Review Article

Alteration of cellular metabolism in cancer cells and its therapeutic prospects

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Abstract

Transformation of a normal cell into a cancerous phenotype is essentially backed by genetic mutations that trigger several oncogenic signaling pathways. These signaling pathways rewire the cellular metabolism to meet the bioenergetic and biomass requirement of proliferating cell, which is different from a quiescent cell. Although the change of metabolism in a cancer cell was observed and studied in the mid-20th century, it was not adequate to explain oncogenesis. Now, equipped with a revolution of oncogenes, we have a genetic basis to explain the transformation. Through several studies, it is clear now that such metabolic alterations not only promote cancer progression but also contribute to the chemoresistance of cancer. Targeting specific enzymes and combinations of enzymes can improve the efficacy of cancer therapy and help to overcome the therapeutic resistance.

Keywords: Cancer cell, metabolic alterations, therapeutics

INTRODUCTION

The relationship between cancer and altered cellular metabolism has been deciphered decades ago. However, the importance of tumor metabolism has dwindled over the past due to limited knowledge of tumorigenesis. It's only after the oncogenic revolution, the interest in tumor cell metabolism and signaling pathways is being renewed, and presently metabolic reprogramming is considered as a hallmark of cancer.[1]

The three basic requirements of cancer to sustain are rapid growth and proliferation of cancer cells, the capability of the tumor cells to evade the normal apoptotic pathways, thus favoring survival and unrestricted entry of the tumor cells into cell cycle progression even in the absence of growth signals. All these requirements are met by the tumor cell by acquiring a phenotype as a result of various host cell mutations that combine to alter the metabolic pathways. Many of these adaptations are also seen in rapidly proliferating normal cells, in which they represent appropriate response to physiological growth signals as opposed to constitutive cell autonomous adaptations.[2,3] In case of cancer, this adaptability should also work in a stressful microenvironment which is deficit in nutrients.[4]

Energy and biomass requirement of proliferating cells are more than that of normal cells. In tumor cells when nutrients are abundant, the requirements are met by reprogramming pathways to increase acquisition and

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utilization of nutrients, and in case of nutrient stress, the metabolism is rewired to compensate for the scarcity of one nutrient by filling the metabolite pool with another nutrient.[5-7]

In this review, we will discuss the alterations in cellular metabolism that the transforming cells undergo to sustain and proliferate in all nutrient conditions. Further, how these alterations can produce opportunity to target specific enzymes or combination of enzymes will also be discussed.

NUTRIENT SUFFICIENCY STATE

In the presence of abundant nutrient, oncogenic RAS stimulates the uptake and utilization of glucose[8,9] by activation of PI3K pathway [Figures 1 and 2].[10] PI3K pathway is one of the most commonly altered pathways in many human cancers. Apart from oncogenic RAS, this pathway is also activated by mutation in the tumor suppressor gene PTEN[11-13] or by aberrant signaling

from receptor tyrosine kinase.[14] The activation of PIK3 in turn activates the downstream effectors AKT1 which strongly stimulates signaling through mammalian target of rapamycin (mTOR) by inhibiting tuberin.[15] At molecular level, mTOR indirectly causes metabolic changes by activating transcription factors such as hypoxia-inducible factor 1 (HIF1). HIF1 is a heterodimer that is stabilized in hypoxia.[16] However, it can also be activated in normal oxygen concentration by oncogenic signaling pathways such as PI3K.[17,18] HIF1, once activated, amplifies transcription of gene encoding glucose transporter (GLUT1) and most glycolytic enzymes, increasing influx of glucose into cell.[19]

High expression of MYC collaborates with HIF1 inactivation of glucose receptors and glycolytic enzymes.[20,21] Increased MYC also enhances glutamine uptake and metabolism.[22,23] Glutamine has an added advantage of providing its two nitrogen atoms for biomass synthesis.[24]
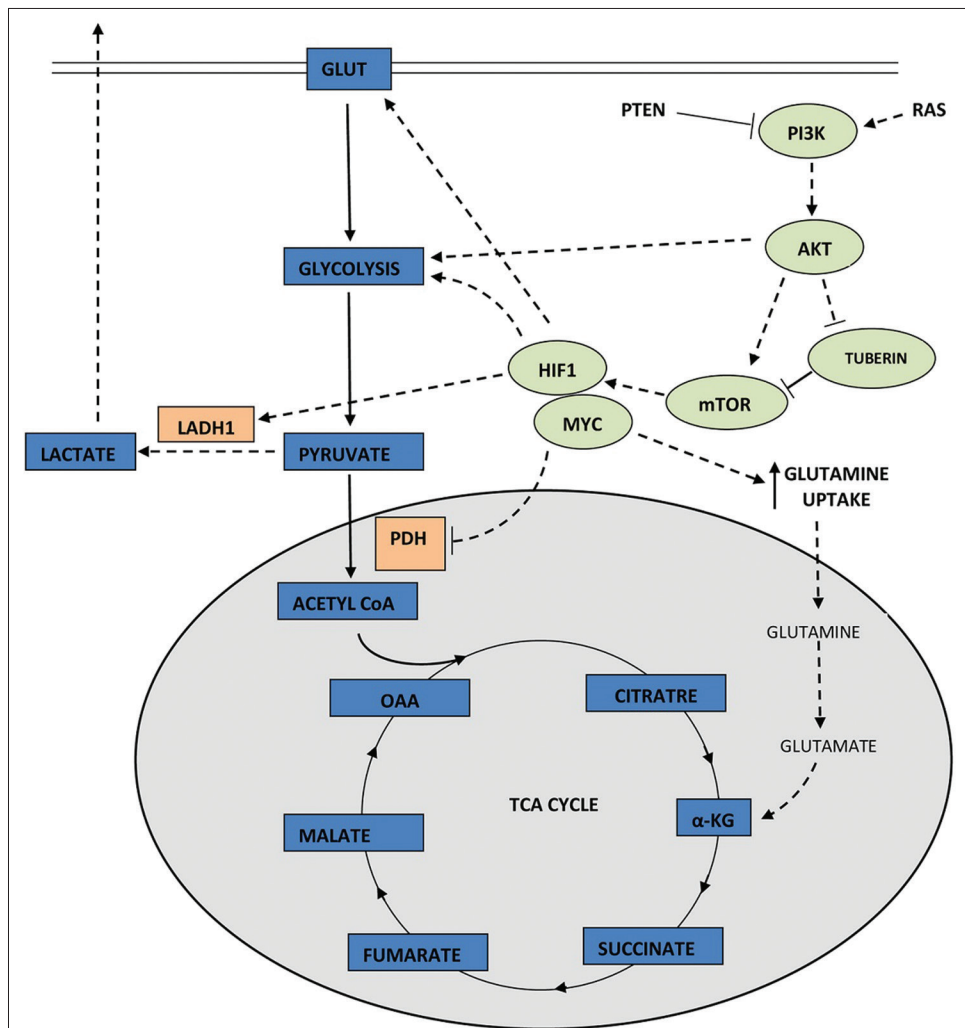
In terms of adenosine triphosphate (ATP) generation,

Figure 1: Glucose metabolism (represented by solid lines) in a quiescent cell
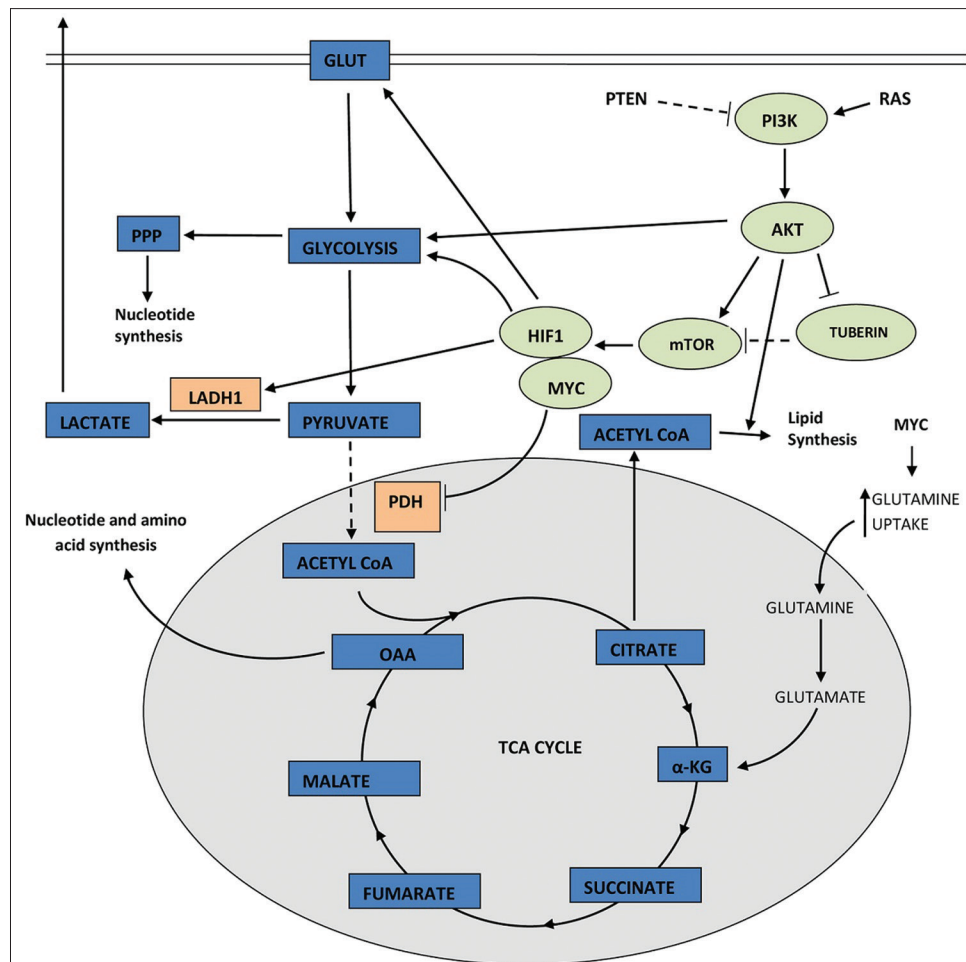
Figure 2: Genetic mutations of gene such as RAS and PTEN can trigger several oncogenic signaling pathways such as PI3K and mammalian target of rapamycin that in turn upregulate glucose transporters and enzymes that catalyze various steps of glycolysis, thus favoring aerobic glycolysis

one of the most characteristic metabolic alterations occurring in tumor cells is shift from ATP generation through oxidative phosphorylation to ATP generation through glycolysis, even in the presence of normal oxygen concentration (Warburg effect).[25] Although the ATP generation by this pathway is faster, it is inefficient in terms of number of ATPs produced per glucose molecule. Earlier it was hypothesized that this shift might an adaptation to defective mitochondria.[26] However, it was later appreciated that mitochondrial defects are rare,[27-29] and oxygen consumption of tumor cells remains same as that of normal cell. The faster rate of ATP production by this pathway can justify such switch, but this is possible only in an environment where nutrient supply is surplus. The most recent theory regarding the switch believes that aerobic glycolysis provides a biosynthetic advantage for tumor cells by a high flux of substrate through glycolysis.[2,3]

AKT1, which is a downstream effector of PI3K activation, is an important driver of tumor glycolytic phenotype. It phosphorylates and activates glycolytic enzymes such as hexokinase and phosphofructokinase 2.[15,30]

The subsequent activation of HIF1 decreases the flow of glucose-derived pyruvate into tricarboxylic acid (TCA) cycle[31-33] by activation of pyruvate dehydrogenase kinase which inactivates mitochondrial pyruvate dehydrogenase complex. Oncogenic MYC activates lactate dehydrogenase A that catalyzes the conversion of pyruvate to lactate.[20,21] Recently, ^{13}C-nuclear magnetic resonance spectroscopy measurements have shown that glioblastoma cells in culture convert as much as 90% of glucose and 60% of glutamine they acquire into lactate or alanine.[34]

ADAPTATION TO NUTRIENT STRESS

For most of the mammalian cells in culture, glucose and glutamine are the only two molecules that are catabolized appreciably to meet most of the energy and biomass requirement of cell.[2] Tumor cell deprived of these nutrients are supposed to die of starvation. On the

contrary, nutrient deprivation has been correlated with poor survival of patients[35] suggesting that scarcity of nutrient makes the cancer cell stronger. This may be attributed to the biochemical alterations leading to acquisition of necessary plasticity of cancer cells that is required to reprogram metabolism [Figure 3] in response to different nutritional conditions.

In most of the cancer cells, oxaloacetate (OAA) in TCA cycle is supplied by glutamine which compliment with acetyl-CoA from glucose. In case of glucose deprivation, carbon from glutamine has been seen to be rerouted to acetyl CoA in some cancers.[5] Similarly, glutamine deficiency can induce metabolic pathway changes. One of such changes is a loss of citrate synthase. Citrate synthase condenses OAA to acetyl-CoA to maintain TCA cycle.[35] However, in scarcity of glutamine, OAA is shunted toward asparagine formation to support cell survival.[36] Expression of asparagine synthetase has been seen to be associated

with poor prognosis of glioma and neuroblastoma,[37] suggesting that maintenance of asparagine pool may provide an advantage to tumor cells.

In RAS expression, cancer, glutamine stimulates macropinocytosis.[38] In this process, extracellular matrix is captured and internalized. This allows starving cell to generate pools of glutamine and other amino acids supply to TCA cycle.[38] This process must be highly controlled as hyperactive macropinocytosis can lead to cell death in a process previously misidentified as autophagic cell death.

Along with scavenging on extracellular matrix, autophagic degradation of macromolecules is also active in cancer cells.[39-42] During autophagy, the organelles and the macromolecules are degenerated to produce small molecule nutrients to feed intermediary metabolism.[38,41,43] This process has been seen to be crucial in tumor growth and survival of cancer cells in some RAS driven tumor.[44]
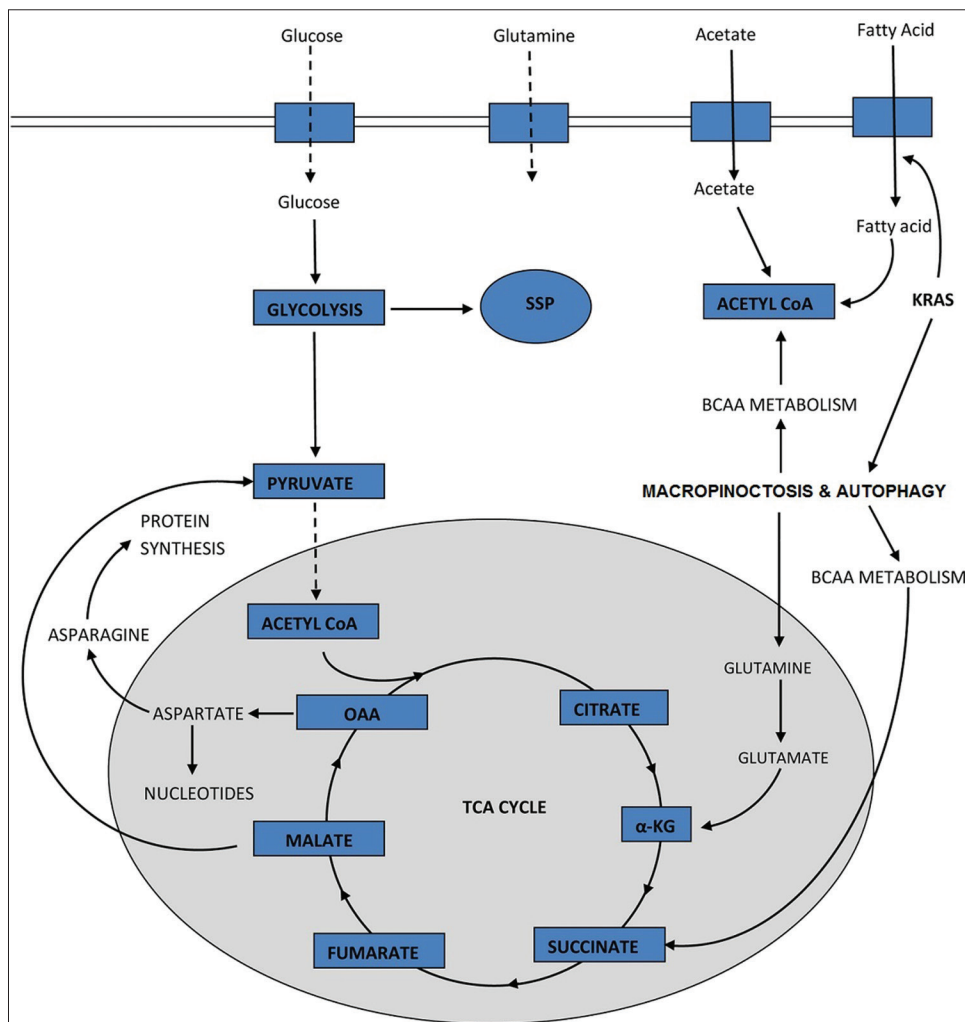

Figure 3: Reprogramming of metabolism in cancer cells, deprived of glucose and glutamine (reduced intake represented by dotted lines). Pool of amino acids and tricarboxylic acid cycle intermediates, required are maintained by activating pathways that promote autophagy, macropinocytosis and scavenging fatty acids

In the presence of oxygen and abundant nutrients, cell synthesizes fatty acids *de novo*.[45,46] However, under nutrient stress, scavenging extracellular lipids become an adaptive mechanism for the cell.[47,48] Scavenging instead of synthesizing spares the cell from the need to supply carbon to pentose phosphate pathway (PPP) for NADPH production which is required for lipid synthesis. In case of ovarian cancer, a cooperative mechanism exists between stromal cell and cancer cell. The stromal cells have been found to provide fatty acid to tumor cells. When ovarian cancer cells were cocultured with adipocytes, the transfer of fatty acid from adipocytes to tumor cells triggered activation of adenosine monophosphate-activated protein kinase and fatty acid oxidation leading to enhanced cell proliferation.[49]

Deprivation of glucose or glutamine leads to activation of serine synthesis pathway (SSP) in tumor cells.[50] By this pathway, serine and glycine can be synthesized from glutamine by a process "reverse glycolysis."[51] Serine driven one carbon metabolism produces reducing equivalent (NADPH) with a comparable importance to PPP.[52] Serine binding has also been related to activation of PKM2 enzymatic activation.[53] M2 is an isoform of pyruvate kinase which is present in self-renewing cells such as embryonic and adult stem cells.[54] It is found to be expressed in many tumor cells and therefore might be a useful biomarker for early detection of tumors.[4,55-57] Unlike the other isoform PKM1, it is usually found inactive and is inefficient at promoting glycolysis.[58-60] Due to its nature of inhibiting Warburg effect and ATP production, which is unfavorable for tumor progression, presence of PKM2 in cancer cells was ignored for several years. However, recent work has produced evidences that PKM2 exerts a regulatory contribution to SSP.[61] Further work is required to understand the regulatory cascade completely.

Acetate is one of the smallest molecules available as nutrient in mammals. It is converted to acetyl-CoA by acetyl-CoA synthetase. Although acetate has a very low concentration in circulating fluid,[62] it can be taken up by tumor cells and oxidized.[63] However, the primary role of acetate utilization is still to be evaluated.

Depending on the tumor microenvironment, the tumor cells rewire their metabolism to ultimately direct the available nutrient into the synthesis of new biomass while maintaining adequate level of ATP for survival.

THERAPEUTIC PROSPECTS

Over the past few decades, hundreds of genes have been identified that are mutated in cancer. Many of these genetic alterations that are known to promote cancer lead to a single converging metabolic phenotype that is characterized by reorganization of metabolic pathway in such a way that biosynthesis of macromolecules and ATP production to support cell survival are well balanced.[26,64,65] As all cancer cells are dependent on this alteration of metabolism, these altered pathways represent attractive therapeutic targets.[66,67] Further, effective agents targeting many of the common driver mutation in cancer are not available. For example, mutation of RAS or dysregulated expression of MYC is frequent events in human cancer, yet no specific therapies exist to treat cancers based on either genetic event, and many RAS-driven cancers are nonresponsive to existing therapies.[68,69] RAS-mutant cells are dependent on sufficient glucose uptake,[70] and MYC-dependent cells have a particular reliance on glutamine metabolism.[22,23,71] Small molecule inhibitors that disrupt glucose intake and metabolism has been found to decrease the growth of tumors that are derived from cells driven by these oncogenes in preclinical models.[70,72,73] Targeting metabolism may also be synergistic with many of the existing therapies such as kinase inhibitor and cytotoxic therapies which act by impairing glucose metabolism.[2,74,75]

Cancer development, progression and treatment outcomes are linked to whole body metabolism. Obesity, hyperglycemia and insulin resistance are all associated with an increased risk of developing cancer and worse clinical outcomes in patients suffering from cancer.[76-80] Insulin and insulin-like growth factor, which are capable of activating signaling pathways that drive cell growth, are increased in circulation of individuals suffering from obesity and insulin resistant. This suggests that obesity and insulin resistance promote cancer at least in part by activating pathways that drive cell growth.[77] These same signaling pathways also drive nutrient uptake into cells.[78] Further, elevated levels of glucose alone can promote increased glucose uptake in some cells, and lower glucose levels are seen to be associated with better cancer treatment outcomes.[81-84] Hence, antidiabetic drugs are being explored for anticancerous activity. Retrospective clinical studies have shown decreased cancer mortality rate in patients on metformin.[85,86] Metformin lowers level of glucose and insulin by inhibiting gluconeogenesis.[87] Other antidiabetic drugs which act by other mechanisms such as increasing the level of insulin in blood may worsen the clinical outcome in cancer.

Glutamine is an important nutrient source for cancer cell, and some cancers are addicted to glutamine. This increased reliance of some cancers on glutamine makes glutamine a prospective therapeutic target. Small molecules such as 2-amino-(2,2,1)-heptane-2-carboxylic acid that inhibits glutamine transporters have been shown

to slow proliferation and tumor growth.[88,89] Another potential therapeutic target is glutaminase, the enzyme that catalyzes the conversion of glutamine to glutamate. The growth of transformed cells can be selectively inhibited by targeting glutaminase activity.[90,91] Molecules such as bis-2-(5-phenylacetamido-1,2,4-thiodiazol-2-yl) ethyl sulfide have been shown to successful in inhibiting glutaminase.[91,92]

Although biochemistry and metabolism of transforming cells are extensively studied for the past few decades, targeting cancer metabolism for therapy of cancer still remains as a challenge. As normal proliferating cells have same metabolic requirements as cancer cells, so finding a therapeutic window is difficult. Sometimes, it is assumed that a therapeutic window is obtained by chemotherapeutic agents because cancer cell proliferates more rapidly than normal cells. However, this is not always true. Proliferative cells of gut can have cell cycle as frequently as 10 h[93,94] and hematopoiesis in human can generate 2 million red blood precursors per second.[95] Like the cancer cells, rapidly proliferating cells of immune system rely on aerobic glycolysis[96-98] and glutamine metabolism.[99,100] Another challenge in targeting cancer metabolism is the metabolic flexibility of the transformed cell. Cancer cells often have a remarkable ability to shift fuel source when deprived of favored metabolic pathways.[101-103]

CONCLUSION

The present cancer therapeutics that target DNA synthesis are not found to be promising because instead of a single tumor-specific metabolism, several metabolic programming exists that promotes proliferation of cancer cell. A better understanding of how metabolism is altered in specific genetic contexts that lead to cancer will guide to formulate strategies to target specific enzyme or combination of enzyme in cancers.

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Conflicts of interest
There are no conflicts of interest.

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