Topic: Reviews of Recent Advances in Research and Treatment for Gastroenterological Malignancies

Cancer metabolism in gastrointestinal cancer

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ABSTRACT

Cancer cells exhibit altered glucose metabolism, mitochondrial dysfunction, anaerobic glycolysis and upregulation of the pentose phosphate pathway (PPP). Recent genetic and metabolic analyses have provided insights into the molecular mechanisms of genes that are involved in the alteration of cancer metabolism and tumorigenesis. Hypoxic induced factor 1 regulates the reciprocal relationship between glycolysis and oxidative phosphorylation, and p53 also modulates the balance between the glycolytic pathway and oxidative phosphorylation. Mitochondria function in cancer differs from that in normal cells owing to mutations of mitochondrial DNA and alterations of metabolism. Overexpression of transcription factors, metabolite transporters and glycolytic enzymes is observed and associated with poor prognosis, and it may be associated with chemoradiotherapy resistance in multiple cancer cell types. The PPP plays a critical role in regulating cancer cell growth by supplying cells with ribose-5-phosphate and nicotinamide adenine dinucleotide phosphate for detoxification of intra-cellular reactive oxygen species (ROS), reductive biosynthesis and ribose biogenesis. ROS levels increase during carcinogenesis owing to metabolic aberrations. This review discusses alterations of mitochondrial metabolism, anaerobic glycolysis, the PPP and control of ROS levels by the endogenous anti-oxidant system in cancer, as well as the novel small molecules targeting these enzymes or transporters that exert anti-proliferative effects.

Key words: Anti-oxidants, cancer metabolism, mitochondria, pentose phosphate pathway, reactive oxygen species, Warburg effect

Introduction

In 1926, Otto Warburg found the conversion of glucose to lactic acid in the presence of adequate oxygen as a specific metabolic abnormality of cancer cells.[1,2] Warburg further hypothesized that cancer results from a defect of mitochondrial metabolism that leads to aerobic glycolysis. The role of dysfunctional glucose metabolism in cancer is now firmly established. Recent genomic and proteomic research has provided insights into the molecular mechanisms of cancer metabolism.

Two main pathways generate adenosine triphosphate (ATP) required for cell proliferation and survival. The first is glycolysis, which metabolizes glucose to pyruvate in the cytoplasm to produce a net two ATP molecules from each glucose molecule. The other is the tricarboxylic acid (TCA) cycle, which uses pyruvate formed from glycolysis to donate electrons via nicotinamide adenine dinucleotide (NADH) (reduced form of NADH) and flavin adenine dinucleotide (FADH2) (reduced form of FADH2) to the respiratory chain complexes in mitochondria. The electron transfer system generates 36 ATP molecules per glucose across the mitochondrial inner membrane. Under limited oxygen conditions, such as muscles under prolonged exercise, pyruvate is not used in the TCA cycle and is converted into lactic acid by lactate dehydrogenase (LDH) in a process termed anaerobic glycolysis.

Recent genetic and metabolic analyses have provided insights into the molecular mechanisms of the genes that contribute to anaerobic glycolysis and tumorigenesis. The direct mechanistic links between activated oncogenes and altered glucose metabolism are regulated by phosphoinositide 3-kinase (PI3K),[3] Akt,[4] p53,[5,6] AMP-activated protein kinase (AMPK),[3,7] c-Myc and hypoxia-inducible factor (HIF). The c-Myc and HIF-1A transcription factors target many of the same glycolytic enzyme genes, including hexokinase 2 (HK2),

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pyruvate kinase type M2 (PKM2), LDH-A and pyruvate dehydrogenase kinase, isozyme 1 (PDK-1).

The pentose phosphate pathway (PPP) is a major pathway for glucose catabolism. The PPP directly or indirectly provides reducing power to fuel the biosynthesis of lipids and nucleotides and sustains anti-oxidant responses to support cell survival and proliferation. Abnormal respiratory metabolic pathways influence energy balance and the reactive oxygen species (ROS) balance in cancer cells. The increase in ROS generation from metabolic abnormalities and oncogenic signaling in cancer cells triggers a redox adaptation response to maintain ROS levels below the toxic threshold. Cancer cells would be increasingly dependent on the anti-oxidant system.

In this review, significant molecular insights into mitochondrial metabolism, anaerobic glycolysis and the PPP in cancer are discussed. We also review the control of ROS levels by the endogenous anti-oxidant system and the therapeutic strategies targeting cancer metabolism.

Mitochondria in Cancer Cells

As the main energy producers, mitochondria produce ATP using the TCA cycle and oxidative phosphorylation. However, they also generate ROS during this process, which are harmful to the cell if produced in excess. In addition, mitochondria play a crucial role in the regulation of cell death pathways and intra-cellular Ca^{2+} homeostasis. Mitochondria activate apoptosis by regulating the release of pro-apoptotic proteins from the mitochondrial intermembrane to the cytosol, and they also play a crucial role in non-apoptotic cell death.[8] Key regulators related to cell death in the mitochondria are frequently altered in cancer cells,[9] and the function of mitochondria in cancer cells is different from that in normal cells.[10]

The mitochondrial mechanism in cancer cells is different from that in normal cells using oxidative phosphorylation. In oxidative phosphorylation, ATP synthesis requires significant amounts of oxygen, which leads to the continuous production of ROS such as superoxide anion, organic peroxide and hydrogen peroxide.[11] If the redox regulating system does not eliminate the generated ROS, the excessive ROS may cause cellular damage. Mitochondria have redox defense systems for the elimination of hydrogen peroxide. Glutathione (GSH) and glutathione peroxidases require nicotinamide adenine dinucleotide phosphate (NADPH) for the elimination of H_2O_2 and other peroxides generated in the mitochondria. The mitochondrial complex V (ATP synthase) produces ATP from ADP and inorganic phosphate. As an anti-oxidant defense system, peroxiredoxin (Prx) 3, Prx5, superoxide dismutase 2 (SOD2) and thioredoxin 2 eliminate ROS produced in mitochondria.[12,13] Prx3 knockout (KO) mice exhibit metabolic dysregulation and induction of oxidative damage,[14] thioredoxin 2 KO mice show an embryonic lethal phenotype[15] and SOD2 KO mice die within 3 weeks of birth because of mitochondrial oxidative damage and severe neurodegeneration.[16,17]

Mutations in mitochondrial DNA (mtDNA) occur at a high frequency in human tumors. Tumor mtDNA somatic mutations range from severe insertions/deletions and chain termination mutations to mild missense mutations. A total of 190 tumor-specific somatic mtDNA mutations have been reported and 72% of them are also mtDNA sequence variants found in the general population. They include 52% tumor somatic mRNA missense mutations, 83% tRNA mutations, 38% rRNA mutations and 85% control region mutations. Germline mtDNA mutations at nucleotides 10,398 and 16,189 have been associated with breast cancer,[18] esophageal cancer[19] and endometrial cancer.[20] The mtDNA conferring high metastatic potential contained G13997A and 13885insC mutations in the gene encoding NADH dehydrogenase sub-unit 6. These mutations produced a deficiency in respiratory complex I activity and were associated with overproduction of ROS.[21] Severe mutations can inhibit oxidative phosphorylation, increase ROS production and promote tumor cell proliferation; milder mutations may permit tumors to adapt to new environments.[22]

Recent investigations have revealed that p53 can modulate the balance between the glycolytic pathway and mitochondrial oxidative phosphorylation.[23] The key component in this regulation is the gene encoding synthesis of cytochrome c oxidase 2 (SCO2), in conjunction with the SCO1 protein. Analysis of potential p53 target genes that can influence mitochondrial function showed that SCO2, but not SCO1, was induced in a p53-dependent manner. SCO2 is critical for regulating the cytochrome c oxidase (COX) complex, the major site of oxygen use and is required for the assembly of COX.[24] Mutation of p53 in tumor cells leads to inhibition of mitochondrial respiration as a result of COX deficiency and a shift of cellular energy metabolism toward glycolysis. Inhibition of glycolysis by glucose withdrawal leads to the activation of p53. Under conditions of cellular stress, activation of p53 could increase SCO2 expression and stimulate mitochondrial respiration and ATP production. Another newly discovered target of p53 is TP53-induced glycolysis and apoptosis regulator (TIGAR). Expression of TIGAR lowered fructose-2,6-bisphosphate levels in cells, resulting in the inhibition of glycolysis while stimulating NADPH generation through the pentose phosphate shunt.[25] The expression of TIGAR in primary tumors is significantly correlated with standardized uptake values max, and low expression of TIGAR may predict a worse clinical outcome in patients with non-small cell lung cancer.[26]

HIF-1 plays an important role in the upregulation of enzymes stimulating glucose use. Recent investigations demonstrated that HIF-1 suppresses mitochondrial function in tumor cells and modulates the reciprocal relationship between glycolysis and oxidative

phosphorylation. The balance between glycolysis and oxidative phosphorylation is controlled by the relative activities of two enzymes: pyruvate dehydrogenase (PDH) and LDH. The activity of PDH is negatively controlled by PDK-1, and HIF-1 can inactivate PDH by inducing PDK-1. Inactivation of PDH leads to suppression of mitochondrial respiration.[27,28] HIF-1 also stimulates expression of LDH-A, which facilitates the conversion of pyruvate into lactate,[10] which decreases use of pyruvate by mitochondria and suppresses mitochondrial respiration. In addition, HIF-1 can also modulate COX expression. Under hypoxic conditions, the sub-unit composition of COX is changed to optimize its activity. The expression of the COX4-2 sub-unit is increased and optimizes the activity of COX under aerobic conditions.[24]

Another important consequence of the glycolytic shift in tumor cells is their acquired resistance to apoptotic cell death. The two major apoptotic pathways include the extrinsic (receptor-mediated) pathway and the intrinsic pathway. The extrinsic pathway engages initiator pro-caspase-8, which activates pro-caspase-3 and other effector caspases. The intrinsic pathway involves permeabilization of the outer mitochondrial membrane (OMM) followed by the release of cytochrome c and other proteins from the intermembrane space of mitochondria. Permeabilization of the OMM is considered to be a crucial event during the early phase of the apoptotic process. Multiple proteins, including B-cell lymphoma 2 (Bcl-2) family,[29,30] hexokinase,[31,32] Akt[33,34] and loss of p53,[35,36] support the glycolytic shift. These proteins render tumor mitochondria less susceptible to the permeabilization of the OMM and the mitochondrial pathway of apoptosis.

Alteration of Protein Expression in the Warburg Effect

Cancer cells exhibit altered glucose metabolism, which is described by the increased uptake of glucose and the conversion of glucose to lactate in cancer cells under adequate oxygen tension. HIF-1A and c-Myc transcription factors cooperatively induce a transcriptional program for glycolysis by targeting many glycolytic enzyme genes, including HK2, PKM2, LDH-A and PDK-1. Key regulatory sub-units of HIF include HIF-1A and endothelial PAS domain protein 1 (EPAS1; HIF-2), and these proteins are differentially overexpressed in cancer cells.[37,38] Many studies demonstrated that HIF-1A positive expression was significantly associated with poor outcome of diverse human cancers.[38-43] Low expression of HIF-1A may be associated with a favorable outcome of 5-fluorouracil (5-FU)-based adjuvant chemotherapy in gastric cancer patients.[44,45] High expression of HIF-2A was associated with poor survival in gastric cancer patients,[46] but not colorectal cancer (CRC) patients.[42,47] The MYC protein affects the expression of approximately 15% of the genes in the human genome,[48] and thus MYC deregulation may result in alterations in various biological pathways involved in cancer initiation and progression.[49] The expression of MYC genes is often elevated or deregulated in human neoplasms, and c-Myc seems to be at the crossroads of several important pathways and processes involved in carcinogenesis. MYC overexpression and promoter hypomethylation may have a role in the gastric carcinogenesis process. MYC deregulation was mainly associated with poor prognostic features.[50]

The GLUT family proteins are glucose transporter-like proteins that have been well characterized. The 14 GLUTs are categorized into three classes based on sequence similarity: Class 1 (GLUTs 1-4 and 14); Class 2 (GLUTs 5, 7, 9 and 11) and Class 3 (GLUTs 6, 8, 10, 12 and HMIT).[51] Several studies have been published on GLUT family members, especially GLUT 3,[52-54] but GLUT 1 has been the main focus of the investigation.[55-57] GLUT 1 comprises 492 amino acid residues and possesses a single N-linked glycosylation site at N45,[58] and its crystal structure has been reported recently.[59] GLUT 1 is transcriptionally regulated by HIF-1A[60] and c-Myc.[61] A recent investigation showed that GLUT 1 was upregulated in cells with KRAS or BRAF mutations,[62] and GLUT 1 expression in CRC cells was positively correlated with FDG accumulation and KRAS/BRAF mutation.[63] MAPK signaling induces phosphorylation of Ser 37 in PKM2, and nuclear-phosphorylated PKM2 then induces c-Myc expression, resulting in the upregulation of GLUT 1.[64] Overexpression of GLUT 1 in a mammary tumor cell line with low levels of endogenous GLUT 1 results in both a decrease in apoptosis and an increase in proliferation.[65]

Hexokinases catalyze the phosphorylation of glucose to glucose-6-phosphate (G6P). This is the first and rate-limiting step in glucose metabolism. HK2 is one of four members of the hexokinase family. The hexokinase isoenzymes (HK1, HK2, HK3 and glucokinase) are structurally similar; however, only HK1 and HK2 are functionally similar. HK2, but not HK1, is overexpressed in several cancer types compared with normal tissue, and overexpression of HK2 was reported in hepatocellular carcinoma (HCC).[66-68] HK2 localizes to the outer membrane of the mitochondria and is the major hexokinase isoform expressed in cancer cells.[69]

PK is a glycolytic enzyme that catalyzes a reaction generating pyruvate and ATP from phosphoenolpyruvate and ADP. Four isoforms of PK (L, R, M1 and M2) are present in mammals. Splicing of PKM is regulated by splicing repressors, and the expressions of those repressors are induced by MYC oncoprotein.[70,71] M2 is expressed in embryonic cells, adult stem cells and cancer cells and is necessary for aerobic glycolysis and that this metabolic phenotype provides a selective growth advantage for cancer cells *in vivo*.[72] Mutation of the S37 ERK phosphorylation site in PKM2 blocked translocation of PKM2 to the nucleus,[64] which suggested

that PKM2 moves into the nucleus as a monomer. Tumor cells have multiple ways to regulate PKM2 for cell growth and survival, including controlling PKM2 expression, localization, post-translational modification and allosteric regulation. PKM2 also has non-metabolic functions as a transcriptional coactivator and protein kinase. PKM2 is considered an attractive target for cancer treatment.[73] Further studies are needed before inhibitors and activators of PKM2 can be used as therapeutic interventions.[74]

PDK regulates PDH, which links glycolysis to the TCA cycle by reversible phosphorylation. Phosphorylation of PDH by PDK inactivates PDH and halts pyruvate use in the TCA cycle.[75] Four PDK isoforms have been verified in human tissue, and the expression of these isoforms was organ specific. PDK-1 positivity was associated with poor prognosis in gastric cancer;[76] however, expression of PDK-1 was decreased in colon cancer compared to normal tissue. PDK-3 expression was detected in colon cancer, and PDK-3 positivity was associated with poor prognosis.[77] Only a few studies have reported the relation between PDK positivity and prognosis, and the clinical significance of PDK expression has remained unclear. LDH is a tetrameric enzyme comprising two major sub-units, A and/or B, resulting in five isozymes (A4, A3B1, A2B2, A1B3 and B4) that can catalyze the forward and backward conversion of pyruvate to lactate. LDH-A (LDH-5, MLDH or A4), which is the predominant form in skeletal muscle, kinetically favors the conversion of pyruvate to lactate, controlling the conversion of pyruvate to lactate of the cellular glycolytic process.[78] Many studies have shown that human cancers have higher LDH-A levels compared with normal tissues.[79] Previous studies showed that 661 intestinal-type gastric cancer (ITGC)[80] and 128 CRC[81] specimens with high LDH-A expression are associated with poor prognosis. LDH-A is specifically phosphorylated at Y10 in various cancer cell lines, head and neck squamous cell carcinoma (SCC), lung cancer, breast cancer and prostate cancer cells and by diverse oncogenic tyrosine kinases, including FGFR1, ABL, JAK2 and FLT.[82] LDH-A reduction using si-RNA for LDH-A can suppress the tumorigenicity of ITGC cells[80] and HCC.[83]

The Pentose Phosphate Pathway

The PPP is a major pathway for glucose catabolism. Glucose is a common fuel for multicellular organisms, entering cells through GLUTs and then being phosphorylated by HK to form G6P. G6P can be further metabolized by both the glycolytic pathway and the PPP.[84] The PPP generates ribose 5-phosphate (R5P), a critical sub-strate for nucleotide synthesis. The PPP plays a critical role in regulating cancer cell growth by supplying cells with not only R5P but also NADPH for detoxification of intra-cellular ROS, reductive biosynthesis and ribose biogenesis.

Fructose-6-phosphate is isomerized to G6P in cells, and this accumulated G6P is diverted into the PPP, an alternative metabolic pathway that can provide substrates for the later steps in glycolysis. Glucose-6-phosphate dehydrogenase (G6PD) is mediated by various signals, and it acts as a sensor of cellular $NADP^+$ levels. Increased $NADP^+$ activates G6PD by competing with NADPH for binding to this enzyme (G6PD), and determines the amount of NADPH by controlling the metabolism of glucose via the PPP.[85] The increased flow through the PPP lowers apoptosis because of an increased generation of reduced GSH and removal of ROS in cells.[25] Elevated levels of G6PD in association with higher levels of PPP-derived metabolites suggest a prominent role of this pathway in metabolic alterations of human cancer.[86,87] G6PD inhibition decreases cancer cell survival, NADPH levels and increases production of ROS, suggesting that the PPP plays an important role in the regulation of redox homeostasis.[88,89] G6PD is associated with adriamycin resistance in breast cancer cells using proteomics analysis.[90]

The PPP is positively regulated by K-ras^{G12D},[91] PI3K,[91] mTORC1,[92] Tap73,[93,94] HSP27,[95] SREBP,[92] the ataxia-telangiectasia mutated kinase, protein kinase A, NADP and glycolytic inhibition (TIGAR,[25] PKM2 and PGAM). The PPP is negatively regulated by p53, PTEN,[96] AMPK,[3] cyclic adenosine monophosphate, cyclic AMP-response element modulator and aldosterone.[97] TAp73, the transcriptionally competent isoform of the p53 family protein p73, was identified as a transcriptional regulator of G6PD.[94]

The PPP is a well-established metabolic pathway, but the mechanism that activates the PPP has yet to be identified. TIGAR, a target of p53, inhibits glycolysis and diverts the carbon flux into the PPP, resulting in the passive promotion of PPP activity. NADPH production pathway is targeted by nuclear factor E2 p45-related factor 2 (Nrf2).[98] Nrf2, a bZIP transcription factor, plays a central role in the regulation (basal and/or inducible expression) of phase 2 genes by binding to the anti-oxidant response element in their promoters. A previous study focused on the cytoprotective aspect of the PPP by analyzing NADPH production as reducing equivalents for ROS elimination.[99] The PPP genes are strongly activated by Nrf2 in proliferating cells in which the PI3K-Akt pathway is active, and increased expression of the PPP genes contributes to cell proliferation.[98]

Under basal conditions, Keap1 binds to the ETGE and DLG motifs in Nrf2 and recruits Nrf2 to the Keap1-Cul3-E3 ubiquitin ligase complex, leading to ubiquitination and subsequent degradation of Nrf2. Oxidative stress or electrophiles can cause a conformational change in the Keap1-Cul3-E3 ubiquitin ligase by acting on specific cysteine residues in Keap1.[100] These changes disrupt Nrf2-Keap1 binding at

the DLG domain, resulting in stabilization of Nrf2 and translocation of free Nrf2 to the nucleus.[101] Nrf2 is aberrantly accumulated in many types of cancer, and its expression is associated with a poor prognosis in patients.[102-106] In addition, Nrf2 expression is induced during the course of drug resistance in gastric cancer,[107] CRC[108] and esophageal SCC.[109]

ROS and Energy Metabolism in Cancer Cells

Oxygen free radicals are highly reactive with biological molecules, including DNA, proteins and lipids. The free radical reaction could cause oxidative modification of these biomolecules and alter their functions. Mitochondria generate ROS that are thought to augment intra-cellular oxidative stress. In all cells, the majority of ROS are by-products of mitochondrial respiration. Approximately, 2% of the molecular oxygen consumed during respiration is converted into the superoxide anion radical, the precursor of most ROS. Mitochondria possess at least nine known sites that are capable of generating superoxide anion, a progenitor ROS.[110] A mild increase in the level of ROS may result in transient cellular alterations, whereas a severe increase of ROS in cells could cause irreversible oxidative damage, leading to cell death.[111] In normal cells, the ROS level is tightly controlled by the endogenous anti-oxidant system. However, energy metabolism and ROS homeostasis in cancer cells are different from those in normal cells. During the transition phases from normal tissue to invasive carcinoma, ROS levels increase because of metabolic aberrations.[112]

Severe accumulation of cellular ROS under various endogenous and exogenous stress stimuli may induce fatal damage in cells that have inadequate stress responses or adaptation. In cancer cells, ROS stress may induce adaptive stress responses, including activation of redox-sensitive transcription factors, such as nuclear factor κB and Nrf2. These responses lead to an increase in the expression of ROS-scavenging enzymes, such as SOD and glutathione (GSH), elevation of survival factors such as Bcl-2 and MCL1, and inhibition of cell death factors, such as caspases.[111,113,114] ROS-mediated DNA mutations or deletions promote genomic instability and thus induce an additional mechanism for stress adaptation. All these events contribute to the survival of cells with high levels of ROS and maintain cellular viability.[115] As these transcription factors also have roles in regulating the expression of genes that are responsible for proliferation, senescence evasion, angiogenesis and metastasis, and thus the redox adaptation processes may promote cancer development.[116,117] The increase in GSH during the redox adaptation can enhance the export of certain anti-cancer drugs and their inactivation. This altered drug metabolism, together with enhanced cell survival, may render cancer cells more resistant to chemotherapeutic agents.[113,118,119] Activation of oncogenes, aberrant metabolism, mitochondrial dysfunction and loss of functional p53 are intrinsic factors known to cause increased ROS production in cancer cells.[111] In chemotherapy, 5-Fluorouracil (FU) generates mitochondrial ROS via a p53-dependent pathway.[120] Tumor cells which adapt to oxidative stress by increasing the production of SOD2, Prx1 and Bcl-2 are resistant to 5-FU.[121] Products of oxidative stress can slow cell-cycle progression of cancer cells, cause cell-cycle checkpoint arrest and interfere with the ability of anti-cancer drugs to kill cancer cells.[122] The capacity of some chemotherapeutic agents to cause an imbalance in ROS levels offers a therapeutic opportunity for treating cancer.

Considering that cancer cells have increased ROS levels, they may be selectively sensitive to the damaging effects of further increasing ROS. Cancer cells frequently have increased expression of anti-oxidants to maintain homeostasis. Inhibiting anti-oxidants to expose cancer cells to endogenously produced ROS may be a therapeutic approach.[123] In support of this model, several small molecule screens have identified compounds that specifically inhibit the growth of transformed cells. Piperlongumine increases ROS and apoptotic cell death in both cancer cells and normal cells engineered to have a cancer genotype, irrespective of p53 status, with little effect on dividing primary normal cells.[124] Beta-phenylethyl isothiocyanate (PEITC) is a natural compound found in consumable cruciferous vegetables with chemopreventive activity. PEITC increases ROS and selectively kills cancer cells.[125] Malignant cells are often resistant to conventional anti-cancer drugs. These cells are under intrinsic ROS stress, so using small molecules that induce ROS to kill such malignant cells may exert a therapeutic effect.

Cancer Treatment

Novel small molecules targeting metabolic regulators and glycolytic enzymes have been reported to exert anti-proliferative effects.[126] Phloretin, a natural product with GLUT inhibitory activity found in apples and pears, exerts anti-tumor effects in HCC and color cancer cell lines.[127,128] The WZB117 small molecule inhibitor of GLUT 1 was effective in inhibiting cancer cell growth both *in vitro* and *in vivo*.[129] The widely used 3-bromopyruvate (3-BrPA)[130] depletes cellular ATP. A previous study showed that 3-BrPA inhibits HK2 expression and exhibits anti-proliferative effects when combined with daunorubicin in CRC cell lines[131] and when combined with protein disulfide isomerase in HCC cell lines.[132] DCA, a PDK-1 inhibitor, has reduced lactate production and increased responsiveness to 5-FU in MKN45 cells[76] and CRC cell lines.[133] DCA treatment exerts anti-proliferative effects and sorafenib resistance in HCC cell lines *in vivo*.[134] Oxmate, a LDH inhibitor, combined with phenformin, has exhibited cytotoxic effects in diverse cancer cell lines, including colon cancer.[135] Future studies should examine whether

inhibitors of glycolytic enzymes and metabolite transporters are effective in preclinical or clinical settings and evaluate adverse effects and feasibility for clinical practice.

The Nrf2 transcription factor is an important modifier of cellular responses to oxidative stress. Stable RNAi-mediated knockdown of Nrf2 in human colon cancer cells suppressed tumor growth in a xenograft model with a reduction in blood vessel formation and VEGF expression. The Nrf2-inhibited cancer cells failed to accumulate HIF-1A protein under hypoxic conditions.[136] HIF plays a crucial role in cellular adaptation to hypoxia and regulates the expression of genes responsible for glucose metabolism, angiogenesis and cell survival. Conventional anti-cancer therapies typically target actively dividing cells near the vasculature, though they function poorly in hypoxic regions.[137] Cells in hypoxic regions are relatively quiescent, and these cells also tend to be refractory to agents targeting rapidly proliferating cells.[138-140]

Novel therapeutic agents targeting the resistant hypoxic zones may provide additional anti-tumor activity and clinical benefit when combined with conventional treatments. Tirapazamine is a bioreductively activated, hypoxia-selective anti-tumor agent of the benzotriazine series; it is 35-450 times more cytotoxic to hypoxic cells than to well-oxygenated cells.[141] Standard cisplatin chemoradiotherapy plus tirapazamine has not been superior to cisplatin chemoradiotherapy in either progression-free survival or overall survival in locally advanced cervix cancer.[142] TH-302 is a novel therapeutic agent and a hypoxia-activated, cytotoxic prodrug with a 2-nitroimidazole component designed to release the DNA cross-linker bromo-isophosphoramide mustard when reduced by intra-cellular reductases in the setting of severe hypoxia.[143] The phase II study by Borad *et al.*[144] evaluated treatment of TH-302 in patients with either locally advanced or metastatic pancreatic cancer and found that the addition of TH-302 to gemcitabine resulted in a near doubling of progression-free survival and objective response with acceptable toxicity.

Many anti-cancer treatments regulating ROS levels have been demonstrated. NOV-002 is a GSH disulfide mimetic that alters the intra-cellular GSH/GSSG ratio by increasing GSSG levels via the induction of S-glutathionylation.[145] NOV-002 modulates signaling pathways involved in tumor cell proliferation and metastasis and enhances anti-tumor immune responsiveness. NOV-002, in combination with neoadjuvant AC in patients with HER-2 negative breast cancer, was well tolerated and resulted in a favorable pCR rate in a phase II study.[146] Sulfasalazine inhibits xCT (a cystine/glutamate transporter) and reduces the intra-cellular transport of cysteine required for GSH synthesis.[147] Sulfasalazine in combination with conventional anti-cancer agents may be an effective therapy for refractory pancreatic cancer[148] and small cell lung cancer.[149] The small molecule 968 is identified to block glutaminase activation and inhibit the growth of cancer cells, and this enzyme shows potential as a therapeutic strategy against cancer.[150]

Conclusions and Perspective

This review describes recent investigations in mitochondrial metabolism, anaerobic glycolysis and the PPP in cancer. We also discussed the control of ROS levels by the endogenous anti-oxidant system. Key regulators related to cell death in the mitochondria are frequently altered in cancer cells, and mitochondria in cancer differ functionally and structurally from those of normal cells. Mitochondria dysfunction in cancer is associated with the activation of oncogenes and inactivation of tumor suppressors. Recent genetic and metabolic analyses have revealed the molecular mechanisms of genes that are involved in cancer metabolism and tumorigenesis. The alterations of gene expression in glycolysis are associated with poor prognosis and may be associated with chemoradiotherapy resistance. The PPP is positively regulated by oncogenes and regulates cancer cell growth by supplying cells with R5P and NADPH. Direct regulators that activate the PPP have been identified. ROS levels are increased during carcinogenesis from metabolic aberrations. Cancer cells frequently have increased expression of anti-oxidants to maintain homeostasis. Anti-cancer agents targeting ROS status may exert therapeutic effects. Novel small molecules targeting metabolite transporters, glycolytic enzymes and ROS status have been reported. However, further studies should examine whether these inhibitors are useful in cancer therapy and evaluate adverse effects and feasibility for use in clinical practice.

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Conflicts of interest

There are no conflicts of interest.

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