**Metabolism of tumor cells**

Tumor is a genetic disease that involves changes in the genome and consists of several complicated events. Changes that directly transform a cell into a tumor cell include **the loss of function of tumor suppressor genes**, **the enabling function of oncogene activation**, and **other mutations that affect gene stability**. Especially mutations of genes responsible for mitotic recombination and chromosomal segregation. The main characteristics that a cell needs to become a malignant (tumor) cell: **unlimited replicative potential**, **angiogenesis**, **avoidance of apoptosis**, **independent growth** (does not depend on growth signals), **non-responsiveness to anti-growth signals**, **tissue invasion** and **metastasis**. These characteristics are the subject of intensive study. In addition to these six, two new features appear: **avoiding the immune system and reprogramming the energy metabolism**. Besides energy, tumor cells require building block molecules, NADPH and NADH cofactors for housekeeping, growth and proliferation in a changing microenvironment. Tumor cells undergo metabolic reprogramming, which involves changes in the metabolic fluxes, to satisfy large demands for ATP, NADPH, NADH and carbon skeletons. Replication requires the cell to duplicate its genome, proteins, and lipids in order to divide into daughter cells. Increased proliferation of tumor cells requires the reorganization of metabolic pathways (tumor cell metabolism).

Tumor metabolism has been studied even before the discovery of oncogenes and tumor suppressor genes. Metabolic activities are altered in tumor cells compared to non-tumor (normal) cells. Changes in the metabolism of tumor cells enable the acquisition and maintenance of malignant properties. Knowing how to reprogram metabolism that later benefits tumor characteristics and how to use metabolic changes for therapeutic purposes is a key issue in this field of oncology. Increased rates of glycolysis with high lactate production in tumors were noted by Otto Warburg. Tumor cells can reprogram their metabolism based on changes in the microenvironment (acidity and availability of substrates and oxygen). Tumor cells enhance glycolysis and glutaminolysis to meet their ATP and NADPH requirements, respectively. Fatty acid and nucleotide biosynthesis mainly uses glucose as a carbon source. Krebs cycle is mainly filled with glutamic carbons, for which mitochondria are essential. A better understanding of metabolic reprogramming may lead to the identification of important checkpoints that may aid in diagnosis or may be therapeutic targets. Altered metabolic activity enables anabolic growth during nutrient-replete conditions, catabolism enabling cell survival during nutrient-limited conditions, and strengthening of redox homeostatic systems to counteract the metabolic effects of oncogene activation, loss of tumor suppressor genes, and other stresses. Detecting and evaluating changes in metabolic activities, makes possible to predict tumor development and provide opportunities to prevent tumor progression. One of the first examples of changes in the metabolism of tumor cells is the Warburg effect or aerobic glycolysis. Physiological reaction to hypoxia in normal tissues is glycolysis. Otto Warburg observed that tumor cells constitutively take up glucose and produce lactate, regardless of oxygen availability, this allows the high metabolic demands of proliferating cells to be met.

*Figure 1. Metabolism of normal and tumor cells*

Despite the genetic and histological heterogeneity of tumors, malignancy utilizes the anabolism, catabolism, and redox balances implied for basic cell functions. The general activation of these pathways may reflect their regulation by signaling pathways that are commonly disrupted in tumor cells. Normal cells upon stimulation with growth factors, activate phosphatidyl-inositol 3-kinase (**PI3K**) consequently downstream **AKT** pathway and the mammalian target of rapamycin (**mTOR**), thus initiating a strong anabolic program that involves increased glycolytic flux and fatty acid synthesis through the activation **of hypoxia-inducing factor-1 (HIF-1)** and **sterol regulatory element-binding protein** **(SREBP).** Tumor cells often have mutations that enable strong activation of PI3K-AKT-mTOR signaling that is minimally dependent on growth factors. A large number of well-characterized oncogenes and tumor suppressor genes utilize this signaling. Another tumor possibility, a dysregulated **MYC** gain-of-function pathway enabled by chromosomal translocations, gene amplification, and single nucleotide polymorphisms. Control of the MYC oncogene results in the expression of many genes that enable anabolic growth, fatty acid synthesis, glutaminolysis, serine metabolism, and mitochondrial metabolism. **Kras** oncogene, frequently mutated in lung, colon and pancreatic cancers, can take over the physiological functions of PI3K and MYC and thus affect tumorigenicity. Tumor suppressor genes, such as **p53**, can regulate metabolism. The TP53 gene that codes for p53 may be missing or mutated in half of cancers. As we already know, tumor suppressor p53 participates in cell cycle arrest, DNA repair, aging, apoptosis, but also loss of p53 will increase glycolytic flux and thus promote anabolism and redox balance, thus facilitating tumorigenesis.

Tumor cells are in hypoxia, up to 2% oxygen, due to rapid proliferation that always exceeds angiogenesis. Metabolic adaptation to hypoxia is established by the HIF-1 protein, which induces genes involved in increasing glycolytic flux. Some tumors constitutively activate HIF-1 by different mechanisms:

* Hyperactivation of mTORC1
* Loss of VHL gene (Von Hippel–Lindau, tumor suppressor gene)
* Accumulation of ROS
* Accumulation of succinate and fumarate TCA cycle metabolites, caused by SDH (succinate dehydrogenase) and FH (fumarate hydratase) mutations.

Coordinated induction of metabolic pathways that support tumorigenesis through a combination of deregulation of PI3K-AKT-mTOR signaling pathways, loss of tumor suppressors and activation of oncogenes reduces the need for mutations or amplifications in metabolic enzymes. This is why we rarely find examples of metabolic enzyme deregulation by genetic mutations.

One rare example is increased expression of **phosphoglycerate dehydrogenase** **(PHGDH)**. PHGDH catalyzes the conversion of the glycolytic intermediate 3- phosphoglycerate to 3-phosphohydroxypyruvate in the first step of the serine biosynthesis pathway. Serine metabolism supplies methyl groups to the one-carbon and folate pools contributing to nucleotide synthesis, methylation reactions, and **NADPH** (reduced nicotinamide adenine dinucleotide phosphate) production. Inhibiting serine biosynthesis by silencing PHGDH in cells with high levels of this enzyme results in growth suppression, and the enzyme displays oncogenic properties in gain of function assays. Other examples of mutational deregulation of metabolic enzymes are those that generate oncometabolites.

Otto Warburg’s hypothesis that cancer cells take up glucose and generate a substantial amount of lactate in the presence of ambient oxygen due to impaired mitochondrial function led to the widely held misconception that cancer cells rely on glycolysis as their major source of ATP. Cancer cells exhibit aerobic glycolysis due to activation of oncogenes, loss of tumor suppressors, and up-regulation of the PI3K pathway, and that one advantage of high glycolytic rates is the availability of precursors for anabolic pathways. Warburg’s observation that tumors display a high rate of glucose consumption has been validated in many human cancers with fluorodeoxyglucose positron emission tomography, which uses a radioactive glucose analog to image glucose uptake in tumors and adjacent normal tissue. Nevertheless, many studies have demonstrated that the great majority of tumor cells have the capacity to produce energy through glucose oxidation (the process by which glucose-derived carbons enter the TCA cycle and are oxidized to CO2, producing ATP through oxidative phosphorylation). Furthermore, limiting glycolytic ATP production by inhibiting the activity of pyruvate kinase fails to prevent tumorigenesis, suggesting that the major role of glycolysis is not to supply ATP. Moreover, mitochondrial metabolism is necessary for cancer cell proliferation and tumorigenesis. Thus, despite their high glycolytic rates, most cancer cells generate the majority of ATP through mitochondrial function, with the likely exception of tumors bearing mutations in enzymes involved in mitochondrial respiration (for example, SDH and FH). Nevertheless, cells harboring mutations in FH or SDH still rely on mitochondrial metabolism by metabolically rewiring themselves to provide the necessary TCA cycle intermediates and ROS for cell proliferation. In addition to pyruvate derived from glycolysis, fatty acids and amino acids can supply substrates to the TCA cycle to sustain mitochondrial ATP production in cancer cells. Breakdown of fatty acids (β-oxidation) in the mitochondria generates acetyl-CoA and the reducing equivalents NADH and FADH2, which are used by the ETC to produce mitochondrial ATP. The amino acid glutamine can generate glutamate and subsequently [α](https://en.wiktionary.org/wiki/%E1%BD%A5%CF%81%CE%B1#Ancient_Greek)-ketoglutarate to fuel the TCA cycle through a series of biochemical reactions termed glutaminolysis. Furthermore, the BCAAs isoleucine, valine, and leucine, which are elevated in plasma of patients with pancreatic cancers, can be converted into acetyl-CoA and other organic molecules that also enter the TCA cycle. The metabolic flexibility afforded by multiple inputs into the TCA cycle allows cancer cells to adequately respond to the fuels available in the changing microenvironment during the evolution of the tumor. A combination of the local tumor microenvironment and oncogenic lesions is likely to dictate the fuel used by mitochondria to sustain tumor growth.

Solid tumors have significant heterogeneity of perfusion, many tumor cells are found in environments that are not rich in nutrients and oxygen, so they have to adapt to such conditions. They have developed multiple mechanisms to maintain mitochondrial functions. For example, the mitochondrial ETC is able to function optimally even at an oxygen concentration of only 0.5%. Tumor cells under hypoxic conditions (<2% O2) can use glutamine for oxidative ATP production. Kras-driven pancreatic cancer cells under conditions that are not rich in nutrients use proteins scavenged from the extracellular space to produce glutamine and other amino acids to fuel the TCA cycle for cell survival and growth. Under condition not rich in nutrients, tumor cells reduce their demand for ATP, adapting to the microenvironment.

**mTOR** kinase drives the energy-demanding growth of tumor cells. mTOR kinase is inhibited when amino acid and oxygen levels decrease. Decreased mTOR activity increases autophagic flux. In oncogenic Kras- or Braf-driven non–small-cell lung cancer (NSCLC) cells, autophagy provides an intracellular glutamine supply to sustain mitochondrial function. To survive the hypoxic tumor microenvironment, cancer cells also diminish their demand for ATP by decreasing highly demanding ATP-dependent processes, such as running the Na/K-dependent adenosine triphosphatase. If diminishing ATP demand is not sufficient to maintain ATP/ADP ratio, the rise in ADP activates adenylate kinase, a phosphotransferase enzyme that buffers the fall in ATP levels by converting two ADP molecules into adenosine 5´-monophosphate (AMP) and ATP. The rise in AMP during nutrient deprivation triggers the activation of AMP kinase (AMPK), which activates catabolic pathways like fatty acid oxidation to stimulate ATP production. In conditions of metabolic stress, certain Ras-driven cancer cells scavenge lipids to support ATP production. Ovarian cancer cells use fatty acids from neighboring adipocytes to fuel mitochondrial ATP production.

*Figure 2. Metabolic pathways under nutrient-replete and nutrient-deprived conditions*

Biosynthetic or anabolic pathways are an essential aspect of cancer metabolism because they enable cells to produce the macromolecules required for replicative cell division and tumor growth. As a general theme, these pathways involve the acquisition of simple nutrients (sugars, essential amino acids…) from the extracellular space, followed by their conversion into biosynthetic intermediates through core metabolic pathways like glycolysis, PPP (pentose phosphate pathways), TCA cycle (Krebs Cycle), and nonessential amino acid synthesis, and finally the assembly of larger and more complex molecules through ATP-dependent processes. The three macromolecular classes most commonly studied in cancer metabolism are proteins, lipids, and nucleic acids, which comprise approximately 60, 15, and 5% of the dry mass of mammalian cells, respectively. Evidence indicates that biosynthesis of all three classes is under the control of the same signaling pathways that govern cell growth and are activated in cancer via tumorigenic mutations, particularly PI3K-mTOR signaling.

Protein biosynthesis is strictly regulated and requires access to essential and non-essential amino acids. Tumor cells and other cells under growth factor signaling express surface transporters that allow them to take up amino acids from the extracellular space. Thus, protein synthesis is ensured, as well as the activity of the mTOR signaling pathway, primarily mTORC1. mTORC1 is stimulated by the presence of amino acids and activates protein synthesis by acting on translation and ribosome biogenesis. Tumor cells take up glutamine and convert it into glutamate, which they use to synthesize non-essential amino acids. In the absence of nutrients, the cell has access to a large number of catabolic pathways to break down macromolecules. Intracellular proteins and other macromolecules are degraded by autophagy. Macropinocytosis allows cells to internalize proteins and other components of the extracellular milieu and deliver them for degradation via the endocytic pathway.

Tumor cells rapidly produce fatty acids for membrane synthesis, lipidation reactions, and signaling. Fatty acid synthesis requires acetyl-CoA and reducing power in the form of cytosolic NADPH. Transcription of genes involved in fatty acid synthesis is regulated by the transcription factor SREBP-1. SREBP-1 regulates not only the enzymes needed to convert acetyl-CoA into fatty acids but also the enzymes of the PPP and pathways required to convert acetate and glutamine into acetyl-CoA. This transcription factor therefore regulates genes encoding proteins that catalyze or facilitate fatty acid synthesis. In cancer cells with constitutively high rates of fatty acid synthesis, additional mechanisms help keep SREBP-1 in a transcriptionally active state. mTORC1 signaling, via its effector **S6 kinase (S6K)**, activates a transcriptional program that includes both SREBP-1 and the related protein SREBP-2, which regulates transcription of genes in sterol biosynthesis. Both SREBP-1 and SREBP-2 are required for mTORC1-mediated cell proliferation. Fatty acids and lipids can also be obtained from the extracellular space, for membrane synthesis. PI3K signaling activates fatty acid uptake and suppresses fatty acid oxidation.

Purine and pyrimidine nucleotides are required for synthesis of RNA and DNA. The purine and pyrimidine bases are constructed from various nonessential amino acids and methyl groups donated from the one-carbon/folate pool. The TCA cycle contributes oxaloacetate, which is transaminated to aspartate, an intermediate required to synthesize both purine and pyrimidine bases. Conversion of ribonucleotides to deoxynucleotides by ribonucleotide reductase requires a source of NADPH. Well-characterized mechanisms of feedback inhibition exist to prevent excessive accumulation of nucleotides, and mutations interrupting these mechanisms can produce pathological accumulation of nucleotide intermediates (for example, precipitation of uric acid crystals in gout). Clearly, nucleotide biosynthesis is a targetable vulnerability in cancer cells because nucleoside analogs and antifolates have been a mainstay of chemotherapeutic regimens for decades.

**Redox balance**

ROS are intracellular chemical species that contain oxygen and include the superoxide anion (O2−), hydrogen peroxide (H2O2), and the hydroxyl radical (OH·). The mitochondria and cytosolic NADPH oxidases (NOXs) produce O2− from the one-electron reduction of oxygen. O2− is converted into H2O2 by the enzymatic activity of superoxide dismutase 1 or 2, which are localized to the cytosol or mitochondrial matrix, respectively. H2O2 is subsequently detoxified to water by the enzymatic activity of mitochondrial and cytosolic peroxiredoxins (PRXs), which, as a consequence, undergo H2O2-mediated oxidation of their active-site cysteines. Thioredoxin (TXN), thioredoxin reductase (TrxR), and the reducing equivalent NADPH reduce oxidized PRXs to complete the catalytic cycle. Glutathione peroxidases (GPXs) can also convert H2O2 to water in the mitochondrial matrix and cytosol through H2O2-mediated oxidation of reduced glutathione (GSH). Glutathione reductase (GR) and NADPH reduce oxidized glutathione (GSSG) back to GSH. Additionally, catalase, an abundant antioxidant in peroxisomes, can detoxify H2O2 to water without any cofactors. However, in the presence of ferrous or cuprous ions, H2O2 can become OH· and quickly cause the oxidation of lipids, proteins, and DNA, resulting in cellular damage. NADPH is required to maintain multiple antioxidant defense systems. The cytosol has multiple sources of NADPH generation, including the oxidative PPP, malic enzyme 1, IDH1, and one-carbon metabolism. NADPH generation in the mitochondria, in part, is controlled by one-carbon metabolism and IDH2.

ROS have been thought of as lethal metabolic byproducts of cellular respiration and protein folding. However, studies over the past two decades have unveiled a previously underappreciated role of ROS in cellular signaling. Low levels of ROS, particularly H2O2, can reversibly oxidize the cysteine residues of proteins to positively regulate cell proliferation and cellular adaptation to metabolic stress. As H2O2 levels increase, however, cell death signaling pathways are initiated, and H2O2 is converted to OH·, which can directly damage DNA, proteins, and lipids. Cancer cells have an increased rate of spatially localized mitochondria- and NOX-dependent ROS production compared to normal cells. This allows for the proximal activation of signaling pathways [PI3K and mitogen-activated protein kinase/extracellular signal–regulated kinase (MAPK/ERK)] and transcription factors [HIF and nuclear factor kB (NF-kB)] necessary for tumorigenesis. The cancer cell–specific increased rate of spatially localized ROS production is due to a combination of oncogenic lesions and the tumor microenvironment. The activation of oncogenes, PI3K signaling pathway induction, and hypoxia (low-oxygen levels) stimulate the increased rate of ROS production from the mitochondria and NOXs in cancer cells. Thus, mitochondria-targeted antioxidants and NOX inhibitors can prevent cancer cell proliferation, hypoxic activation of HIF, tumorigenesis, and metastasis.

The increased localized ROS in cancer cells, which activates signaling pathways and transcription factors to promote tumorigenesis, needs to be buffered from reaching levels of ROS that incur cellular damage by the increased expression of antioxidant proteins. Thus, cancer cells have higher levels of ROS scavenging enzymes than normal cells, preventing ROS-mediated activation of death-inducing pathways like c-Jun N-terminal kinase (JNK) and p38 MAPK and oxidation of lipids, proteins, and DNA, resulting in irreversible damage and cell death. One mechanism by which cancer cells increase their antioxidant capacity is by activating the transcription factor nuclear factor (erythroid derived 2)–related factor-2 (**NRF2**). Specifically, NRF2 is activated following disruption of the interaction of NRF2 with its binding partner Kelch-like ECH-associated protein 1 (KEAP1). Critical cysteine residues within KEAP1 can undergo oxidation, succination, and glutathionylation, thereby inhibiting the KEAP1-NRF2 interaction, leading to the proteasomal degradation of NRF2. Additionally, NRF2 activation can occur independently of KEAP1. Once activated, NRF2 induces the transcription of many antioxidant proteins including GPXs and TXNs as well as enzymes involved in GSH synthesis and cysteine import through the cysteine/glutamate antiporter. Furthermore, to maintain the antioxidant capacity of GPXs and TXNs, NADPH is required. NRF2 plays an important role in activating enzymes that increase cytosolic NADPH levels. NRF2 also regulates the serine biosynthesis pathway, generating NADPH in the mitochondria, which is critical for redox balance under hypoxic conditions. Therefore, inactivating NRF2 or disabling antioxidant proteins in cancer cells would allow for the accumulation of excessive amounts of ROS to levels that initiate toxicity and reduce tumorigenesis. During tumorigenesis and metastasis, redox homeostasis is required. An emerging model of redox balance is that as a tumor initiates, the metabolic activity of cancer cells is increased, resulting in an increase in ROS production and subsequent activation of signaling pathways that support cancer cell proliferation, survival, and metabolic adaptation. Accordingly, to prevent toxic levels of ROS, tumor cells increase their antioxidant capacity to allow for cancer progression. The harsh tumor microenvironment increases ROS levels due to hypoxia, and the low glucose levels limit flux through the cytosolic oxidative PPP, thus decreasing cytosolic NADPH levels. Cells in these nutrient-deprived conditions activate AMPK to increase NADPH levels by stimulating PPP dependent NADPH and diminishing anabolic pathways, such as lipid synthesis, that require high levels of NADPH. ROS-dependent signaling and increased mitochondrial respiration are also necessary for tumor metastasis. However, when tumor cells detach from a matrix, they encounter high levels of ROS that incur cellular damage and require activation of adaptive ROS-mitigating pathways to survive and grow. The ability to up-regulate antioxidant proteins and increase flux through NADPH-producing metabolic pathways enables distant metastasis to occur. These findings suggest that perhaps disabling antioxidant capacity in cancer cells to raise ROS levels might be beneficial in preventing metastasis.

**Therapy based on a tumor metabolism**

Inhibition of some metabolic enzymes is likely to be systemically toxic because of their physiological functions in normal tissues. Normal proliferating cells, such as immune cells and stem cells, also reprogram their metabolism in a manner similar to cancer cells. Metabolic inhibitors should likely not interfere with the adaptive immune system. Enhanced nucleotide and DNA synthesis in tumor cells is targeted by antifolates (methotrexate and others). One approach is to target a metabolic enzyme in a deregulated pathway specific to cancer cells. Many of the genetic and pharmacologic interventions on metabolic enzymes have been conducted using human cancer cells subcutaneously injected into athymic mice. An emerging theme is that cancer cells display metabolic plasticity and can alter their metabolic profile during the course of tumorigenesis and metastasis. Thus, it is conceivable that cancer cells could develop resistance to inhibition of a particular metabolic pathway by expressing alternate protein isoforms or up-regulating compensatory pathways. Therefore, a rational cancer therapeutic strategy should involve targeting multiple metabolic pathways simultaneously or targeting a particular metabolic pathway in combination with therapies against oncogenic or signaling pathways.

Glycolysis was an early attractive target for cancer therapy given the clinical observation that many tumors exhibit a significant increase in glucose uptake compared with adjacent normal tissue. LDH-A, a metabolic enzyme that converts pyruvate (the final product of glycolysis) to lactate, was identified as the first metabolic target of the oncogene MYC. Genetic or pharmacologic inhibition of LDH-A has been shown to diminish MYC-driven tumors in xenograft models. Inhibition of LDH-A leads to the regression of established tumors in genetically engineered mouse models of NSCLC (Non-small cell lung cancer) without systemic toxicity. Genetic ablation of LDH-A also delays the progression of myeloid leukemia. Increased expression of LDH-A, specifically in MYC-mutant cancer cells, may prove to be an attractive target. Another potential therapeutic target is the glycolytic protein HK2. Many tumor cells overexpress HK2, and preclinical mouse models of genetically engineered NSCLC and breast cancer demonstrate that HK2 inhibition delays tumor progression. Lactate can inhibit cytotoxic T cells, so LDH-A inhibition may cooperate with immune checkpoint inhibitors to unleash host inflammatory T cells that will specifically attack tumor cells. Lactate can also reprogram macrophages to promote tumorigenesis.

Another potential glucose-dependent target is PHGDH, an enzyme in the de novo serine synthesis pathway. High levels of PHGDH have been found in a subset of human melanoma and breast cancers, and these cancer cells require PHGDH for their growth in vitro. Serine starvation in mice diminishes tumorigenicity of p53-null cancers. De novo synthesis or exogenous uptake of serine can enter the mitochondria where SHMT2 converts it into glycine to generate folate intermediates. In many cancer types, SHMT2 expression is elevated and correlates with a poor prognosis. Furthermore, the transcription factors MYC and HIF induce SHMT2 under hypoxia to promote survival. Currently, it is not known whether targeting PHGDH, SHMT2, or other enzymes in the one-carbon metabolism pathway would be effective in delaying or regressing tumor progression in genetically engineered, PDX, or syngeneic mouse models of cancer without incurring systemic toxicity. However, given the importance of one-carbon metabolism in supporting the anabolic needs of cancer cells and its up-regulation in cancer cells, it is likely that this pathway is needed for in vivo tumor progression.

Mitochondrial metabolism has also emerged as a key target for cancer therapy, in part, due to the revelation that the antidiabetic drug metformin is an anticancer agent. Numerous epidemiological studies first suggested that diabetic patients taking metformin, to control their blood glucose levels, were less likely to develop cancer and had an improved survival rate if cancer was already present. Laboratory based studies have also provided evidence that metformin may serve as an anticancer agent. Biochemists recognized that metformin reversibly inhibits mitochondrial complex I. Recent studies indicate that metformin acts as an anticancer agent by inhibiting mitochondrial ETC complex I. Specifically, metformin inhibits mitochondrial ATP production, inducing cancer cell death when glycolytic ATP levels diminish as a result of limited glucose availability. Metformin also inhibits the biosynthetic capacity of the mitochondria to generate macromolecules (lipids, amino acids, and nucleotides) within cancer cells. The remarkable safety profile of metformin is due to its uptake by organic cation transporters (OCTs), which are only present in a few tissues, such as the liver and kidney. Certain tumor cells also express OCTs to allow the uptake of metformin. However, in the absence of OCTs, tumors would not accumulate metformin to inhibit mitochondrial complex I. Ongoing clinical trials using metformin as an anticancer agent should assess the expression levels of OCTs to identify the tumors with highest expression, which are likely to be susceptible to metformin. Moreover, it is not clear whether the current antidiabetic dosing of metformin used in clinical trials allows for metformin accumulation to levels necessary to inhibit mitochondrial complex I in tumors. Thus, it is possible that metformin at doses higher than those currently used for diabetes might be more efficacious without causing toxicity. Like metformin, the biguanide phenformin exhibits anticancer properties by inhibiting mitochondrial complex I.

Another potential therapeutic strategy to inhibit mitochondrial metabolism in certain tumors would be to use autophagy or glutaminase inhibitors. Autophagy provides amino acids, such as glutamine, that fuel the TCA cycle in NSCLC and pancreatic cancers, and short-term autophagy inhibition has been shown to decrease tumor progression without incurring systemic toxicity in mouse models of NSCLC. Some tumors are addicted to using glutamine to support TCA cycle metabolism even in the absence of autophagy; thus, glutaminase inhibitors can reduce tumor burden in these models. An alternative approach is to target acetate metabolism. Although a major function of the mitochondria is to provide acetyl-CoA to the cell, cancer cells can also use acetate to support cell growth and survival during metabolic stress (hypoxia or nutrient deprivation). The cytosolic enzyme acetyl-CoA synthase 2 (ACCS2), which converts acetate to acetyl-CoA, is dispensable for normal development; thus, ACCS2 is a promising target of acetate metabolism. ACCS2 knockout mice do not display overt pathologies, but genetic loss of ACCS2 reduces tumor burden in models of hepatocellular carcinoma. Human glioblastomas can oxidize acetate and may be sensitive to inhibitors of this process. Thus, targeting metabolism with inhibitors of autophagy, acetate metabolism, and other pathways that supply key metabolic intermediates may be efficacious in some contexts.

Because mitochondrial inhibitors are unlikely to be effective cancer therapies as single agents, combination therapy is likely the best approach. For example, the use of metformin with the current clinical PI3K inhibitors, which reduce glucose uptake and glycolysis, is one approach that would impair both sources of ATP within cells. Targeted therapies against oncogenes such as KRAS, BRAF, and NOTCH1 kill a large majority of cancer cells but ultimately yield resistant cells that exhibit an increased sensitivity to inhibitors that impair mitochondrial metabolism. Cancer-initiating cells also have increased sensitivity to mitochondrial inhibitors, adding further evidence that inhibiting mitochondrial metabolism may suppress tumor recurrence.

To balance the increased production of ROS, during tumorigenesis and metastasis, tumor cells must increase their antioxidant capacity. One of the approaches in therapy can thus be targeting redox metabolism, selectively targeting the antioxidant capacity of tumor cells, which would result in an increase in ROS levels and consequent elimination of tumor cells. The reducing equivalent of NADPH is necessary for the maintenance of antioxidant systems. The cytosol has multiple sources of NADPH generation, including the oxidative PPP, malic enzyme 1, IDH1, and one-carbon metabolism. By contrast, NADPH generation in the mitochondria is controlled in part by one carbon metabolism and IDH2. Many of these NADPH-generating systems are critical for normal cell survival and function. Nevertheless, there are two NADPH-generating systems that may serve as potential therapeutic targets. It is estimated that 400 million people worldwide are deficient in **G6PDH**, an enzyme in the oxidative PPP that converts NADP+ to NADPH. However, certain tumors rely on this pathway as a major source of cytosolic NADPH; therefore, it may be therapeutic to disable this pathway and induce oxidative stress and diminish tumor growth. Moreover, RNA profiling of metabolic enzymes identified the mitochondrial one-carbon metabolism protein **MTHFD2**, which can generate NADPH, as being highly expressed in 19 different cancer types but not in normal adult proliferating cells. Loss of MTHFD2 in cancer cells increases ROS levels and sensitizes the cells to oxidant-induced cell death in vitro. An interesting approach to depleting NADPH levels and increasing ROS is to administer high doses of **vitamin C** (ascorbate). Vitamin C is imported into cells through sodium-dependent vitamin C transporters, whereas the oxidized form of vitamin C, **dehydroascorbate** (**DHA**), is imported into cells through glucose transporters such as GLUT1. When the cell takes up DHA, it is reduced back to vitamin C by glutathione (GSH), which consequently becomes GSSG. Subsequently, GSSG is converted back to GSH by NADPH-dependent GR. Because the blood is an oxidizing environment, vitamin C becomes oxidized to DHA before being taken up by the cell. Thus, high doses of vitamin C diminish the tumorigenesis of colorectal tumors that harbor oncogenic KRAS mutations and express high levels of GLUT1 by depleting the NADPH and GSH pools and consequently increasing ROS levels to induce cancer cell death. Vitamin C administered at high doses intravenously is safe in humans and, in conjunction with conventional paclitaxel-carboplatin therapy, demonstrated a benefit in a small number of patients. Additional strategies to diminish GSH include the administration of buthionine sulfoximine, an irreversible inhibitor of g-glutamylcysteine synthetase, which can be safely administered to humans and is efficacious in preclinical tumor models. Moreover, glutathione is a tripeptide consisting of cysteine, glutamate, and glycine. Thus, decreasing glutamate levels using glutaminase inhibitors or diminishing cysteine levels by preventing extracellular cysteine (two linked cysteine molecules) uptake can also raise ROS levels in cancer cells to induce cell death.

It must also be considered that stem cells are also sensitive to ROS levels. To determine which antioxidant pathways are upregulated due to high ROS production in tumor cells. Many tumors use the NRF2 pathway to maintain redox balance, targeting this pathway may provide a therapeutic opportunity. Superoxide dismutase 1 (SOD1) is overexpressed in NSCLC, inhibition of SOD1 eliminates human NSCLC cells and reduces tumor cell burden in mouse models of NSCLC. Knockout mice that lack the genes for NRF2 and SOD1 develop normally, so short-term inhibition of these pathways may be an effective way to eliminate tumor cells.