Metabolic Reprogramming in Thyroid Cancer

Sang-Hyeon Ju¹, Minchul Song¹, Joung Youl Lim¹, Yea Eun Kang¹,², Hyon-Seung Yi¹,², Minho Shong³

¹Division of Endocrinology and Metabolism, Department of Internal Medicine, Chungnam National University Hospital; ²Department of Internal Medicine, Chungnam National University College of Medicine; ³Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Korea

Thyroid cancer is a common endocrine malignancy with increasing incidence globally. Although most cases can be treated effectively, some cases are more aggressive and have a higher risk of mortality. Inhibiting RET and BRAF kinases has emerged as a potential therapeutic strategy for the treatment of thyroid cancer, particularly in cases of advanced or aggressive disease. However, the development of resistance mechanisms may limit the efficacy of these kinase inhibitors. Therefore, developing precise strategies to target thyroid cancer cell metabolism and overcome resistance is a critical area of research for advancing thyroid cancer treatment. In the field of cancer therapeutics, researchers have explored combinatorial strategies involving dual metabolic inhibition and metabolic inhibitors in combination with targeted therapy, chemotherapy, and immunotherapy to overcome the challenge of metabolic plasticity. This review highlights the need for new therapeutic approaches for thyroid cancer and discusses promising metabolic inhibitors targeting thyroid cancer. It also discusses the challenges posed by metabolic plasticity in the development of effective strategies for targeting cancer cell metabolism and explores the potential advantages of combined metabolic targeting.

Keywords: Thyroid neoplasms; Tyrosine kinase inhibitors; Drug resistance, neoplasm; Metabolic networks and pathways; Immunotherapy

INTRODUCTION

Thyroid cancer is a common endocrine malignancy that originates from the thyroid gland. The incidence of thyroid cancer has been increasing globally in the past few decades, with an estimated 567,000 new cases and 41,000 deaths in 2020 [1]. The exact causes of thyroid cancer remain elusive, but several risk factors have been identified. The most well-established risk factor for thyroid cancer is exposure to ionizing radiation, particularly during childhood [2]. Other factors that have been associated with an increased risk of thyroid cancer include a family history of the disease, the presence of certain variants in susceptibility genes, such as WD repeat domain 77 (WDR77), BRO1 domain and CAAX motif containing (BROX), hyaluronan binding protein 2 (HABP2), and SLIT-ROBO Rho GTPase activating protein 1 (SRGAP1) for papillary thyroid cancer (PTC), and forkhead box E1 (FOXE1) for both PTC and follicular thyroid cancer (FTC), and certain benign thyroid conditions such as goiter and thyroid nodules [2,3]. PTC is the most common type of thyroid cancer, accounting for 80% to 85% of cases. FTC is the second most common type, representing 10% to 15% of cases, followed by medullary thyroid cancer (MTC) and anaplastic thyroid cancer (ATC), which account for 3%–5% and 1%–2% of cases, respectively [4]. Most cases of thyroid cancer have a favorable prognosis and can be effectively treated, but some cases are more aggressive and have a higher risk of mor-
tality. Consequently, there is a need to identify new targets and develop novel therapeutic approaches to improve the management of thyroid cancer.

Inhibition of RET and BRAF kinases has been explored as a potential therapeutic strategy for the treatment of thyroid cancer, particularly in cases of advanced or aggressive disease. Studies before 2010 reported RET/PTC rearrangements in approximately 10% to 70% of sporadic PTC cases, while recent next-generation sequencing studies have shown a prevalence of up to 7% [5]. RET/PTC rearrangements activate downstream signaling pathways, including the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/AKT pathways, that promote cell proliferation and survival [4,6]. Sorafenib and lenvatinib were the first multi-kinase inhibitors approved for progressive radioiodine refractory differentiated thyroid cancer (DTC), both targeting vascular endothelial growth factor receptors (VEGFRs) and platelet-derived growth factor receptors, with lenvatinib additionally covering fibroblast growth factor receptors and RET [7]. More recently, RET-selective inhibitors (selercatinib and pralsetinib) were approved for advanced or metastatic RET fusion-positive thyroid cancer in 2020, and showed overall response rates of 79% and 89%, respectively [8,9]. Neurotrophic tyrosine receptor kinase (NTRK) fusions are found in about 2% of thyroid cancers [10]. NTRK inhibitors (larotrectinib and entrectinib) were approved for metastatic NTRK fusion-positive solid tumors in 2018, and showed an overall response rate of 79% [11]. Despite the promising response rate of RET-selective inhibitors and NTRK inhibitors, acquired resistance involving on-target or off-target mechanisms has been reported [12,13]. BRAF, a serine/threonine kinase, is mutated in 30% to 80% of PTC cases, with a review published in 2005 reporting an average mutation rate of 44%, and is predominantly found in the classic and tall-cell subtypes [6,14]. The most common mutation is V600E, which results in constitutive activation of the MAPK pathway. Several BRAF kinase inhibitors, such as vemurafenib and dabrafenib, have been approved for the treatment of metastatic melanoma harboring the V600E mutation. These inhibitors have also shown activity in clinical trials for the treatment of BRAF-mutated thyroid cancer, although response rates have varied [15,16]. The main limitation of BRAF kinase inhibitors is the development of resistance, which may occur through activation of alternative signaling pathways, such as the PI3K/AKT pathway [17]. In 2018, a combination of BRAF/MEK inhibitors (dabrafenib and trametinib) was approved for ATC, showing an overall response rate of 67% [18].

Although the inhibition of RET and BRAF kinases has shown therapeutic efficacy in some cases of thyroid cancer, the development of resistance and toxicity highlight the need for further research to identify new targets related to metabolic remodeling and develop alternative therapeutic approaches. Thyroid cancer cells undergo complex metabolic remodeling to support their growth and proliferation, and targeting metabolic pathways may offer new therapeutic opportunities. Dysregulation of regulatory signaling pathways and interactions with the tumor microenvironment (TME) contribute to the induction and coordination of metabolic pathways in thyroid cancer [19]. Moreover, cancer stem cells play a critical role in the metabolic rewiring of thyroid cancer cells [20]. However, resistance mechanisms that arise from dysregulated metabolism and metabolic crosstalk can limit the effectiveness of targeted therapies. Therefore, developing precise strategies to target thyroid cancer cell metabolism (CCM) and overcome resistance is a critical area of research for advancing thyroid cancer treatment.

**ALTERED THYROID CANCER CELL METABOLISM**

**Metabolic dependencies in thyroid cancer cells**

In general, cancer cells exhibit altered metabolism, which is characterized by increased glucose uptake, a greater reliance on glycolysis, and changes in amino acid metabolism and the tricarboxylic acid (TCA) cycle (Fig. 1). Moreover, cancer cells often rely on the pentose phosphate pathway (PPP) to support nucleotide synthesis and antioxidant defense [21,22].

Glycolysis is a critical metabolic pathway that provides cancer cells with energy and building blocks for biosynthesis. Cancer cells upregulate glucose transporters (GLUTs) and glycolytic enzymes to increase glucose uptake and glycolytic flux. This phenomenon, known as the Warburg effect, is a hallmark of cancer metabolism [23]. The Warburg effect allows cancer cells to rapidly generate adenosine triphosphate (ATP) and produce intermediates for the biosynthesis of nucleotides, amino acids, and lipids. Moreover, the Warburg effect leads to the accumulation of metabolic intermediates that can promote cell growth and proliferation through various signaling pathways [23]. A hallmark of thyroid cancer metabolism is the upregulation of GLUTs, such as GLUT1 and GLUT3, which facilitate the uptake of glucose into cancer cells [24]. In addition, thyroid cancer cells exhibit increased expression of key glycolytic enzymes, including hexokinase 2 (HK2), phosphofructokinase (PFK), and lactate dehydrogenase (LDH), which contribute to a higher level
Fig. 1. Metabolic reprogramming in thyroid cancer and therapeutic resistance. (A) Metabolic reprogramming of thyroid cancer is illustrated. Glucose import is increased by higher levels of glucose transporter 1 (GLUT1) and GLUT3 in the cell membrane. Glycolysis is upregulated by the elevated expression of hexokinase 2 (HK2) and the rate-limiting enzyme of glycolysis, phosphofructokinase-1 (PFK-1). Increased lactate dehydrogenase (LDH) convert pyruvate into lactate, which is exported to the tumor microenvironment via monocarboxylate transporter 4 (MCT4). The final product of glycolysis, pyruvate, is converted into acetyl coenzyme A (acetyl-CoA) in oxygen-enriched conditions, and enters the tricarboxylic acid (TCA) cycle in the mitochondria. Citrate, an intermediate of the TCA cycle, could be exported to the cytoplasm via mitochondrial citrate carrier (CIC) and used for fatty acid synthesis. During glycolysis, the shunt pathways, including the pentose phosphate pathway (PPP) and serine synthesis pathway, are activated to produce ribose-5-phosphate (R5P) and nicotinamide adenine dinucleotide from PPP and serine and nicotinamide adenine dinucleotide from the serine synthesis pathway. The serine synthesis pathway is closely connected to one-carbon metabolism by the serine hydroxymethyltransferase (SHMT) enzyme. The amino acid transporters, L-type amino acid transporter 1 (LAT1) and alanine-serine-cysteine transporter 2 (ASCT2), are upregulated in thyroid cancer cells. The imported glutamine enters the mitochondria via glutamate carrier 1 (GC1) and is hydrolyzed by glutaminase to yield glutamate, which is converted into α-ketoglutarate (α-KG) to enter the TCA cycle. (B) The pathologic signaling pathways and related metabolic reprogramming in thyroid cancer cells that induce resistance to therapies. G6P, glucose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase; F6P, fructose-6-phosphate; F1,6BP, fructose 1,6-bisphosphate; 3-PG, 3-phosphoglycerate; THF, tetrahydrofolate; meTHF, 5,10-methylenetetrahydrofolate; EAA, essential amino acids; PI3K, phosphoinositide 3-kinase; mTOR, mammalian target of rapamycin; MAPK, mitogen-activated protein kinase; HIF-1α, hypoxia-inducible factor 1α; ATC, anaplastic thyroid cancer; RAI, radioactive iodine.
of glycolytic flux [25]. Studies have shown that the activation of oncogenic signaling pathways, such as the PI3K/AKT/mammalian target of rapamycin (mTOR) pathway and the MAPK pathway, can drive the upregulation of glycolytic enzymes in thyroid cancer cells [26]. Moreover, the hypoxic TME, which is a common feature of solid tumors, can also upregulate glycolysis in thyroid cancer cells by stabilizing hypoxia-inducible factor 1α (HIF-1α), a transcription factor that promotes the expression of glycolytic enzymes [27]. Refractory thyroid cancer refers to thyroid cancer that remains unresponsive or progresses despite RAI therapy, which is typically administered after initial surgical treatment [28]. Glycolysis has been implicated in the development of refractory thyroid cancer, as it plays a critical role in the metabolic adaptation of cancer cells to treatment-induced stress [29]. A study found that refractory thyroid cancer cells exhibited higher rates of glycolysis than sensitive cells, and that this metabolic adaptation was associated with increased HK2 and LDH expression [30]. In addition, the activation of the PI3K/AKT/mTOR pathway has been implicated in the development of resistance to RAI therapy in thyroid cancer, and this pathway is known to upregulate glycolytic enzymes [31].

Amino acid metabolism also plays an essential role in CCM. Cancer cells upregulate amino acid transporters and amino acid metabolic enzymes to support the increased demand for protein synthesis and other cellular processes. Moreover, amino acids can also act as signaling molecules and promote cancer cell survival and growth through various signaling pathways. For example, the amino acid glutamine is an important source of energy and carbon for cancer cells, and it also promotes the activation of the mTOR signaling pathway, which is critical for cancer cell proliferation [32]. In thyroid cancer, increased amino acid transport and metabolism have been shown to contribute to the growth and survival of cancer cells [33]. Specifically, increased expression of the amino acid transporter L-type amino acid transporter 1 (LAT1) has been observed in thyroid cancer, facilitating the uptake of essential amino acids, such as leucine, for use in protein synthesis and energy production [34]. The activation of the mTOR signaling pathway, which is commonly observed in thyroid cancer, promotes amino acid metabolism by upregulating amino acid transporters and amino acid metabolic enzymes [35]. ATC is an aggressive and highly lethal form of thyroid cancer that is associated with significant alterations in amino acid metabolism. A study found that ATC cells exhibited increased expression of several amino acid transporters, including LAT1, and increased rates of amino acid uptake and metabolism [36]. This increased amino acid metabolism was associated with the activation of the mTOR signaling pathway and the upregulation of amino acid metabolic enzymes, such as glutaminase (GLS) and alanine transaminase [37,38].

The TCA cycle is a central metabolic pathway that generates ATP and provides precursors for biosynthesis. In cancer cells, the TCA cycle is often dysregulated, with some intermediate metabolites diverted to support biosynthesis or other metabolic pathways [39]. For example, the intermediate citrate is exported from the mitochondria and used for fatty acid synthesis, which is critical for the formation of cellular membranes [40]. Studies have shown that the activation of oncogenic signaling pathways, such as the MAPK pathway, can drive the upregulation of enzymes involved in fatty acid synthesis in DTC cells [41]. Moreover, dysregulation of the TCA cycle has been shown to contribute to the development of resistance to targeted therapies, such as BRAF inhibitors [42]. ATC cells exhibit increased expression of several key enzymes in the TCA cycle, including pyruvate dehydrogenase and citrate synthase; this upregulation contributes to the increased production of ATP and nicotinamide adenine dinucleotide phosphate (NADPH), which supports cancer cell proliferation and survival [43].

Glutamine is a non-essential amino acid that plays a critical role in CCM. Glutamine is a major source of energy and carbon for cancer cells, and it is also involved in the synthesis of nucleotides, amino acids, and lipids. Glutaminolysis is the process by which glutamine is converted to α-ketoglutarate, which can enter the TCA cycle and contribute to energy production and biosynthesis. In DTC, glutamine metabolism and glutaminolysis have been shown to be dysregulated, with cancer cells exhibiting increased expression of glutamine transporters and enzymes involved in glutaminolysis, such as GLS [44,45]. Moreover, the activation of the mTOR signaling pathway, which is commonly observed in DTC, promotes glutamine metabolism by upregulating amino acid transporters and metabolic enzymes [46]. In ATC, glutamine metabolism and glutaminolysis are also dysregulated, with cancer cells exhibiting increased expression of glutamine transporters and enzymes involved in glutaminolysis [26]. A study found that ATC cells displayed increased expression of the glutamine transporter alanine-serine-cysteine transporter 2 (ASCT2) and the GLS isoform glutaminase C, contributing to the increased utilization of glutamine for energy production and biosynthesis [47]. Moreover, the upregulation of glutaminolysis has been implicated in the development of resistance to chemotherapy in ATC [48].

Finally, the PPP is a metabolic pathway that generates NADPH and ribose-5-phosphate for nucleotide synthesis and
antioxidant defense. The PPP plays a critical role in CCM, and alterations in PPP activity have been observed in thyroid cancer, including DTC and ATC [26]. Upregulation of the PPP has been shown to contribute to cancer cell proliferation and survival in DTC. A study found that DTC cells exhibited increased expression of enzymes involved in the PPP, including glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, which contributed to the increased production of NADPH and ribose-5-phosphate [49]. Moreover, the upregulation of PPP activity has been shown to be associated with the activation of the PI3K/AKT/mTOR pathway, which is commonly observed in DTC. Alterations in PPP activity have also been observed, with cancer cells exhibiting increased expression of enzymes involved in the PPP in ATC. ATC cells displayed increased expression of glucose-6-phosphate dehydrogenase and transketolase, contributing to the increased production of NADPH and ribose-5-phosphate [26]. Moreover, the upregulation of PPP activity has been implicated in the development of therapeutic resistance in ATC, as PPP activity can provide cancer cells with antioxidant defense mechanisms to protect against chemotherapyproduced oxidative stress [49].

**Metabolic reprogramming by oncogenes and tumor-suppressor genes**

Tumors are highly heterogeneous, but metabolic reprogramming in cancer cells seems to involve a common set of pathways to support anabolism, catabolism, and redox homeostasis. The PI3K/Akt/mTOR pathway is the central regulator of cellular energetics and metabolism and is responsible for increasing glycolysis and fatty acid synthesis via HIF-1α and sterol regulatory element-binding protein (SREBP) activation, respectively [50,51]. Malignant tumors co-opt this network, with mutations in upstream receptor tyrosine kinases, the PI3K catalytic subunit, downstream Akt kinase, and negative regulator phosphatase and tensin homolog (PTEN) being frequently observed in cancer. Hypoxia is also a frequent feature of tumors, as rapid proliferation often exceeds angiogenesis. To adapt to hypoxia, tumors upregulate HIF-1α signaling, which is a downstream effector of the PI3K/Akt/mTOR pathway [52]. Activation of the HIF-1α pathway in cancer cells leads to increased glycolysis, which can deplete glucose levels and reduce energy stores, resulting in increased intracellular adenosine monophosphate (AMP)/ATP levels [43]. This, in turn, activates the AMP-activated protein kinase (AMPK)-liver kinase B1 (LKB1) pathway, which stimulates catabolic pathways that produce ATP, mainly through upregulating oxidative phosphorylation (OXPHOS) and mitochondrial biogenesis [53]. AMPK also increases cellular levels of nicotinamide adenine dinucleotide (NAD+), which activates sirtuin 1 (SIRT1) and downstream targets such as peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) and forkhead box O (FOXO) transcription factors, promoting mitochondrial biogenesis and activity [54]. These mechanisms maintain energy stores and promote efficient ATP production in cancer cells. Studies have shown that the \( \text{BRAF}^{\text{V600E}} \) mutation can activate the MAPK/extracellular signal-regulated kinase (ERK) pathway, which in turn upregulates genes involved in glycolysis and glutaminolysis, leading to an increase in glucose and glutamine uptake and utilization in PTC cells [44]. In addition, the \( \text{BRAF}^{\text{V600E}} \) mutation has been shown to promote the expression of HIF-1α, a transcription factor that regulates cellular responses to hypoxia. HIF-1α can activate genes involved in glycolysis, angiogenesis, and apoptosis, and has been implicated in the development and progression of several cancers, including thyroid cancer [55]. \( \text{BRAF}^{\text{V600E}} \) also promotes the expression of GLUT1, which increases glucose uptake in thyroid cancer cells. This increased glucose uptake and utilization support the high energy demands of rapidly proliferating cancer cells [56].

Studies have shown that \( \text{RET}/\text{PTC} \)-positive PTCs display alterations in metabolic pathways, including increased glucose uptake and glycolysis, as well as increased lipid synthesis and uptake. The TPC-1 (human papillary thyroid cancer) cell line, a PTC cell line driven by the \( \text{RET}/\text{PTC1} \) rearrangement that expresses the coiled-coil domain containing 6/rearranged during transfection (CCDC6/RET) fusion protein, demonstrates elevated levels of HIF-1α and its downstream targets, promoting glycolysis [57]. Furthermore, \( \text{RET}/\text{PTC1} \) and \( \text{RET}/\text{PTC3} \) have been shown to increase the expression of phosphoinositide-dependent kinase 1 (PKD1), which inhibits the activity of pyruvate dehydrogenase, a key enzyme in mitochondrial metabolism. This results in a shift towards aerobic glycolysis, leading to increased lactate production and secretion, which can contribute to the acidification of the TME and promote tumor growth [58].

Driver oncogenes play a critical role in the metabolic reprogramming of thyroid cancer. The oncogenic activity of c-MYC, which is encoded by the \( \text{MYC} \) gene, has been reported to promote aerobic glycolysis by upregulating lactate dehydrogenase A, GLUT1, and glycolytic enzymes including PFK-1 and enolase [59]. c-MYC also facilitates the uptake and catabolism of glutamine, which is a significant source of energy for cancer cells. The expression of genes essential for glutamine metabolism, including GLS, glutamine synthetase (GLUL), and the glutamine cell-entry transporter ASCT2 (SLC1A5), is induced
by c-MYC [60].

Mutations in RAS oncogenes have been reported in 12%–16% of PTCs, predominantly in follicular-variant PTCs, 25%–30% of FTCs, and 20%–40% of poorly differentiated thyroid cancers (PDTCs), ATCs, and even follicular adenoma [6,61]. Among the RAS oncogenes, NRAS is the most commonly mutated isoform, followed by HRAS and KRAS [62]. The activation of the RAS signaling pathway can lead to changes in metabolic reprogramming in cancer cells [63]. An integrative multi-omics analysis of PTC with the BRAFV600E mutation, FTC with RAS mutation, and ATC found that one-carbon metabolism and pyrimidine metabolism were upregulated in both PTC and FTC, and to a greater extent in ATC. All subtypes of thyroid cancer exhibited increased expression of serine hydroxymethyltransferase 2 (SHMT2), a key enzyme in one-carbon metabolism. In that study, compared to PTC, FTC showed enrichment in branched-chain amino acid degradation and the TCA cycle, exhibiting shared but distinct metabolic features [64].

Specifically, KRAS mutations have been shown to enhance glucose uptake and glycolysis in an FTC cell line, potentially promoting tumor growth and survival [65]. Additionally, KRAS activation has been linked to increased glutamine metabolism and the diversion of glucose-derived carbon to support nucleotide biosynthesis [66]. Overall, RAS activation in thyroid cancer may contribute to metabolic remodeling, which supports tumor growth and survival.

Resistance to tyrosine kinase inhibitors

Despite the progress in cancer treatment and the availability of multimodal therapy for advanced thyroid cancer, the emergence of resistance remains a major obstacle contributing to treatment failure. This section delves into how metabolic reprogramming in cancer cells contributes to therapeutic resistance in refractory and undifferentiated thyroid cancer.

Resistance to cell signaling pathway inhibitors

The treatment-induced metabolic adaptation of many cancers is a mechanism of therapeutic resistance, especially in oncogene-addicted tumors treated with tyrosine kinase inhibitors (TKIs). This resistance is often accompanied by a metabolic switch to OXPHOS for survival, contributing to treatment failure and cancer progression. For example, treatment of epidermal growth factor receptor (EGFR)-mutant non-small cell lung cancer (NSCLC) with osimertinib, a third-generation TKI, led to acquired resistance with glycolytic suppression and a metabolic switch to OXPHOS, as observed in gefitinib-treated EGFR-mutant NSCLC and vemurafenib-treated BRAF-mutant melanoma [67]. In these cases, OXPHOS inhibition restored sensitivity to TKI therapy, prolonged survival, and reduced the tumor burden in vivo [67].

The relationship between OXPHOS and TKI resistance has been attributed to various mechanisms. For instance, BRAF-mutant melanomas treated with the BRAF inhibitor vemurafenib or the MEK inhibitor selumetinib exhibit microphthali-

ma-associated transcription factor (MITF) signaling and elevated expression of the mitochondrial master regulator PGC-1α [68]. This results in a PGC-1α-mediated induction of an OX-

PHOS gene program and mitochondrialgenesis. Additionally, signal transducer and activator of transcription 3 (STAT3) signaling has been proposed as another mechanism underlying the upregulation of OXPHOS in response to pathway-targeted therapy [69]. Various oncogene-addicted cancer cells engage in a positive feedback loop leading to STAT3 activation, which limits drug response. Non-canonical STAT3 signaling via GRIM-19 (gene associated with retinoid-interferon-induced mortality)-dependent import of STAT3 into the mitochondria increases the activity of complexes I and II of the electron transport chain and OXPHOS, leading to TKI therapeutic resistance [70].

Metabolic remodeling in thyroid cancer may affect the effectiveness of sorafenib and vemurafenib. In a phase 3 clinical trial, sorafenib improved progression-free survival in progressive RAI-refractory DTC by 5 months compared to placebo [71]. In a phase 2 clinical trial, patients with recurrent or metastatic PTC refractory to RAI and positive for the BRAFV600E mutation were treated with vemurafenib. Patients who were previously treated with a VEGFR multi-kinase inhibitor exhibited shorter progression-free survival and overall survival compared to those naive to multi-kinase inhibitor therapy [72]. Tumor cells may adapt to treatment-induced stress by altering their metabolism, potentially leading to the development of resistance. In vitro studies have demonstrated that sorafenib can induce metabolic changes in thyroid cancer cells, such as increased glucose uptake and lactate production, which may contribute to drug resistance [73]. Additionally, sorafenib has been shown to induce autophagy, a process wherein cells degrade and recycle cellular components, thereby promoting tumor cell survival under metabolic stress conditions [74]. Similarly, vemurafenib has also been shown to induce metabolic changes in melanoma cells, such as increased glycolysis and decreased OXPHOS, potentially contributing to drug resistance [42]. Moreover, in thyroid cancer cells harboring the BRAFV600E mutation, vemurafenib-induced metabolic changes were associated with the upregulation of glutaminoly-
sis, a metabolic pathway that utilizes glutamine to produce energy [43]. These findings suggest that metabolic remodeling may play a significant role in the development of resistance to sorafenib and vemurafenib in thyroid cancer.

Studies have suggested that restoring metabolic remodeling may potentiate the effectiveness of anti-cancer therapies in thyroid cancer. For example, glycolytic inhibition using 2-deoxyglucose (2-DG) sensitized ATC cells to cisplatin chemotherapy and external beam radiation [75]. Dichloroacetate, an inhibitor of pyruvate dehydrogenase kinase, suppresses glycolysis and thereby exhibits an anti-proliferative effect on ATC cells [76]. In PTC and ATC cell lines, aurora-A, a member of the aurora serine/threonine kinase family, induces glycolysis by activating 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3), and the inhibition of aurora-A with its selective inhibitor, alisertib, has been shown to improve the efficacy of sorafenib in both PTC and ATC cells [77]. Moreover, in vitro studies have demonstrated that metabolic modulators such as metformin and phenformin that inhibit complex I of the mitochondrial electron transport chain make vemurafenib and sorafenib more effective [78-80]. These findings suggest that restoration of metabolic remodeling could be a promising strategy to increase the effectiveness of sorafenib and vemurafenib in the treatment of thyroid cancer.

**METABOLIC CROSSTALK WITH THE TUMOR MICROENVIRONMENT**

The TME refers to the local environment surrounding a tumor, which includes a complex network of various cell types (such as fibroblasts, immune cells, and endothelial cells [ECs]), extracellular matrix, signaling molecules, and physical factors (Fig. 2). These cells undergo metabolic reprogramming in the thyroid cancer microenvironment (Table 1). The TME plays a crucial role in tumor initiation, growth, invasion, and metastasis, as well as in determining the response to therapy [81]. Tumor cells can actively modify and interact with their microenvironment, leading to the creation of an immunosuppressive and pro-tumor environment that supports cancer progression. Understanding the dynamics of the TME and the interactions between tumor cells and their surroundings is essential for the development of effective cancer therapies.

---

**Fig. 2.** Metabolic reprogramming induced by genetic alterations and interactions with the tumor microenvironment in thyroid cancer. Thyroid cancer cells manifest distinct metabolic changes, such as elevated glycolysis (the Warburg effect) and alterations in crucial metabolic pathways, contributing to therapeutic resistance and oncogenic progression. These metabolic shifts are influenced by genetic alterations, including the *BRAF*<sup>V600E</sup> mutation, *RET/papillary thyroid cancer (PTC)* rearrangements, *MYC* overexpression, and *RAS* mutations. The tumor microenvironment (TME), comprising diverse cellular components such as cancer-associated fibroblasts (CAFs), extracellular matrix (ECM), endothelial cells, and immune cells, plays a pivotal role in tumor progression and response to therapy. A dynamic metabolic crosstalk within the TME is essential for tumor development. The metabolic reprogramming of immune cells significantly affects their anti-tumor activity. Understanding these complex interactions is crucial for developing targeted cancer therapies. OXPHOS, oxidative phosphorylation; PPP, pentose phosphate pathway; NK, natural killer; TAM, tumor-associated macrophage.
Cancer-associated fibroblasts

Solid tumors often have a hypoxic and hypoglycemic core due to rapid growth, resulting in a nutrient-poor environment that hinders tumor growth. Tumor cells overcome this limitation by reprogramming stromal cells in the TME. Cancer-associated fibroblasts (CAFs) are a key stromal component that provides metabolic support to tumor cells, thereby facilitating tumor initiation, growth, invasion, and dissemination. This is achieved through metabolic reprogramming of CAFs, which release energetic substrates into the TME, a phenomenon known as “tumor-feeding” [82]. Several modes of tumor-feeding have been proposed, including the “reverse Warburg effect.” In this mode, CAFs undergo metabolic reprogramming toward a glycolytic phenotype, while the associated cancer cells are reprogrammed toward OXPHOS [83]. CAFs produce lactate, which is exported via the monocarboxylate transporter-4 (MCT4) into the TME and taken up by tumor cells via the MCT1 transporter. In advanced PTC, MCT4 expression was found to be higher than in low-stage disease [84].

Cancer-associated fibroblasts

Solid tumors often have a hypoxic and hypoglycemic core due to rapid growth, resulting in a nutrient-poor environment that hinders tumor growth. Tumor cells overcome this limitation by reprogramming stromal cells in the TME. Cancer-associated fibroblasts (CAFs) are a key stromal component that provides metabolic support to tumor cells, thereby facilitating tumor initiation, growth, invasion, and dissemination. This is achieved through metabolic reprogramming of CAFs, which release energetic substrates into the TME, a phenomenon known as “tumor-feeding” [82]. Several modes of tumor-feeding have been proposed, including the “reverse Warburg effect.” In this mode, CAFs undergo metabolic reprogramming toward a glycolytic phenotype, while the associated cancer cells are reprogrammed toward OXPHOS [83]. CAFs produce lactate, which is exported via the monocarboxylate transporter-4 (MCT4) into the TME and taken up by tumor cells via the MCT1 transporter. In advanced PTC, MCT4 expression was found to be higher than in low-stage disease [84]. CAFs upregulate glycolysis-related enzymes, such as HK2 and 6-phosphofructokinase liver type (PFKL), which is thought to occur via signaling with platelet-derived growth factor receptor-β (PDGF-β) and α-SMA, α-smooth muscle actin; GLUT1, glucose transporter 1; PTC, follicular thyroid cancer; AhR, aryl hydrocarbon receptor; STAT, signal transducer and activator of transcription; MCT4, monocarboxylate transporter 4; ATC, anaplastic thyroid cancer; PDGFR-β, platelet-derived growth factor receptor-β; α-SMA, α-smooth muscle actin; GLUT1, glucose transporter 1; MCT4, monocarboxylate transporter 4; ATC, anaplastic thyroid cancer; PDGFR-β, platelet-derived growth factor receptor-β; α-SMA, α-smooth muscle actin; GLUT1, glucose transporter 1; FTC, follicular thyroid cancer; AhR, aryl hydrocarbon receptor; STAT, signal transducer and activator of transcription; NKG2D, natural killer group 2, member D; NKp46, natural killer cell p46-related protein; PGE2, prostaglandin E2; EP, prostaglandin E2 receptor; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; NF-κB, nuclear factor κB; IFN-γ, interferon-γ; GPR81, G-protein-coupled receptor 81; mTOR, mammalian target of rapamycin; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; PKM2, pyruvate kinase M2; TNF-α, tumor necrosis factor-α; IL, interleukin; ROS, reactive oxygen species; NOX2, nicotinamide adenine dinucleotide phosphate oxidase 2; NADPH, nicotinamide adenine dinucleotide phosphate.
which fuels the TCA cycle and supports energy production in cancer cells [85]. Apart from the direct release of metabolites into the TME, stromal cells have also been reported to fuel cancer metabolism by releasing metabolites carried in exosomes. These CAF-derived exosomes fuel cancer metabolism by supplying amino acids, lipids, and TCA cycle intermediates [86]. Similarly, in the context of thyroid cancer, CAFs release factors that activate the Src/Akt pathway in quiescent fibroblasts around ATC cells, enhancing CAF markers and GLUT1 expression. These factors also increase proliferation and invasiveness, and induce the epithelial-to-mesenchymal transition in FTC cells, thereby promoting tumorigenic alterations in thyroid cancer (Table 1) [87].

Metabolic remodeling in thyroid cancer can affect CAFs and the TME. The metabolic reprogramming of cancer cells can induce changes in the TME, leading to the activation of CAFs and promoting tumor growth and invasion. CAFs, in turn, can also reprogram their metabolism to support the energy needs of cancer cells, further contributing to the altered metabolism of the TME. This metabolic shift can also promote the secretion of cytokines and growth factors by CAFs, further modulating the TME and promoting tumor progression [85]. Therefore, targeting the metabolic crosstalk between cancer cells, CAFs, and the TME may offer a potential therapeutic strategy for thyroid cancer.

**Endothelial cells**

ECs play an important role in tumor angiogenesis by providing oxygen and nutrients to cancer cells. The metabolic signatures of ECs are altered in cancer, and the vascular EC function is modulated by metabolites [88]. Tumor paracrine signaling promotes a glycolytic phenotype in ECs by upregulating surface GLUT1 and PFKFB3, which activates PFK-1 to further increase glycolysis [89]. Lactate, which is enriched in the TME, triggers tube formation in ECs via HIIF-1α-dependent nuclear factor κB (NF-κB) activation [90]. Amino acids also play a role in EC proliferation and migration. Glutamine is required for TCA cycle anaplerosis and non-essential amino acid synthesis, and depletion of glutamine or inhibition of GLS1 causes vessel sprouting defects due to impaired EC proliferation and migration [46]. Glycine and serine are also required for vascular endothelial growth factor (VEGF) signaling and mitochondrial function in ECs. Finally, fatty acids supply the carbon needed for deoxynucleotide triphosphate (dNTP) synthesis during EC sprouting, and altered CCM may affect fatty acid availability to support EC proliferation [91]. Overall, these changes in EC metabolism may contribute to the pathogenesis and progression of thyroid cancer.

**TUMOR IMMUNE MICROENVIRONMENT**

**T cells**

The immune system plays a crucial role in tumor development and progression, with bidirectional crosstalk that can either inhibit or promote tumor growth (Fig. 2). An effective immune response depends on rapid adaptation to stimuli through metabolic reprogramming of immune cells [92,93]. During T cell activation, there is a metabolic shift from predominantly OXPHOS in resting T cells to increased glycolysis in activated T cells, facilitated by the upregulation of GLUTs and glycolytic enzymes [94]. This metabolic switch, known as the Warburg effect and similar to the reprogramming observed in cancer cells, enables rapid ATP production, supporting T cell proliferation, cytokine secretion, and effector functions. Additionally, activated T cells increase the uptake and utilization of amino acids and fatty acids for the biosynthesis of proteins, nucleotides, and lipids necessary for cell division and differentiation. However, in the TME, tumor cells can compete with T cells for essential nutrients, such as glucose and glutamine, which can impair T cell metabolism and function [95]. Additionally, the high production of metabolites by tumor cells, such as lactate and kynurenine, can create an immunosuppressive environment and induce the polarization of immunosuppressive T cell subsets, such as Tregs, exhausted T cells, and myeloid-derived suppressor cells (MDSCs) [86,96]. Overall, altered metabolic reprogramming in cancer can both deprive T cells of essential nutrients for anti-tumor activity and induce polarization of immunosuppressive T cell subsets, leading to T cell dysfunction and impaired anti-tumor immunity, and allowing the cancer to evade the immune system and proliferate.

**Natural killer cells and neutrophils**

Altered metabolic reprogramming in cancer can impair the function of natural killer (NK) cells and neutrophils, which are key components of the innate immune system, by inducing nutrient scarcity and producing cancer metabolites that impair anti-tumor immunity. The TME creates an immunosuppressive environment that inhibits the function of NK cells and neutrophils mediated by TGF-β, interleukin 10 (IL-10), and prostaglandin E2 (PGE2) released from tumor cells and other cells within the TME [97]. Furthermore, recent studies have shown that NK cells and neutrophils can also undergo metabolic reprogramming in response to the TME. For example, in the TME,
NK cells can shift their metabolism towards glycolysis, which impairs their cytotoxic function [98]. Kynurenine secreted by thyroid cancer cells enters NK cells via aryl hydrocarbon receptor (AhR) and activates the STAT1 and STAT3 pathways, reducing the expression of activating receptors, natural killer group 2, member D (NKG2D) and natural killer cell p46-related protein (NKP46) [99]. Concurrently, PGE2, also secreted by thyroid cancer cells, binds to prostaglandin E2 receptor subtypes 2 and 4 (EP2 and EP4) and inhibits the MAPK/ERK and NF-κB pathways, leading to decreased cytotoxicity and interferon-γ (IFN-γ) production (Table 1) [100]. Similarly, neutrophils in the TME also undergo metabolic reprogramming toward an immunosuppressive phenotype that supports tumor growth and progression [101].

**Tumor-associated macrophages**

The relationship between tumor-associated macrophages (TAMs) and cancer cells is complex, with both influencing each other’s behavior. TAMs can either potentially eliminate tumor cells or promote their survival, proliferation, metastasis, angiogenesis, and immune suppression. The specific impact of TAMs on tumor progression depends on their reprogramming within the TME, influenced by factors including hypoxia, local mediators (such as cytokines and growth factors), and metabolic products from cancer or other immune and stromal cells. One key factor driving this reprogramming is lactate, which activates the G-protein-coupled receptor 81 (GPR81) receptor on TAMs, initiating a series of events that activate the AKT1/mTOR pathway, leading to increased aerobic glycolysis (Table 1) [102]. Another study has reported that TAMs in thyroid cancer undergo a distinct metabolic shift, involving increased fatty acid synthesis, elevated glycolysis, and the release of cytokines, including both pro-inflammatory (tumor necrosis factor-α and IL-6) and immunosuppressive (IL-10) types [103,104]. These findings highlight the profound impact of metabolic reprogramming on TAMs, emphasizing their significant role in the TME.

**PD-1 and CTLA-4 signaling and the immune checkpoint blockade on metabolic pathways**

The immune checkpoints programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) negatively regulate T cell function, limiting T cell activation to prevent excess inflammation and tissue damage [105]. In cancer, programmed death-ligand 1 (PD-L1) and PD-L2 ligands are upregulated on cancer cells to dampen anti-tumor immunity. Engagement of PD-1 by its ligands results in the recruitment of protein tyrosine phosphatases such as Src homology region 2 domain-containing phosphatase-2 (SHP2), which antagonizes downstream pathways required for metabolic reprogramming of activated T cells, including PI3K/Akt, Ras, ERK, Vav, and phospholipase C gamma (PLCγ) [106]. PD-1/PD-L1 signaling is known to suppress glycolysis and promote lipolysis and fatty acid oxidation, and anti-PD-1/PD-L1 and anti-CTLA-4 antibodies have shown to upregulate glycolysis and promote IFN-γ production [107,108]. Based on this understanding, the combination of PD-1 blockade therapies with interventions targeting cellular metabolism has the potential to enhance the effectiveness of anti-tumor immune responses. Overall, the function of PD-1 and CTLA-4 in antagonizing key metabolic pathways in T cells provides a mechanism by which tumors limit anti-tumor immunity and highlights the potential for metabolic invigoration of T cells by immune checkpoint blockade.

PD-L1 expression has been found to be higher in ATC than in other subtypes, and the tumor mutational burden is generally higher in ATC [109,110]. Different subtypes of thyroid cancer also have different immune infiltrates in the TME, with DTC having a lower immune infiltration dominated by regulatory T cells (Tregs) and fewer cytotoxic T cells, whereas ATC and MTC show robust immune infiltrates, including Tregs, MDSCs, and TAMs [111,112]. However, clinical trials with immunotherapy have shown limited efficacy, and there is a need for further investigation into new strategies, validation of predictive biomarkers, and better population selection for clinical trials in thyroid neoplasm [113].

**Resistance to immunotherapies**

Immunotherapy, while successful in treating several types of cancers, has shown comparatively limited efficacy in thyroid cancer [112]. This reduced effectiveness of immunotherapy in thyroid cancer is due to multiple contributing factors that lead to immune evasion and immunosuppression. One key aspect is poor tumor immunogenicity, a consequence of tumor editing, where tumors evolve to escape immune detection. Another contributing factor is the upregulation of immune checkpoint ligands, often exacerbated by the dysregulated expression of key metabolic enzymes such as pyruvate kinase M2 (PKM2) [114]. Additionally, the effectiveness of immunotherapy is compromised by the lack of tumor-infiltrating T cells due to the release of immunosuppressive cytokines and upregulation of immune checkpoint ligands induced by CAFs [115]. Moreover, CAFs are glycolytically active and release metabolic fuels into the TME, thereby feeding adjacent cancer cells and reprogramming...
cellular metabolism toward OXPHOS [82]. Finally, metabolic reprogramming of cancer cells not only affects the tumor’s mutation rate and antigenicity, but also contributes to an immunosuppressive tumor environment through nutrient competition with immune cells and production of cancer metabolites. These metabolic changes significantly impact both the behavior of the tumor cells and the effectiveness of immunotherapy in thyroid cancer.

**THERAPEUTIC OPPORTUNITIES TARGETING METABOLIC REPROGRAMMING**

Recent studies have suggested that targeting dysregulated cancer metabolism and the metabolic interplay of cancer cells, TME, and cancer stem cells can overcome therapeutic resistance [20]. The following sections highlight promising strategies that target altered pathways of metabolism, alone or in combination with other available anti-cancer therapies. These strategies include targeting glycolysis, glutamine metabolism, fatty acid metabolism, mitochondrial metabolism, and the TME. Metabolic inhibitors and subtypes of thyroid cancer to which they have been applied *in vitro or in vivo* are shown in Table 2 [75,116-140].

**Glycolysis inhibitors**

Some studies have shown that thyroid cancer cells exhibit higher levels of glucose metabolism and glycolytic enzymes than normal thyroid cells, suggesting that targeting glycolysis may be a viable therapeutic strategy [26]. Additionally, some research indicates that inhibiting glycolysis can sensitize thyroid cancer cells to radiation therapy, a common treatment for thyroid cancer [75]. However, limited experimental evidence supports the use of glycolysis inhibitors for thyroid cancer, specifically ATC.

In a broader context, inhibitors of glycolysis present a promising strategy for cancer treatment. HK2, because of its role in maintaining high glycolytic rates, has emerged as a potential target. Known HK2 inhibitors such as 2-DG, 3-bromopyruvate, and lonidamine have been identified. Notably, 2-DG has shown promising outcomes as a single-agent therapy in phase I/II clinical trials [141,142]. PFKFB3, a significant regulator of glycolysis that is often upregulated in cancers, has inhibitors (e.g., PFK15 and PFK158) that have displayed anti-neoplastic prop-

<table>
<thead>
<tr>
<th>Target metabolic pathway</th>
<th>Metabolic inhibitors</th>
<th>Drugs</th>
<th>Preclinical data</th>
<th>Clinical data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycolysis</td>
<td>HK2 inhibitor</td>
<td>2-DG</td>
<td>PTC, with doxorubicin or sorafenib [128]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PTC and FTC, with metformin [129]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-BP</td>
<td>ATC [75]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ATC [130]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PDTC [131]</td>
<td></td>
</tr>
<tr>
<td>Oxidative phosphorylation</td>
<td>OXPHOS inhibitor</td>
<td>Metformin</td>
<td>PTC [116-123]</td>
<td>DTC, with RAI (NCT03109847)</td>
</tr>
<tr>
<td></td>
<td>- Biguanides</td>
<td></td>
<td>PTC, with etoposide and epirubicin [132]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FTC [124]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ATC [125]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ATC, with pioglitazone [133]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ATC, with doxorubicin and cisplatin [134]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ATC, with vemurafenib [135,136]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ATC, with sorafenib [137]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ATC, MTC, and FTC [138]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PTC and PDTC [126]</td>
<td></td>
</tr>
<tr>
<td>Glutaminolysis</td>
<td>Glutaminase inhibitor</td>
<td>BPTES</td>
<td>PTC [127,139]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CB-839</td>
<td>PTC [127,140]</td>
<td></td>
</tr>
</tbody>
</table>

HK2, hexokinase 2; 2-DG, 2-deoxyglucose; 3-BP, 3-bromopyruvate; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; ATC, anaplastic thyroid cancer; PDTC, poorly differentiated thyroid cancer; OXPHOS, oxidative phosphorylation; MTC, medullary thyroid cancer; DTC, differentiated thyroid cancer; RAI, radioactive iodine.
Inhibiting OXPHOS can be beneficial for cancer treatment, particularly in combination with other therapies.

**Biguanides**

There is evidence suggesting that inhibitors of OXPHOS, including the biguanides metformin and phenformin, may have anti-tumor effects in various cancer types, including thyroid cancer [116-126,146]. These inhibitors target mitochondrial respiratory chain complexes and have been shown to inhibit mTOR, reduce cellular proliferation, and delay resistance to conventional cancer therapies. Metformin has been studied extensively and found to be a useful adjuvant agent in preventing cancer relapse, particularly in prostate cancer and colorectal cancer [147]. However, concerns remain about whether it can reach sufficiently high concentrations to inhibit OXPHOS in vivo due to reduced uptake in some tumor types. Phenformin is nearly 50 times as potent as metformin and has intrinsic pharmacokinetic properties that may overcome the concentration issue. It has demonstrated more potent inhibition of cell proliferation in multiple tumor types and has been proposed to delay treatment resistance to conventional cancer therapies [148]. However, phenformin is associated with a higher incidence of lactic acidosis, which has limited clinical studies into its effectiveness as a cancer therapy.

Metformin, a drug commonly used to treat type 2 diabetes mellitus (T2DM), has been studied for its potential anti-cancer effects, but its impact on thyroid cancer remains a matter of debate. Preclinical studies have revealed the multifactorial functions of metformin: activation of AMPK and thereby inhibition of its downstream target mTOR, suppressing cellular growth and proliferation; inhibition of mitochondrial glycerophosphate dehydrogenase (mGPDH) to downregulate glycolytic flux; and inhibition of NF-κB to reduce cell proliferation, angiogenesis, and inflammation [149]. Clinical studies have suggested that metformin may have a role as an adjuvant therapy to reduce the growth of benign and malignant thyroid neoplasms, particularly in patients with metabolic diseases presenting insulin resistance [150]. Insulin resistance increases the risk of thyroid cancer. Zhao et al. [151] recently conducted a meta-analysis of 14 articles including 2,024 cases and 1,460 controls, studying the association between insulin resistance and thyroid cancer. They found that patients with thyroid cancer had higher levels of homeostatic model assessment–insulin resistance than those without thyroid cancer, and concluded that insulin resistance and hyperinsulinemia increase the risk of thyroid cancer, with an odds ratio (OR) of 3.16 (95% confidence interval [CI], 2.09 to 4.77). Further research is needed to fully understand the effectiveness of metformin for controlling thyroid cancer.

Several clinical observational trials have identified metformin as a protective factor against DTC. Tseng et al. [152] observed in Taiwanese patients with T2DM that ever-users of metformin had an adjusted hazard ratio of 0.683 (95% CI, 0.598 to 0.78; \( P < 0.0001 \)) for cancer development compared with never-users. They reported that a decreased risk could be observed with a cumulative duration of 9 months of metformin use or a cumulative dose of 263,000 mg. Similarly, in a retrospective cohort study in the Korean population, Cho et al. [153] found that metformin use had a hazard ratio of 0.69 (95% CI, 0.60 to 0.79; \( P < 0.001 \)) for thyroid cancer, and the effect was stronger with a higher cumulative dose (>529,000 mg) or a longer use (>1,085 days).

However, the results of an observational study with limited statistical power showed that the use of metformin was not associated with a decreased risk of thyroid cancer, nor was the use of other antidiabetic drugs such as sulfonylurea, insulin, or thiazolidinediones [154]. In 1,229 cases and 7,374 matched controls, the adjusted OR for the risk of thyroid cancer-associated with ever-use of metformin was 1.48 (95% CI, 0.86 to 2.54). The highest relative risk estimate was observed in long-term (>30 prescriptions) users of metformin (adjusted OR, 1.83; 95% CI, 0.92 to 3.65), based on a limited number of 26 exposed cases. The study found that neither a T2DM diagnosis (adjusted OR, 1.17; 95% CI, 0.89 to 1.54) nor diabetes duration >8 years (adjusted OR, 1.22; 95% CI, 0.60 to 2.51) altered the risk of thyroid cancer.

The conflicting results of observational studies on the association between metformin use and the risk of thyroid cancer suggest the need for further research in the form of randomized controlled trials to establish a definitive link between the two. Such trials could provide more reliable evidence regarding the effectiveness of metformin as a preventative measure against thyroid cancer.
Complex I inhibitors

IACS-010759 is an experimental drug that has been developed as an OXPHOS inhibitor. It targets the mitochondrial respiratory complex I, which is a key component of the OXPHOS pathway, thereby inhibiting OXPHOS. No published studies have investigated the effectiveness of IACS-010759, specifically in the treatment of thyroid cancer. However, preclinical studies have suggested that targeting OXPHOS with IACS-010759 may have potential therapeutic benefits in various types of cancer, including breast, lung, and pancreatic cancer [155]. Therefore, it is possible that IACS-010759 may also be effective in thyroid cancer, but further research is needed to evaluate its potential as a treatment option for this particular cancer type.

Recent studies have suggested that tumor cell oxidative metabolism is a barrier to PD-1 immunotherapy, and that radiotherapy could overcome PD-1 resistance in NSCLC. In this context, researchers investigated the efficacy of a combination treatment with IACS-010759 and radiotherapy against PD-1 resistance in NSCLC [156]. In vitro and in vivo experiments with anti-PD-1-sensitive and anti-PD-1-resistant NSCLC xenografts showed that the PD-1-resistant model utilized OXPHOS to a significantly greater extent than the PD-1-sensitive model, and that radiotherapy increased OXPHOS. The combination of radiotherapy and IACS-010759 promoted anti-tumor effects in the PD-1-resistant model, and triple therapy with IACS-010759, radiotherapy, and anti-PD-1 increased abscopal responses and prolonged the survival time [156]. These findings suggest that OXPHOS inhibition as part of a combinatorial regimen with radiotherapy is a promising strategy to overcome PD-1 resistance in NSCLC.

Glutamine blockade

Several inhibitors have been developed to target the glutamine uptake transporter ASCT2. One widely-used pharmacological agent to inhibit ASCT2 in preclinical studies is the L-glutamine analogue, l-γ-glutamyl-p-nitroanilide (GPNA). Inhibition of ASCT2 with GPNA has demonstrated the ability to decrease lung cancer cell growth and viability by blocking glutamine-dependent mammalian target of rapamycin complex 1 (mTORC1) signaling [157]. In a study of NSCLC patients, ASCT2 expression was found to be a significant prognostic marker and a potential diagnostic marker for glutamine-dependent NSCLC. However, due to the toxicity of ASCT2 inhibitors in healthy cells, progress in bringing these inhibitors into clinical use has been slow.

Glutaminolysis is a metabolic pathway that involves the mitochondrial enzyme GLS in the production of α-ketoglutarate (α-KG) for the replenishment of TCA cycle intermediates. Small molecule inhibitors such as BPTES, CB-839, and compound 968 can selectively inhibit GLS isoforms not commonly expressed in normal cells, making it possible to target cancer cells while reducing toxicity to normal cells [158]. Among these inhibitors, CB-839 is the most advanced and demonstrates greater bioavailability, selectivity, and potency than BPTES [159]. Early-phase studies showed that CB-839 is safe and well-tolerated in solid tumors with promising signs of clinical activity in multiple tumor types including triple-negative breast cancer, NSCLC, and mesothelioma. However, no specific biomarker for patient selection for GLS inhibition has been established, but tumor GLS overexpression and the specific GLS1 variant that is overexpressed have been evaluated. In particular, the glutaminase C splice variant of GLS1 is sensitive to GLS inhibition by CB-839.

A recent study confirmed the glutamine dependency of PTC cells and found that GLS, an enzyme involved in glutaminolysis, was aberrantly overexpressed in PTC tissues and cells. The inhibition of GLS, using both pharmacological and genetic methods, suppressed glutaminolysis, reduced mitochondrial respiration, and impaired the viability, migration, and invasiveness of PTC cells [127]. Notably, inhibition of GLS also deactivated the mTORC1 signaling pathway, leading to autophagy and apoptosis [160]. These findings suggest that GLS-mediated glutamine dependency could be a promising therapeutic target for PTC.

CONCLUSIONS

In conclusion, the metabolic plasticity of cancer cells poses a major challenge in developing successful CCM-targeting strategies. Targeting inherent metabolic dependencies in isolation has failed due to the ability of tumors to reprogram their metabolism and upregulate a separate compensatory pathway upon inhibition of a particular pathway, leading to therapeutic resistance. Therefore, researchers have explored combinatorial strategies involving dual metabolic inhibition and metabolic inhibitors in combination with targeted therapy, chemotherapy, and immunotherapy to overcome this challenge. These combinations have shown promising advancements in clinical trials. Moreover, the “synthetic lethality” approach, which involves targeting cancer cells with a specific genetic defect while sparing normal cells with a different genetic makeup, has shown potential for targeting cancer cells with genetic or epigenetic alterations that make
them dependent on a specific metabolic pathway or nutrient. For example, targeting GLS in PTC cells that are glutamine-dependent and have aberrant overexpression of GLS may be a potential therapeutic target. Despite the challenges posed by metabolic plasticity, significant progress has been made in developing successful CCM-targeting strategies, and the potential benefits of combinatorial metabolic targeting continue to be explored in clinical trials. Ongoing research into CCM-targeting strategies and a further understanding of the mechanisms underlying metabolic plasticity in cancer cells will be essential in the development of more effective and targeted therapies for cancer.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

The authors would like to acknowledge Dr. Minho Shong’s research group at the Korea Advanced Institute of Science and Technology and colleagues at Chungnam National University for their contributions to the field of cancer metabolism and for their insights and guidance in the preparation of this review article.

This work was supported by research fund of Chungnam National University.

ORCID

Sang-Hyeon Ju https://orcid.org/0000-0001-7098-4648
Minho Shong https://orcid.org/0000-0002-0247-7115

REFERENCES


44. Coelho RG, Fortunato RS, Carvalho DP. Metabolic reprogramming in thyroid carcinoma. Front Oncol 2018;8:82.


Cancer 2021;20:28.


117. Yu Y, Feng C, Kuang J, Guo L, Guan H. Metformin exerts an antitumoral effect on papillary thyroid cancer cells through...


119. Thakur S, Daley B, Gaskins K, Vasko VV, Boufraqech M, Patel D, et al. Metformin targets mitochondrial glycero-

120. Shen CT, Wei WJ, Qiu ZL, Song HJ, Zhang XY, Sun ZK, et al. Metformin reduces glycometabolism of papillary thy-


122. Ye J, Qi L, Chen K, Li R, Song S, Zhou C, et al. Metformin induces TPC-1 cell apoptosis through endoplasmic reticu-

123. Nozhat Z, Zarkesh M, Baldini E, Mohammadi-Yeganeh S, Azizi F, Hedayati M. Antineoplastic activity of an old natu-


132. Ghavami G, Kiasari RE, Pakzad F, Sardari S. Effect of metformin alone and in combination with etoposide and epirubi-


