Published in final edited form as:

Nat Biotechnol. ; 30(7): . doi:10.1038/nbt.2285.

Therapeutic targets in cancer cell metabolism and autophagy

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Abstract

The metabolism of cancer cells is reprogrammed by oncogene signaling and/or mutations in metabolic enzymes. These metabolic alterations support cell proliferation and survival, but leave cancer cells dependent on continuous support of the nutrients that fuel their altered metabolism. Thus, in addition to core oncogenic pathways, many metabolic enzymes have become targets for novel therapies. Two novel processes- isoform-specific expression of metabolic enzymes and autophagy- have recently been shown to play critical roles in the adaptation of tumor cells to changes in nutrient availability and the cell's ability to sense and adapt to depletion of critical nutrients. These findings suggest that a better understanding of the molecular basis of cancer-associated metabolic changes has the potential to provide insights to enhance cancer therapy.

In the past decades, the development of genomic screening has contributed to the identification of many oncogenes and tumor suppressors which are frequently altered in a variety of tumors. A significant portion of these oncogenic abnormalities are associated with growth signaling pathways. Recently, increasing evidence has suggested that growth signaling pathways directly control cell metabolism, growth and proliferation by regulating metabolic enzymes. Furthermore, individual metabolic enzymes have been reported to be mutated or amplified during tumor progression. Since most cancer cells display and rely on enhanced nutrient utilization, this feature can potentially be explored from a therapeutic perspective. Understanding how metabolic pathways are altered in tumors and how cancer cells benefit from tumor-specific metabolic changes may contribute to the identification of novel therapeutic targets and the development of more effective cancer therapies.

In this review, we discuss studies that elucidate the enzymes contributing to the metabolic reprogramming in cancer cells and their potential as therapeutic targets. In addition, we discuss how cancer cells adapt to bioenergetic challenges by using autophagy as a cell survival strategy and summarize the ongoing efforts to target autophagy in combination with conventional chemotherapy. This review proposes new avenues for cancer therapies in light of the recent progress in understanding tumor metabolism.

Metabolic targets responsible for reprogrammed cancer metabolism

Cancer cells maintain their growth advantage through persistent activation of growth signaling pathways and inactivation of tumor suppressors. Canonical oncogenic signaling

pathways such as PI3K/AKT and mTOR directly reprogram the core carbon metabolism in cancer, leading to increased nutrient uptake, which favors increased macromolecular biosynthesis to support cell proliferation. Indeed, a number of enzymes involved in metabolic alterations are direct targets of oncogenic transcription factors such as Myc and Hypoxia-inducible Factor 1 α (HIF-1 α). Various approaches to target oncogenic signaling pathways have been actively explored and showed great success in clinical trials. The detailed regulatory connection between signaling pathways and metabolic enzymes have been the topic of numerous reviews^{1,2}.

Moreover, emerging evidence suggests that the metabolites derived from altered metabolism also influence oncogenic signaling pathways in a reciprocal manner, and such interaction may be the basis for tumor progression and/or resistance to conventional chemotherapeutic approaches. Accordingly, metabolic alterations involved in cancer progression can become an attractive target for cancer therapy. Table 1 summarizes the enzymes contributing to the metabolic reprogramming in cancer which are targets for clinical trials. In addition, additional metabolic enzymes are being actively investigated for their roles in the progression of various cancers and their potential as therapeutic targets (Figure 1).

Glucose metabolism

As early as in the 1920s the German biochemist Otto Warburg made the observation that tumor cells utilize glucose in a substantially distinct way from normal cells, probably the first evidence for metabolic alterations in cancer³. While normal cells direct glucose to mitochondrial oxidative phosphorylation to generate ATP when oxygen is abundant, tumor cells generally exhibit increased glucose uptake, glycolytic flux and lactate secretion regardless of the oxygen availability. This phenomenon, termed aerobic glycolysis, formed the basis for the development of ¹⁸F-deoxyglucose positron emission tomography (FDG-PET) to image tumor development and regression in patients. The exact role and regulation of aerobic glycolysis is still not fully understood, however it has been suggested that its major benefit to cancer cells is to supply more favorable forms of energetic and anabolic substrates required for massive macromolecular synthesis. The Warburg effect also led to attempts to target glucose metabolism to preferentially eliminate cancer cells.

As a first step, glucose enters the cancer cell via specific transporters. Therefore a straightforward strategy would be to block glucose uptake by cancer cells through inhibition of glucose transporters. However, as there are several isoforms of glucose transporters and most of them have been shown to be highly expressed in cancer cells, thus it will be challenging to develop inhibitors that target all isoforms with an acceptable therapeutic window. At least one small molecule inhibitor (i.e. Silybin/silibinin) of glucose transporters has been developed and clinical trials are ongoing to test its toxicity and efficacy^{4, 5}.

As an alternative strategy, 2-deoxy-D-glucose (2DG), an analogue of glucose, has been tested as an anti-tumor agent. 2DG enters the cells via glucose transporters but is trapped as 2-deoxyglucose-6-phosphate after phosphorylation by hexokinase (HK) and cannot be further metabolized. The accumulation of 2-deoxyglucose-6-phosphate was thought to inhibit glycolytic enzymes and glucose catabolism. Although 2DG was shown to be effective in pre-clinical and clinical studies, the treatment was never fully explored because of concerns related to potential toxicity at high doses⁶. In contrast, recently it was discovered that cancer cells seem to preferentially rely on specific isoforms of glycolytic enzymes. Therefore attention has turned to developing isoform-specific inhibitors which are expected to increase drug specificity to cancer cells and avoid unwanted toxicity to normal cells. Figure 1 describes key metabolic pathways and enzymes being investigated as potential targets for cancer therapy. Examples include the muscle-specific isoform of hexokinase (HK2) and phosphofructokinase 2 (PFK2). Tumor-specific expression of these

isoforms led to the identification of isoform-specific inhibitors which showed significant suppression of tumor growth in pre-clinical studies^{7, 8} (Table 1).

Pyruvate kinase (PK) converts phosphoenolpyruvate (PEP) to pyruvate and generates ATP, which is a rate-limiting step in glycolysis. A splicing variant PKM2, the muscle form of PK, is predominantly expressed in embryonic cells and tumor cells. The glycolytic intermediate, fructose-1,6-bisphosphate (FBP), binds to PKM2 and converts it to an enzymatically active form, whereas the tyrosine kinase signaling pathway inactivates PKM2 through the release of FBP from PKM2⁹. Because most cancer cells exhibit constitutively active tyrosine kinase signaling, the enzyme activity of PKM2 is decreased in cancer cells. This has been proposed to be important for cell proliferation as low activity of PKM2 leads to the accumulation of glycolytic intermediates, many of which are precursors of macromolecular synthesis such as nucleotides and amino acids or can be used for the generation of NADPH¹⁰. Later studies suggested that PEP acts as a phosphate donor for an upstream enzyme phosphoglycerate mutase (PGAM1) in PKM2-expressing cells which further promotes glycolysis as well as biosynthetic processes¹¹. Indeed, it was recently reported that PKM2 cells maintain higher flux to the serine synthetic pathway^{12, 13}. The unique regulation of PKM2-mediated serine synthesis was suggested to be important for tumor growth and mTORC1 signaling, which will be discussed later. Based on these data, a variety of PKM2-specific small-molecule inhibitors or activators have been tested for their therapeutic potential in pre-clinical studies¹⁴⁻¹⁶ and some of them are being tested in clinical trials⁶(Table 1).

As the end product of glycolysis, the regulation of lactate generation and disposal has been extensively studied. Since lactate dehydrogenase (LDH) converts pyruvate to lactate while oxidizing NADH to NAD⁺ to support continued glycolytic flux, this enzyme has been considered as one of the critical targets for suppressing elevated glycolysis in cancer.

An isoform of LDH, LDHA is preferentially expressed in many cancers and is a transcriptional target of Myc and HIF-1 α . RNAi knock-down experiments in various cancer cells showed that LDHA plays an important role in tumor growth¹⁷⁻¹⁹. A natural phenol derivative, Gossypol and its derivatives have been shown to compete with NADH binding to LDH and inhibit LDH activity. Despite the lack of specificity, this agent is being tested in clinical trials for various cancers²⁰. The analogues of Gossypol have been screened for small molecule inhibitors specific for LDHA. Among them, FX11 (3-dihydroxy-6-methyl-7-(phenylmethyl)-4-propylnaphthalene-1-carboxylic acid) was shown to effectively inhibit cancer cell growth *in vitro* and *in vivo* by increasing the levels of oxidative stress²¹. More recently, N-hydroxyindole-based compounds have been identified as isoform-specific inhibitors of LDHA which compete with its substrates pyruvate and the cofactor, NADH²².

Pyruvate dehydrogenase kinase 1 (PDK1) is another transcriptional target of Myc and HIF1 α which appears to play a critical role in many cancers. It inactivates pyruvate dehydrogenase (PDH), which converts pyruvate to acetyl-CoA in the mitochondria. As a result, pyruvate is shuttled from the TCA cycle to produce lactate. Accordingly, specific inhibitors of PDK can block aerobic glycolysis and increase the rate of oxidative phosphorylation. For example, dichloroacetate (DCA), which is widely used for the treatment of lactic acidosis, has been tested in some pre-clinical cancer models and yielded promising results in clinical trials^{23,24} (Table 1), although its mechanism of action requires further investigation as it appears that this pyruvate mimetic compound lacks isoform selectivity among PDKs.

Lactate accumulated inside the cancer cells is exported through the monocarboxylate transporters (MCT) family. Impaired function of MCT leads to significant defects in cancer cell proliferation and tumor growth, indicating that efficient lactate secretion is required for

the optimal growth and proliferation of cancer cells^{25,26}. Moreover, a recent report showed that secreted lactate can be taken up by an isoform of MCT (MCT1) and used as fuel source to support proliferation of neighboring cells which are relatively oxidative, suggesting that in the same tumor, cancer cells with diverse metabolic profiles exchange their metabolites for survival and growth in a symbiotic manner. Several compounds that block the function of MCT 1 are being developed as potential cancer therapeutics²⁷⁻²⁸ (Table 1).

Many of these glycolytic enzyme-targeted compounds have showed synergistic anti-cancer effect in combination with conventional chemo/radiotherapy as well as with pathway targeted agents. At present, although some agents targeting glucose metabolism have shown promise in clinical trials, various enzymes involved in the reactions branching from glucose metabolism which are altered in cancer cells are also actively investigated as novel targets with a great therapeutic potential³⁰ (Figure 1).

Glutamine metabolism

Although the initial investigation of cancer cell metabolism focused primarily on glucose metabolism, it has become appreciated that the metabolism of amino acids and fatty acids is also reprogrammed to provide the building blocks for cancer cell growth and proliferation. Glutamine is the most abundant amino acid in the blood and it is a major source of nitrogen for nucleotides, amino acids and glutathione synthesis. Moreover, in highly proliferative cells glutamine is utilized as a carbon source to replenish the TCA cycle to support cell bioenergetics and anabolic reactions. Moreover, several groups recently proposed that cancer cells grown in hypoxia increase their dependency on glutaminolysis since glutamine-derived α -ketoglutarate (α KG) can undergo reductive carboxylation to produce citrate and lipids³¹⁻³³. Therefore, glutamine metabolism in these cancer cells seems to be an essential pathway and an attractive therapeutic target.

After being taken up, glutamine is converted to glutamate by a mitochondrial enzyme, glutaminase (GLS). It is subsequently converted to α KG by either glutamate dehydrogenase (GDH) or aminotransferases. As an intermediate of the TCA cycle, α KG can function to provide carbon backbones for cellular anabolic reactions³⁴ (Figure 1). Although oncogenic signaling pathways involved in rewiring the glutamine metabolism are not fully understood, multiple reports have suggested that the oncogenic transcription factor Myc controls glutamine catabolism by regulating the expression of glutamine transporters and the enzymes involved in glutaminolysis^{34,35}. In addition, Myc-overexpressing cells are remarkably sensitive to glutamine deprivation, suggesting that these cells are addicted to glutamine^{34,36}. Recently, Myc-induced tumorigenesis was associated with glutamine metabolism as shown by the correlation between Myc levels and metabolic profiling of mouse tumors³⁷.

As a potential target, GLS has two isoforms: glutaminase 1 (GLS1) is thought to be the primary enzyme involved in glutaminolysis, while GLS2 seems to have a different function related to the antioxidant system^{34,35,38}. Recently, GLS1 was identified as a target of the small molecule inhibitor which blocks Rho-GTPase-driven transformation using unbiased high throughput screening³⁹. Moreover, an isoform of GLS1, glutaminase C (GAC) has been considered an important target due to its elevated level in tumors showing glutamine addiction. Recent structure-based studies suggest that its activity is regulated by inorganic phosphate which is highly enriched in mitochondria under hypoxia^{40,41}.

Accordingly, several glutamine analogs including the compound 6-diazo-5-oxo-L-norleucine (L-DON) have been tested as therapeutic agents pre-clinically and clinically. Although the treatment with these agents led to significant inhibition of cancer cell growth *in vitro* and in mouse xenograft studies, these compounds show a high rate of toxicity due to

their lack of specificity⁴². Recently, a GLS1 specific inhibitor, bis-2-(5-phenylacetimido-1,2,4-thiadiazol-2-yl)ethyl sulfide (BPTES) has been identified⁴³ and shown to significantly inhibit cancer cell growth *in vitro* and in mouse tumor model^{37, 44}. As a selective therapeutic target, BPTES may achieve a greater therapeutic window than glutamine analogues as specific inhibition of GLS1 would suppress the tumor relying on glutaminolysis without affecting other important functions of glutamine in normal tissues.

Similarly, another glutaminolysis enzyme, glutamate dehydrogenase (GDH) was shown to be important for glioblastoma cell survival under conditions where glucose catabolism is impaired or the Akt pathway is inhibited⁴⁵. Epigallocatechin gallate (EGCG), a potent and specific inhibitor of GDH, has been shown to block glutaminolysis and sensitize glioblastoma cells to glucose deprivation^{45, 46}. Since glutamine can support cancer cell survival under glucose limitation and hypoxia, the suppression of glutamine catabolism would be synergistic with inhibition of growth signaling pathways related to glucose catabolism.

Serine synthetic pathways

Glycolytic intermediates derived from enhanced glycolysis in cancer cells can be shunted to generate non-essential amino acids, lipids and nucleotides which facilitate cancer cell growth and proliferation. For example, cells expressing PKM2 accumulate the glycolytic intermediate 3-phosphoglycerate (3-PG) which can be shunted to the serine synthetic pathway. As a result, PKM2 cells (i.e. cancer cells) can maintain mTORC1 activity and proliferation in serine-depleted medium while PKM1 cells (i.e. normal cells) cannot^{12, 13}. Interestingly, the rate-limiting enzyme involved in branching glycolysis to the serine synthetic pathways; phosphoglycerate dehydrogenase (PHGDH) was recently found to be amplified in a subset of melanoma and ER-negative breast cancer, suggesting that increased flux into the serine synthetic pathway provides selective advantage to tumor cells. Consistently, overexpression of this gene leads to cellular transformation and RNAi suppression of *PHGDH* leads to inhibition of cancer cell proliferation and transformation^{47, 48}. Therefore PHGDH is an attractive metabolic target for cancer therapeutics together with other enzymes involved in the serine synthetic pathway.

Serine is an important nonessential amino acid which can be used for the synthesis of other amino acids such as glycine and cysteine and the generation of phospholipids. In addition, when serine is metabolized to glycine, tetrahydrofolate (THF) is converted to 5,10-methylene-THF which is a critical step in maintaining folate cycle for nucleotide synthesis⁴⁹ (Figure 1). The dependency of cancer cells to the folate cycle has been demonstrated by the successful development of anti-folate drugs, many of which are widely used in clinics to treat various types of cancer (Table 1). Examples include 5-fluorouracil (5-FU) which inhibits thymidine synthesis and methotrexate which blocks purine synthesis by inhibiting dihydrofolate reductase (DHFR) which converts dihydrofolate (DHF) to tetrahydrofolate (THF) (Table 1).

Autophagy

Context-dependent role of autophagy in cancer

Autophagy is a self-degradation process where cytosolic components and organelles are sequestered in double membrane-bound vesicles and delivered to lysosomes for degradation and recycling (Figure 2). In normal tissue, autophagy maintains cellular homeostasis by clearing damaged organelles or misfolded proteins.

The role of autophagy in cancer is complex and paradoxical. Defects in autophagy such as those resulting from deletions of autophagy genes lead to increased tumorigenesis. Early

studies found mono-allelic deletion of the autophagy gene *beclin1* in various human cancers and mice heterozygous for *beclin1* developed multiple spontaneous malignancies^{50, 51}. Moreover, some gastric cancers were shown to have nonsense mutations of UVRAG (ultraviolet radiation resistance-associate gene), a beclin1-binding autophagy regulator⁵². More recently, mice with systemic mosaic deletion of *atg5* or liver-specific deletion of *atg7* showed high incidence of benign liver adenoma development, suggesting that autophagy serves to suppress tumor initiation⁵³. Furthermore, it was reported that p62, an autophagy substrate protein, is accumulated in autophagy-deficient cells following metabolic stress, which leads to accumulation of damaged mitochondria and elevated oxidative stress and DNA damage⁵⁴.

In contrast, autophagy has been proposed to be a pro-survival process in cells exposed to metabolic stress. It was shown that when growth factor dependent cells were subjected to metabolic stress following growth factor deprivation, these cells utilize autophagy to generate additional bioenergetic substrates to support cell survival⁵⁵. Similar results were seen in established tumors where autophagy was shown to promote cell survival in the center of the tumor, which is likely to be deprived of nutrients and oxygen⁵⁶. In addition, cells genetically deficient for autophagy genes such as *beclin 1* or *atg5* are sensitive to metabolic stress^{57, 58}. Based on recent immunohistochemical analysis of tumor samples, the expression of mammalian homologues of Atg8, GABARAP or LC3 was increased in a variety of tumor tissues compared to normal tissues, and their expression levels correlated with tumor progression and poor diagnostic rate^{59, 60}, indicating that autophagy is actively induced in advanced stages of tumorigenesis.

In addition to the adaptation to primary tumor microenvironment, autophagy has been shown to promote cell survival in extracellular matrix-detached conditions using a three dimensional (3D) mammary cell culture model, suggesting that autophagy is also a critical process for tumor survival during metastasis⁶¹.

Studies using genetically-engineered mouse models of cancer suggested that autophagy plays an indispensable role in tumor development and maintenance, regardless of nutrient availability. Targeted deletion of an autophagy gene FIP200 resulted in significant delay of mammary tumor progression in a mouse breast cancer model induced by the polyoma middle T(PyMT) oncogene⁶². Multiple studies have suggested that autophagy is required for Ras-induced transformation *in vitro* and Ras-driven tumorigenesis⁶³⁻⁶⁵. Moreover, pancreatic cancers known to have Kras mutation showed elevated levels of autophagy necessary for cancer cell growth in an *in vitro* setting⁶⁶.

Despite the increasing evidence supporting the pro-survival role of autophagy in established cancer, the exact mechanism of how autophagy supports energy-demanding cancer cell metabolism still remains largely unknown. One hypothesis is that metabolites derived from autophagic degradation of cellular components might be utilized for the adaptation and growth of cancer cells as bioenergetic and anabolic substrates. Indeed, metabolic analysis showed that autophagy was required to maintain full activities of mitochondrial TCA cycle and oxidative phosphorylation after Ras activation⁶⁵. Moreover, melanoma cell lines established from clinical samples have shown a high dependency on extracellular leucine which could be rescued by autophagy activation⁶⁷.

Another remaining question in the field of autophagy research is what triggers the autophagic response in cells. It is well established that deprivation of essential amino acids activates autophagy through the inhibition of the mTOR pathway. Intriguingly, we and others have described that ammonia, a metabolic byproduct derived from amino acid catabolism including glutaminolysis, also acts as an autophagy inducer. Interestingly, this

form of autophagy is independent of the mTOR-ULK pathway^{68, 69}. These findings suggest that autophagy induction is linked to either the relative lack of nitrogen or excess nitrogen mediated by distinct sensing mechanisms. It remains to be elucidated what the sensor of increased intracellular nitrogen levels is and its implication in cancer development. Since tumors with activated oncogenes such as Myc or Ras have been reported to rely on glutaminolysis to generate bioenergetic fuel^{34, 70}, we can speculate that elevated autophagy shown in these oncogene-driven tumors might be initiated through excess nitrogen sensing mechanisms.

As evident from the above discussion, the role of autophagy in tumor initiation and progression is complex. Autophagy is more likely to have a tumor-suppressing role in the tumor initiation stage, whereas in established tumors, it may function to fulfill the metabolic needs of cancer cells during oncogene activation or nutrient limitation by providing a mechanism to recycle intracellular carbon and nitrogen and thus support tumor cell survival. Clearly, the cellular context of tumors (origin, stage, genetic make-up) plays a role in the outcome of the autophagy response and must be taken into consideration when using autophagy modulation in cancer therapy.

Autophagy as a therapeutic target

Given the critical role of autophagy in tumor progression and maintenance, various pre-clinical and clinical studies have been undertaken or are in progress to develop therapeutic agents targeting autophagy.

As most cancer therapeutic agents inevitably cause cellular stress, autophagy is often activated in cancer cells after drug treatment. Indeed, many of the therapies targeting growth factor signaling- either as single targeted treatment or combinatorial treatment with two agents targeting different pathways -clearly result in autophagy induction (Table 2). For example, several allosteric and catalytic inhibitors of mTOR, PI3K/AKT, and tyrosine kinases signaling and activators of energy sensing pathway (e.g. AMPK), induce autophagy in cells. In addition, various agents targeting cellular processes such as inhibitors of histone deacetylase (HDAC), proteasome, apoptosis and glycolysis have also been suggested to trigger autophagy (Table 2). It was originally proposed that autophagic cell death may be part of the mechanism of action of anti-cancer drugs. Indeed, some combinatorial treatments with the above drugs showed synergistic effect on the induction of cell death which was reduced by knock-down of autophagy genes, suggesting that autophagy induced by chemotherapy contributes to its anti-cancer effect (Table 2). However, it has been suggested that the increased autophagy seen during anti-cancer drug treatment could also be a survival response of the dying cells rather than cause of cell death⁷¹. As many of autophagy inducers have shown limited success in clinical trials, it is necessary to assess the contribution of autophagy in the mechanism of actions of these drugs. Moreover, a growing body of evidence has indicated that stress-induced autophagy in tumor cells might drive cellular resistance against chemotherapy and facilitate tumor dormancy^{72, 73}.

In light of the above data, inhibition of autophagy activity could be a useful approach in combination with conventional chemo/radiotherapy. Indeed, a series of studies have reported that the treatment with targeted agents which induce autophagy, synergized with autophagy inhibitors for anti-cancer effect in *in vitro* settings using numerous cancer cell lines as well as in xenograft models^{74, 75}. Moreover, combination therapies with chemotherapy and autophagy inhibitor are being tested in clinical trials and have showed promising results.

The most used autophagy inhibitors in clinical trials are chloroquine (CQ) and hydroxychloroquine (HCQ). These agents are registered anti-malarial drugs which inhibit

lysosomal acidification and eventually impair autophagosome fusion with lysosomes and degradation⁷⁶. Pre-clinical studies using a Myc-induced mouse lymphoma model suggested that the inhibition of autophagy by treatment with HCQ increased cell death and significantly impaired recurrence of tumors following the treatment with chemotherapy^{77, 78}. Clinical trials involving CQ- and HCQ-mediated autophagy inhibition for cancer therapy are ongoing and listed at <http://www.clinicaltrial.gov>.

In addition to CQ derivatives, a variety of other putative autophagy inhibitors have also been studied for their anti-tumor effect *in vitro* and *in vivo* (Table 2). However, these potential autophagy inhibitors have nonspecific effects in other cellular functions such as endocytosis (e.g. 3-MA) or lysosomal function (e.g. bafilomycin A). Targeting key autophagy proteins would be a more potent and specific approach. Several components of the autophagic machinery are druggable and serve as potential therapeutic targets. These include ULK1/2 serine/threonine protein kinases, the enzymes involved in ubiquitin-like conjugation systems (e.g. E1-like ubiquitination enzyme, Atg7 and E2- like enzymes, Atg3 or Atg10) and cysteine protease, Atg4. Moreover, the class III phosphatidyl inositol 3 (PI3) kinase, Vps34 could be targeted as it is a key autophagy regulator, although normal endocytosis is also regulated by this protein. In addition to pharmacological inhibitors, increasing pre-clinical evidence have supported that gene interference using siRNA against various autophagy proteins can have a synergistic effect in combination with other treatments. Accordingly, deeper understanding of the molecular basis of autophagy induction and execution could identify more cancer therapeutic targets that can be drugged alone or in combination.

Concluding Remarks

As discussed in this review, cancer cells display multiple layers of metabolic alterations affecting cell proliferation and survival. Cancer cell metabolism is reprogrammed by multiple factors including mutations of core oncogenic pathways and tumor suppressors as well as alterations in the microenvironment and stromal cells.

In addition, direct amplification of metabolic enzymes can influence the regulation of cancer growth, indicating that metabolic deregulation can contribute to tumor development. Therefore, defining the mechanism of how cancer cells coordinate their metabolism to support cancer growth and how cancer cells adapt to the tumor microenvironment can provide insights to understanding cancer development.

Understanding the multiple layers of defects in cancer cell metabolism, ranging from oncogene dysregulation, to mutations in specific metabolic enzymes has the potential to identify new targets for cancer therapy. Moreover, investigating the regulatory network of these cellular mechanisms will allow for the development of more effective combinatorial therapeutic approaches.

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Figure 1. Core metabolic pathways and metabolic enzymes suitable as cancer therapeutic targets
Active metabolic pathways in proliferating cells involving glucose and glutamine catabolism are interconnected and linked to macromolecular synthesis and energy balance. Key metabolic enzymes discussed in the text (shown in blue) are actively investigated as therapeutic targets for cancer treatment. Metabolic enzymes targeted by registered agents are shown in Red.

ACL, ATP citrate lyase; α KG, α -ketoglutarate; DHFR, dehydrofolate reductase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; F-2,6-BP, fructose-2,6-bisphosphate; F6P, fructose-6-phosphate; FBP, fructose-1,6-bisphosphate; FH, fumarate hydratase; G6P, glucose-6-phosphate; GLS, glutaminase; HK2, hexokinase 2; IDH, isocitrate dehydrogenase; LDHA, lactate dehydrogenase A; MCT1,4, monocarboxylate transporter 1,4; OAA, oxaloacetate; PDH, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase; PEP, phosphoenolpyruvate; PFK1, phosphofructokinase 1; PFK2, phosphofructokinase 2; PGAM, phosphoglycerate mutase; PHGDH, phosphoglycerate dehydrogenase; PKM2, pyruvate kinase M2 isoform; R5P, ribose-5-phosphate; SDH, succinate dehydrogenase; THF, tetrahydrofolate; TYMS, thymidylate synthase

Figure 2. Modulators of the autophagy pathway

Various growth and nutrient signaling pathways are associated with regulation of autophagy. Following inhibition of mTOR, the ULK/Atg13/FIP200 complex is activated and initiate autophagosome/phagophore formation. The class III PI3 kinase (Vps34)-Atg14L-Beclin 1 complex also regulates the autophagosome nucleation step. To expand the autophagosome membrane, two ubiquitin-like conjugation systems are required for conjugation of LC3 and Atg12 to phosphatidyl ethanolamine (PE) on the autophagosome membrane and Atg5, respectively. Further, the Atg12-Atg5 conjugate interacts with Atg16, presumably located on the surface of the autophagosome membrane. The complete autophagosome fuse with the lysosome to form the autolysosome and cargo molecules enwrapped by autophagosomes are degraded by lysosomal hydrolases and recycled back to the cytoplasm. As shown in red, a variety of pharmacological inhibitors are depicted which modulate distinct steps of autophagy. Some autophagy proteins which enzymatic activity (shown in green and yellow) could be crucial target proteins for modulation of autophagy activity. Potential points where inhibitors could be potentially developed are shown in blank boxes.

Table 1
Potential therapeutic compounds targeting metabolic enzymes of tumors

Compound	Target	Tumor type/Cancer cell types	Clinical stages
Glucose metabolism			
2-DG	Glucose transporter	Prostate cancer	Phase I (terminated)
Silybin/silibinin		Prostate cancer	Phase I/II
2-DG	HK2	Prostate cancer	Phase I (terminated)
Lonidamine			Phase I (Europe)
TLN-232/CAP-23	PKM2	Metastatic RCC, melanoma	Phase II
Gossypol/AT-101	LDHA	Multiple cancers	Phase I/II
Dichloroacetate (DCA)	PDK	Brain cancer	Phase I
		Non-small cell lung cancer (NSCLC)	Phase II
		Head and neck cancer	
AZD3965	MCT1	Advanced solid tumors	Phase I/II
Nucleic acid metabolism			
Methotrexate	Folate cycle (DHFR)	Multiple cancers	Registered
Pemetrexel			
5-Fluorouracil	Thymidine synthesis (TYMS)	Multiple cancers	Registered
Hydroxyurea	Deoxynucleotide synthesis (Ribonucleotide reductase: RNR)	Multiple cancers	Registered
Gemcitabine, Fludarabine	Nucleotide incorporation (DNA polymerase/RNR)	Multiple cancers	Registered
Amino acid metabolism			
L-Asparaginase	Asparagine	Leukemia	Registered
Arginine deaminase (ADI-PEG conjugated)	Arginine	Multiple cancers	Phase II
NAD metabolism			
FK866/APO866	Nicotinamide phosphoribosyl-transferase (NAMPT)	Cutaneous T cell lymphoma (CTCL), B-Cell Chronic Lymphocytic Leukemia (CLL), melanoma	Phase II
CHS828/GMX1777		Metastatic Melanoma, Solid Tumors, Lymphomas	Phase II

Table 2
Autophagy inducers and inhibitors for cancer treatment

Compound	Target	Tumor type/Cancer cell types	References
Autophagy inducers			
Temsirolimus (CCI-779) *	mTORC1 inhibitors	Renal cancer *, Mantle cell lymphoma	79
Everolimus(RAD-001) *		Renal cancer *, Acute lymphoblastic leukemia (ALL)	80
Rapamycin		Glioma, malignant	81
		Chronic myeloid leukemia (CML) *	82
Imatinib *	Tyrosine kinases Inhibitors; KIT, BCR-Abl, PDGFR,	Gastrointestinal stromal tumor *, Chronic myeloid leukemia (CML) *	83
Dasatinib *	EGFR	Glioma *	84
Erlotinib *		Non-small cell lung cancer (NSCLC) *	85
Bortezomib *	Proteasome inhibitors	Multiple myeloma *	86
NPI-0052		Prostate cancer	87
Vorinostat *	HDAC inhibitors	Cutaneous T cell lymphoma (CTCL) *	88, 89
Butyrate, suberoylanilide hydroxamic acid (SAHA)		Multiple cancers, CML	
LAQ824, Panobinostat (LBH589)		Lymphoma	90
Temozolamide *	DNA alkylating agent	Glioblastoma, Metastatic melanoma *	91
GX15-070	Bcl2 inhibitor	Pancreatic carcinoma	92
		Leukemia	93
Arsenic trioxide *		Acute promyelocytic leukemia *	94
		Glioma, malignant	95
Resveratrol	Antioxidant	Ovarian cancer	96
Autophagy Inhibitors			
3-methyladenine (3-MA)	Class III PI3 kinase inhibitor		97-99
		Colorectal cancer,	100
Chloroquine *	Lysosomal pH	Malaria *	89, 101 77, 78
		Glioma, malignant	
		Lymphoma	
Hydroxychloroquine *	Lysosomal pH	Malaria, Lupus, Rheumatoid arthritis *	102 98
		Breast cancer	

Compound	Target	Tumor type/Cancer cell types	References
Bafilomycin A1	Vacuolar-ATPase	Glioma, malignant Breast cancer	98, 103 91 104
Monensin	Lysosomal pH	Glioma, malignant	98, 105 131
Pepstatin A	Lysosomal protease (Cathepsin inhibitor)	Cervical cancer	106

* Compounds with an asterisk, are registered by FDA for designated diseases.