which predominates in all tumor cells studied so far. The strong involvement of the transketolase pathway in tumor cell nucleic acid synthesis is not emphasized in medical textbooks, although it is a crucial process in tumor cell nucleic acid synthesis, proliferation, and growth. Nutritional supplements and prepared food additives that include thiamin need to be reevaluated for cancer patients and for the population as a whole in countries with high cancer rates. The possible role of thiaminase, the natural thiamine-degrading enzyme found abundantly in fish, meat, and vegetables, which seems to be beneficial for cancer patients, needs further investigations. The varying cancer rates between countries where diets containing natural thiaminases are regularly consumed in the form of raw or fermented fish, vegetables, or roasted insects (Asia and Africa) compared with those in countries where thiamin-enriched food products are preferred (United States and Western Europe) need to be explored.

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