Thyroid Diseases, Metformin and the AMP Kinase Pathway

Tiroid Hastalıkları, Metformin ve AMP Kinaz Yolağı

Utku SOYALTIN, Gökhan ÖZGEN*, Taylan KABALAK*

Clinic of Endocrinology, Başakşehir Çam and Sakura City Hospital, İstanbul, TURKEY
*Division of Endocrinology and Metabolism, Ege University Faculty of Medicine, İzmir, TURKEY

Introduction

Thyroid hormone and adenosine monophosphate-activated protein kinase (AMPK) are 2 major determinants of energy balance. Thyroid hormone is known to be a key factor that stimulates energy use in energy balance. It is involved in almost every stage of energy use. The uptake of energy substances into the cell, their conversion to adenosine triphosphate (ATP) in the mitochondria, and the use of ATP in all cellular processes where energy is required are under the stimulating control of tri-iodothyronine and partially 3,5-diido-L-thyronine. A deficiency in thyroid hormone production results in ineffective utilization of energy substrates in the cell, despite their sufficient level. A well-known example of this condi-
tion is severe hypothyroidism or myxedema coma. On the other hand, if there is an energy insufficiency, the cell needs to conserve energy. Energy conservation is achieved by restricting some cellular functions that are not vital. This critical function is carried out by the AMPK enzyme in the cell. Therefore, the physiological function of AMPK activity is equally important (1,2).

As described above, there is a complete contrast between the functions of thyroid hormone and AMPK in cells. To evaluate this aspect better, it is useful to study AMPK in more detail. In this context, we should also investigate the role of the mammalian target of rapamycin complex 1 (mTORC1) pathway.

The mTORC1 Pathway

The mTORC1 pathway is an important pathway in cellular function. The mTORC1 pathway is at the junction of many other signaling pathways. The tuberous sclerosis system [tuberous sclerosis complex 1 (TSC1) and TSC2] suppresses mTORC1, and phosphorylation of this system leads to the activation of mTORC1. mTORC1 plays a direct or indirect role in cell growth and proliferation, fat and protein synthesis, autophagy control (predominantly reduction of autophagy), and cell energy utilization. Thus, the function of mTORC1 is to keep the cells active and alive. This process requires ATP consumption. The carcinogenic effect originating from excessive and continuous stimulation following mutation of some signaling proteins in the mTORC1 pathway is well known (3).

The AMPK Pathway

AMPK is an important enzyme that is activated when ATP levels decrease because of excessive use of ATP and an increase in the AMP/ATP ratio. The activated AMPK significantly restricts energy use and also induces energy recovery pathways in the cell. When AMPK is activated, it suppresses the mTORC1 pathway. In other words, it exerts an antitumorigenic effect. AMPK activation also increases autophagy, mitochondrialogenesis, glucose uptake, and stabilization of mRNA and cell cycle (Figure 1) (4,5). AMPK consists of alpha, beta, and gamma subunits. The activation of AMPK is mainly driven by phosphorylation of the amino acid residue threonine-172 (Thr-172) in the alpha subunit (1). Many compounds stimulate AMPK activation by direct and indirect phosphorylation of Thr-172 (Figure 2). Because hunger and hypoxia will negatively affect ATP synthesis, excessive muscle movements will consume an excess amount of ATP; consequently, the AMP/ATP ratio will increase and phosphorylate AMPK. Apart from the AMP/ATP ratio, calcium/calmodulin-dependent protein kinase kinase-1, liver kinase-B1, metformin, thiazolidinedione, resveratrol, quercetin, and sirtuin are some of the other compounds that increase AMPK activity. 5-Aminoimidazole-4-carboxamide riboside (AICAR) is a direct AMPK stimulant that is widely used in AMPK-related research. Thy-

Figure 1. AMPK directly and indirectly (dephosphorylation of TSC1/TSC2) inhibits the mTORC1 pathway and shows antitumorigenic and energy restrictive effects. It also induces autophagy, mitochondrialogenesis, glucose uptake, and stabilization of mRNA and cell cycle.

AMPK: Adenosine monophosphate-activated protein kinase; TSC: Tuberous sclerosis complex; mTORC1: Mammalian target of rapamycin complex 1; ATP: Adenosine triphosphate.

Figure 2. In addition to the increase in the AMP/ATP ratio, many pathways and drugs activate AMPK by phosphorylating the alpha subunit. T3 also activates AMPK directly or by increasing the use of ATP.

AMPK: Adenosine monophosphate-activated protein kinase; T3: Thyroid hormone; AICAR: 5-Aminoimidazole-4-carboxamide riboside.
Thyroid hormone (T3) activates AMPK directly or indirectly by increasing the AMP/ATP ratio (4,5).

**Thyroid diseases and the AMPK Pathway**

An increased level of AMPK activation in thyrocytes stimulates glucose uptake by activating glucose transporter 1 and decreases iodine uptake by suppressing the function and transcription of Na/I symporter (Figure 3). It also inhibits the proliferative effect of thyroid-stimulating hormone (TSH) on thyrocytes. This relationship is mutually balanced by the inhibitory effect of TSH on AMPK activation (6,7).

TSH and insulin resistance: Both insulin and insulin-like growth factor stimulate the proliferation of thyroid cells and are associated with the pathogenesis of thyroid nodule development. AMPK activity counteracts TSH activity and decreases insulin resistance, which is related to the decrease in thyroid gland size and nodule volume (8,9).

AMPK activity has been shown to increase the incidence of thyroid malignancies (10,11). The extent of increase is also related to the aggressiveness of the malignancy. Cancer cells need a high amount of energy for rapid growth and proliferation. This energy is mainly supplied by glucose, and an increase in AMPK activity leads to high glucose levels in cancer cells. However, if AMPK is overactivated, it shows an antiproliferative effect by suppressing the mTORC1 pathway. Many studies have also shown that AMPK inhibits the cell cycle by stabilizing the expression of the p53 gene. A strong antiproliferative effect of AMPK activation with AICAR application has been demonstrated in many cancers, including breast cancer (6). Continuous PI3K activation has been shown to occur in PTEN mutant mice; this results in a continuous decrease in the mitochondrial respiratory capacity, and the AMPK activity remains proportionally low. Mutant mice were later found to develop thyroid follicular adenoma and thyroid follicular cancers (known as Cowden syndrome in humans). When AICAR was administered to mutant mice, a significant reduction in tumor progression was observed as compared to that in controls (6).

**Metformin and Thyroid Disease**

In 2 retrospective studies, metformin intake was found to be associated with a lower incidence risk of thyroid cancer. These protective effects of metformin were more pronounced in patients who were taking metformin for a longer period and therefore had a higher cumulative dose of metformin (12,13). Klubo-Gwiezdzinska et al. found that patients with diabetes who were being treated with metformin had smaller tumor size and less incidence of extra-thyroid invasion and distant metastasis than non-metformin-treated patients with diabetes and non-diabetic patients with thyroid cancer (14). In a recent study, metformin reduced the tumor growth of thyroid cancer in the metastatic niche of bone by inhibiting osteoblastic receptor activator of nuclear factor kappa-B ligand production in anaplastic thyroid cancer cell lines (15).

Antitumorigenic effects of metformin were mainly associated with the activation of the AMPK pathway. AMPK stimulation by metformin is more pronounced in aggressive types of thyroid tumors. Metformin was found to dose-dependently suppress the growth and migration of cancer cells in an anaplastic cell line (16). In the doxorubicin-resistant HTh74R anaplastic cell line, metformin markedly decreased growth stimulation signaling and showed an additive antimitogenic effect with sorafenib (17).

In addition to mTOR inhibition, AMPK activation inhibits the phosphorylation of...
insulin receptor substrate-1 (IRS-1). IRS-1 transmits signals from insulin-like growth factor 1 receptor to the PI3K/AKT pathway, which is responsible for the mitogenic effect of insulin (18). Metformin also inhibits mitochondrial glycerophosphate dehydrogenase (mGPDH), which plays a role in glycolysis and oxidative phosphorylation. The inhibition of mGPDH activity negatively affects thyroid cancer cell growth by reducing oxidative phosphorylation (19).

Despite these results, metformin is still not recommended for thyroid cancer treatment in clinical practice. The use of metformin might not be necessary because most thyroid cancers (90%) are nonaggressive. Some authors, however, suggest the use of metformin for treating the tall cell, poorly differentiated, and anaplastic thyroid cancer (8). Because metformin intake causes a decrease in iodine uptake, care should be taken for differentiated cancers, which should be treated with radioiodine or through an 1131 whole body scan.

Another thyroid disease group that can be discussed in the context of AMPK and metformin use is nodular goiter. In subjects with Type 2 diabetes mellitus (DM) and insulin resistance, metformin therapy is shown to be associated with smaller thyroid volume, decrease in the number of nodules and nodule size, and lower serum TSH level (20-23). In addition to the activation of AMPK, metformin antagonizes the growth stimulatory effect of insulin and decreases TSH level, which inhibits tumor proliferation in the thyroid gland (8). In a group where metformin treatment was initiated due to insulin resistance, it was found that the nodule volume (as measured by ultrasound) decreased by 30% after 6 months of treatment as compared to the initial values (20). Metformin suppresses TSH levels by changing the affinity and activity of central thyroid hormone receptors. An increase in central dopaminergic tone is another mechanism through which metformin enhances the suppressive effect of thyroid hormones on TSH levels (24-26). Metformin treatment was found to be associated with a decrease in serum TSH levels only in those patients with TSH>2.5-2.95 µU/mL (27,28).

Conclusion
Despite all the positive data, if there is no indication for using metformin for Type 2 DM or insulin resistance, then metformin should not be recommended for treating diffuse goiter. For thyroid malignancies, there is currently no recommendation in guidelines to use metformin as an AMPK activator. The suppressive effect of metformin on TSH levels must be considered for patients undergoing levothyroxine 4 (LT4) therapy, as false dose reduction may cause symptoms of hypothyroidism due to an interruption in demand of LT4 in peripheral tissues.

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References
2. Hardie DG. AMP-activated protein kinase: An energy sensor that regulates all aspects of cell function. Genes Dev. 2011;25:1895-1908. [Crossref] [PubMed] [PMC]


6. Andrade BM, de Carvalho DP. Perspectives of the AMP-activated kinase (AMPK) signalling pathway in thyroid cancer. Biosci Rep. 2014;34:e00105. [Crossref] [PubMed] [PMC]


9. Baser H, Topaloglu O, Tam AA, Evranos B, Alkan A, Sungu N, Dumlu EG, Ersoy R, Cakir B. Higher TSH can be used as an additional risk factor in prediction of malignancy in euthyroid thyroid nodules evaluated by cytology based on Bethesda system. Endocrine. 2016;53:520-529. [Crossref] [PubMed]


13. Shin HS, Sun HJ, Whang YM, Park YJ, Park DJ, Cho SW. Metformin reduces thyroid cancer tumor growth in the metastatic niche of bone by inhibiting osteoblastic RANKL productions. Thyroid. 2021;31:760-771. [Crossref] [PubMed]


22. Alevizaki M. Metformin and the thyroid: Some questions still remain. Clin Endocrinol (Oxf). 2013;78:503-504. [Crossref] [PubMed]


