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Review article

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RECENT ADVANCES IN THE ROLE OF AMP-ACTIVATED PROTEIN KINASE IN METABOLIC REPROGRAMMING OF METASTATIC CANCER CELLS: TARGETING CELLULAR BIOENERGETICS AND BIOSYNTHETIC PATHWAYS FOR ANTI-TUMOR TREATMENT

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Growing data indicate that tumor progression and metastasis is dependent on the reprograming of cellular metabolism. Rapidly growing cancer cells undergo metabolic stress in a harsh microenvironment. AMP-activated protein kinase (AMPK) is an energy sensing factor that regulates bioenergetics and biosynthetic pathways within the cell, but its role under metastasis is in dispute. The best studied phenotype of cancer cells is aerobic glycolysis (the Warburg effect), an increased catabolism of glucose to lactate. However, glycolysis and mitochondrial oxidative phosphorylation may operate simultaneously in cancer cells. Many tumors may switch between these pathways accordingly to the current requirements. The alterations in metabolism of cancer cells combined with the overexpression of oncogenes (c-Myc) and transcription factors (Hypoxia-inducible factor 1α) confer a great advantage to malignant cells to avoid reactive oxygen species induced apoptosis. The determination of the role of AMPK network in metabolic reprogramming of metastatic cancer cells may help to identify the selective molecular targets for efficient anti-cancer therapies. In this review, we discuss the implications of AMPK activation in metabolic reprogramming of cancer cells and we present several potential therapeutic strategies targeting cancer cell metabolism. AMPK activator, biguanide metformin, either alone or in combination with other drugs, may selectively modulate signaling pathways, expresses the chemopreventive potential and can be used in current anti-cancer approaches. However, the ambiguous data suggest that the activation of AMPK may induce multiple effects and thus potential therapeutic anti-cancer approach should be carefully considered in relation to metabolic network of cancer cell signaling and other determinants such tumor stage and origin as well.

Key words: *5'-adenosine monophosphate-activated protein kinase, liver kinase B1, cancer metabolism, Warburg effect, metastasis, reactive oxygen species, mitochondrial oxidative phosphorylation, epithelial-mesenchymal transition, metformin*

INTRODUCTION

The AMP-activated protein kinase (AMPK) is the main energy sensor and a major adaptive kinase within the cell. An increase in the cellular AMP/ATP ratio induces the activation of AMPK, thereby conferring to AMPK complex the function of metabolic checkpoint, regulating the response of the cell to energy demands (1). During periods of energetic stress, AMPK becomes activated with a following shift to inhibition of growth and proliferation of the cell and metabolic oxidative phenotype. However, malignant cells may overcome this checkpoint and due to continuously activated growth signaling pathways they can proliferate even in a hostile microenvironment. Accordingly, several oncogenic mutations and signaling pathways within tumor cell cross-talk with AMPK signaling to uncouple nutrients availability signals from growth pathways, which, in turn, allows cancer cells to proliferate efficiently even under glucose-deficient conditions (2). Currently, numerous studies aim to evaluate, whether AMPK activation may be used to re-couple fuel and growth signals and, finally, if the proliferation of tumor cells can be shut down by manipulation of AMPK (3). Additionally, the recent reports show that AMPK mediates biosynthetic pathways and bioenergetics events, which are crucial for cancer cell survival and metastasis, and underline the role of AMPK signaling in regulation of reprogrammed metabolism of tumors, especially under nutrient deficiency (1-3).

AMP-ACTIVATED PROTEIN KINASE STRUCTURE AND REGULATION

AMPK is a heterotrimeric protein, which is composed of three subunits α, β and γ. Subunit α contains serine-threonine catalytic

kinase domain, as well as autoinhibitory domain and binding sites for β and γ regulatory subunits. The ATP and AMP binding motif can be found in γ subunit, while β subunit stabilizes the whole protein and maintains the correct conformation (4). There are tissue-specific isoforms of each subunit: two of α, two of β and three of γ, yet γ2 and γ3 can occur in different splicing variants. This diversity of isoforms leads to a different level of AMPK specificity and may affect metabolic signal transduction as well (5).

AMPK is regulated allosterically by AMP. The association of AMP to γ regulatory subunit results in a 2.5-fold increase of kinase activity (6). The extent of the enzyme activity increase depends on AMPK subunit composition and is the greatest when the enzyme is composed of α2 and γ2 subunits (7). AMP bounding with γ subunit induces threonine-172 phosphorylation in α subunit by upstream kinases which leads to the conformation change in the kinase domain. This change protects AMPK against dephosphorylation and allows the phosphorylated form to be accumulated within the cell (8). Phosphorylation of threonine-172 in α subunit causes over a 1000-fold increase in AMPK activity and enhances AMPK sensitivity to subtle changes in the cell energy status (9). The upstream kinases, LKB1 (liver kinase B1), CaMKK (Ca^{2+}/calmodulin-dependent protein kinase kinase) or TAK1 (transforming growth factor β-activated kinase 1) directly activate AMPK (10). It has been proven that AMPK can also be directly phosphorylated by ataxia-telangiectasia mutated kinase (ATM), one of the phosphatidylinositide 3-kinases (11). Although threonine-172 is the crucial phosphorylation site for AMPKα, there are more phosphorylation sites in α and β subunits. Serine-487 at α1 subunit can be phosphorylated by AKT kinase (protein kinase B) leading to the inhibition of phosphorylation by upstream kinases at the activating site, threonine-172. This mechanism has been found in tumor cells *in vitro* and leads to restraining the activation of the LKB1-AMPK pathway. Otherwise, the activation of LKB1/AMPK axis would inhibit cell growth and proliferation (12).

As the phosphorylation of threonine-172 activates AMPK, removal of the phosphate group decreases its activity. Several phosphatases regulate the process. *In vitro* studies revealed that AMPK can be dephosphorylated by PP2A and PP2C phosphatase; however, the exact mechanism remains unclear (13). AMPK may be deactivated *via* acetylation, moreover P300 acetyltransferase disables the interaction between LKB1 and AMPK (14). Another type of AMPK regulation is ubiquitination followed by the proteasomal degradation. This process plays important role in cancer development when suppression of AMPK by the cancer-specific MAGE-A3/6-TRIM28 ubiquitin ligase is necessary for sustaining cancer cell viability (15). The AMPK downstream targets are shown in *Fig. 1*.

AMPK ROLE IN METASTATIC PROGRESSION

The mechanisms of AMPK influence on the processes critically relevant to metastasis are poorly recognized. However, current evidence indicates the implication of AMPK signaling in several important aspects of tumor progression, such as epithelial-mesenchymal transition (EMT). EMT can be described as long-lasting morphological and molecular changes that turn an epithelial cell into a mesenchymal one (16, 17). The specific modification of morphology, cellular architecture, adhesion and migration capacity can be observed during EMT. Epithelial cells, that undergo this process, lose contact with adjacent cells, become motile and can migrate through the basement membrane (18). Numerous studies have shown a complex system of

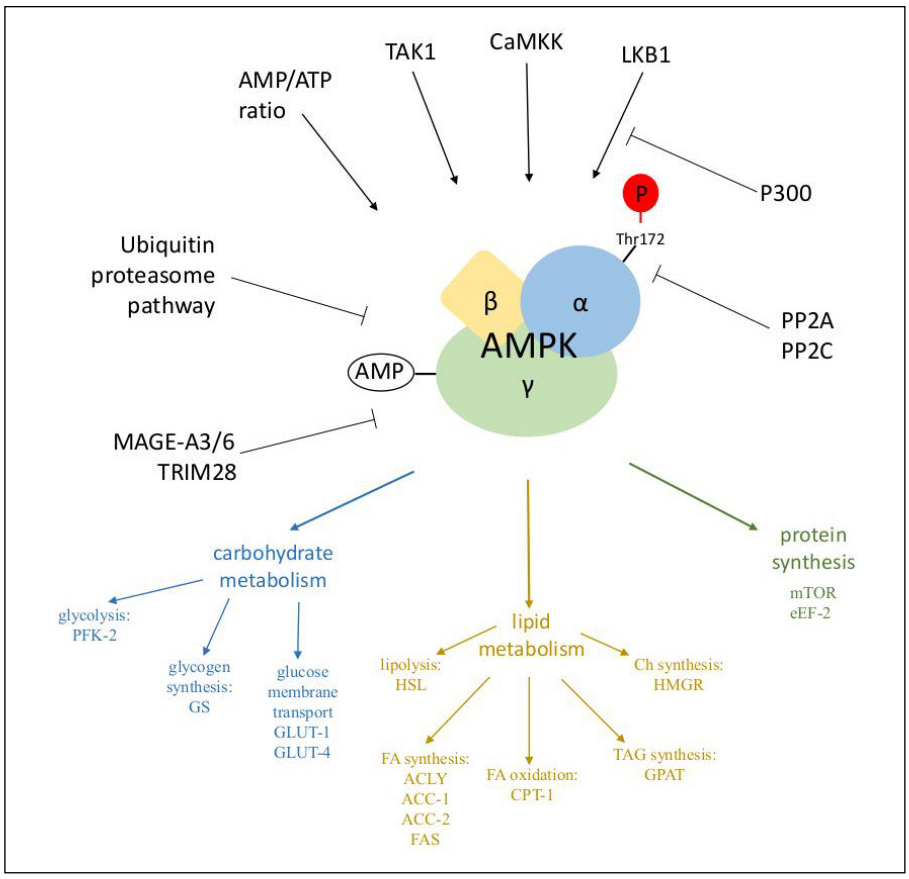

Fig. 1. AMPK acts as the main cellular energy sensor within the cell, which is sensitive to changes in AMP concentration and exert the effect on carbohydrate metabolism, lipid and protein synthesis. AMPK is also activated by its upstream kinases (LKB1, CaMKK or TAK1).

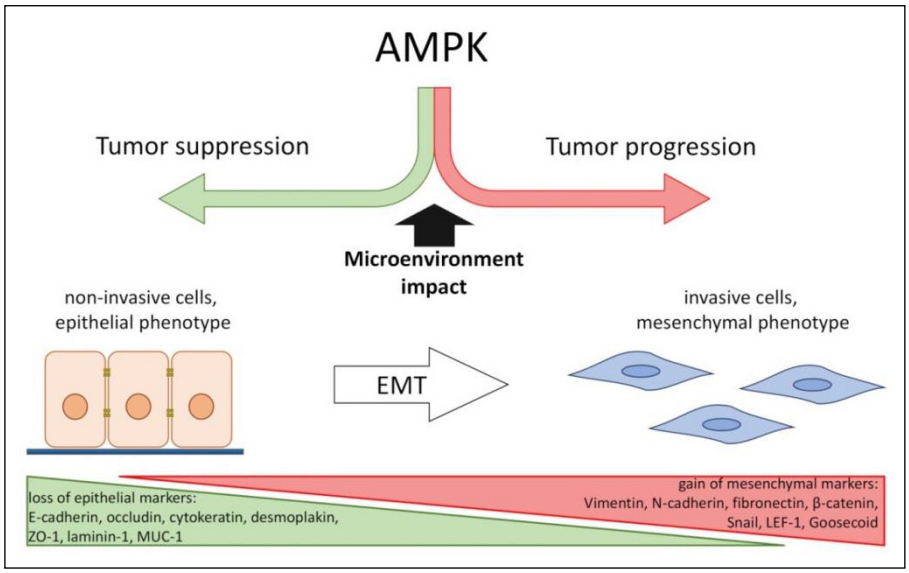

Fig. 2. Dual nature of AMPK in tumorigenesis. AMPK can act as both, a suppressor and promoter of epitehelial-mesenchymal transition (EMT).

interrelationships between AMPK and other important pathways affecting the progression of cancer. Since AMPK is in the crossroads of multiple signaling pathways, there can be found proofs of its involvement in EMT through participation in regulation of cell growth, metabolism and migration (19).

Rattan *et al*. (20) reported that AMPK mediated growth inhibition in both, *in vitro* and *in vivo* experiments. Interestingly, LKB1 independent tumor growth inhibition moderated by AMPK activation in *LKB1-null* MEF cells was also observed. This significant finding underlined the independence of AMPK as a tumor suppressor, since LKB1 was the regulatory point widely described as a main tumor suppressor. However, LKB1 is considered to be a master regulator of cell proliferation and apical-basal polarity and the activation of this protein can efficiently interrupt the implementation of EMT program (21). Growing data indicate, that the interaction of AMPK and LKB1 may be crucial for inhibition of tumor progression (22, 23).

AMPK influences cancer cells migration and invasion *via* various metabolic pathways. AMPK suppresses the activity of HSF1, which acts as a promoter of cancer progression. HSF1 facilitates cell proliferation and migration by enhancing lipid biosynthesis, activating the nuclear factor κB (NF-κB) signaling pathway and mitogen-activated protein kinase (MAPK). Recent research (24) revealed that AMPK activation resulted in inhibition of HSF1 phosphorylation and nuclear translocation, which inhibited EMT implementation. As mentioned previously, AMPK/NF-κB axis is involved in the inhibition of cancer cells invasion and migration through downregulation of MMP-9 expression (25).

One of the most well-known stimuli increasing invasion and migration of tumor cells and the implementation of the EMT program is transforming growth factor β (TGF-β) signaling pathway. TGF-β triggers EMT by promoting expression of EMT associated transcription factors, like Snail and ZEB family, as well as inducing expression effectors like matrix metalloproteinases (MMPs). TGF-β treatment causes changes in cell-to-cell interconnections. The loss of E-cadherin is considered to be a fundamental event of the EMT process. Numerous studies reported inhibition of TGF-β-mediated EMT through AMPK action (26). Activation of AMPK restored E-cadherin gene (*CDH1*) expression and repressed MMPs expression, what resulted in reverting EMT in retinal pigment epithelial (RPE) cells (27). Our studies revealed that AMPK activators, such as metformin and naturally occurred small molecule caffeic acid led to inhibition of TGF-β induced EMT-like changes and acquiring invasive phenotype of cervical cancer cells (28).

On the other hand, a recent study has demonstrated AMPK activation in hypoxia and TGF-β-induced EMT (29). Saxena *et al*. proposed that AMPK may trigger EMT through phosphorylation of transcription factor Twist1. Moreover, the authors hypothesized the possibility of regulation of other EMT associated transcription factors, like Snail and Slug, by AMPK. Accordingly to these findings, the inhibition or depletion of AMPK may led to a reversal of EMT, which is complementary to the postulated necessity of AMPK activation for the induction of EMT. In line with this research, another study revealed that inhibition of AMPK attenuated TGF-β-induced EMT in AML12 cell line. Moreover, TGF-β-induced apoptosis was increased as a result of AMPK inhibition (30).

In conclusion, AMPK may depict a dual nature, it can act as an inhibitor of tumor progression and a promoter as well. Indeed, AMPK activity changes during tumorigenesis, but the context of its action depends on many aspects. Although multiple factors can be pointed out, probably one of the most important is oxidative stress. Considering its role during tumor development and progression, reactive oxygen species (ROS) may be the key players in this process, managing AMPK role in cancer cells. Additionally, the limited availability of nutrients, especially low glucose supply, may regulate generation of ROS within the cell, as will be discussed later.

The various studies show that *via* utilizing the same mechanisms and signaling pathways, AMPK may trigger the opposite effects. Especially, the effects exerted by AMPK in the development and progression of cancer were recognized only in a part. At a current stage of knowledge, the role of AMPK in metastatic progression cannot be unambiguously determined. However, the dual behavior of AMPK in the context of metabolic reprogramming of cancer cells may contribute to a better insight into this issue and undoubtedly requires further intensive research.

REACTIVE OXYGEN SPECIES AS MEDIATORS OF SIGNAL TRANSDUCTION IN CANCER CELLS

ROS are not only destructive molecules for the cells, but they may also be signal transducers in both, normal and cancerous cells. However, within the normal cell, ROS play physiological role as signal mediators, thus their balance is

strictly regulated. In contrast, the increased levels of ROS are proved to exist during cancer development and progression in numerous cancer types (31, 32) and tumor cells themselves enhance antioxidant capacities accordingly to rising ROS levels to protect against oxidative stress (33). On the other hand, the development and progression of cancer lead to metabolic deregulation, which results in increased expression of ROS. Despite these changes, the essence of ROS signaling in cancer cell is the balance between the amount of generated ROS and the antioxidant capacity of the cell (*Fig. 4*). The increased production of ROS leads to the activation of metabolic pathways responsible for increased proliferation, migration, inhibition of apoptosis and the implementation of EMT (34). It should be emphasized that in the state of oxidative stress, the cell manage survival due to the activity of AMPK. At the same time the activity of the AKT (protein kinase B) signaling is limited. Further increase in ROS levels reaches the threshold of cell antioxidant capacity, leading to the activation of mechanisms related to senescence, cell cycle arrest and cell death. In that way the action of AKT surpasses the ability of AMPK to raise the maximum threshold for oxidative stress.

AMP-ACTIVATED PROTEIN KINASE AND AKT CROSS-TALK UNDER GLUCOSE DEPRIVATION

Maintaining of energy homeostasis in cancer cells under glucose deprivation mostly depends on cross-talk between AMPK and AKT. Both proteins, AMPK and AKT, regulate cancer cells metabolism in conditions of metabolic stress; however, AMPK appears to play a pivotal role in the survival of cancer cells in limited glucose supply conditions (*Fig. 3*). AMPK can be regulated by AKT and *vice versa* through direct and indirect phosphorylation, but the essence of this cross-talk is the opposite regulation of the same downstream effectors, mTOR and FOXO (The forkhead box O family of transcription factors) (35). mTOR (Mammalian Target of Rapamycin) is strongly involved in glucose and amino acids metabolism, and it has a direct impact on protein synthesis, autophagy, organelle biogenesis and maintenance (35). mTOR can be found in two different complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1, which is composed of mTOR itself, a regulatory-associated protein of mTOR (Raptor), MLST8 and DEPTOR (36, 37), can promote protein and ribosome synthesis (through phosphorylation of S6K, eIF4E) and inhibit autophagy by inactivation of UNC51like kinase 1 (ULK1) by its phosphorylation at Serine-757. ULK1 is also a target for AMPK but its action promotes autophagy in contrast to mTORC1 (38). mTORC1 activity is negatively affected by AMPK under glucose deprivation that leads to autophagy stimulation as well as the decrease in protein synthesis. As a consequence, ROS production is limited and at the same time the resistance of cancer cell to ROS load raises (*Fig. 4*). mTORC1 activation is moderated by AKT through the inhibition of tuberous sclerosis complex 2 (TSC2) and the inactivation of proline-rich AKT substrate of 40 kDa (PRAS40) (39). Thus, AKT increases ROS production and oxygen consumption, that put cancer cell at risk of exceeding toxic ROS threshold and cell death. The antagonistic regulation pattern also exists for FOXO, the another mutual downstream protein of AMPK and AKT (34). Once activated by AMPK, FOXO upregulates the expression of

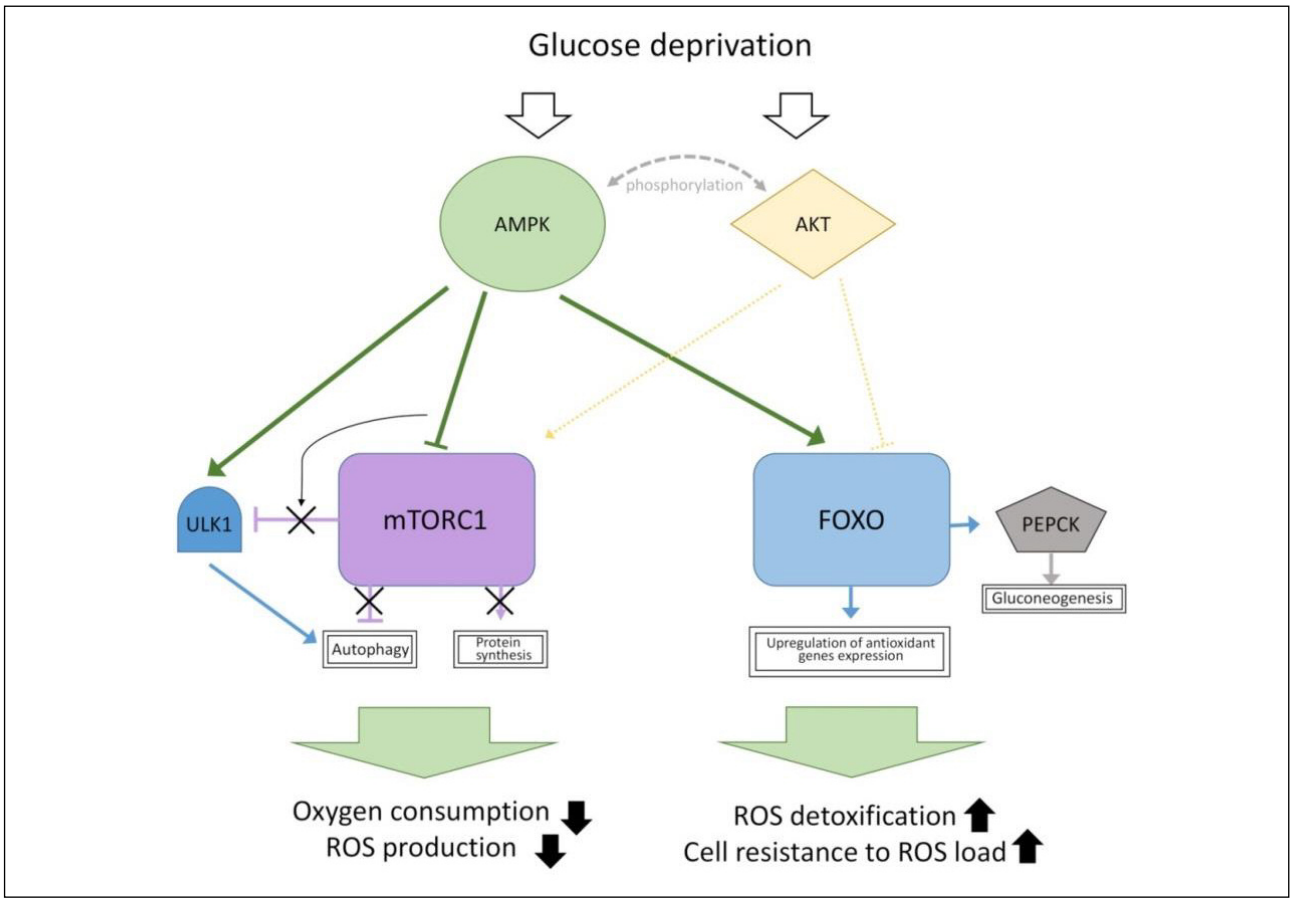

Fig. 3. AMPK and AKT mutual regulation through direct phosphorylation and mTOR/FOXO cross-talk under glucose deprivation. AMPK inhibits mTORC1 complex and activates FOXO, while AKT exerts the opposite effect on mTORC1 and FOXO activities.

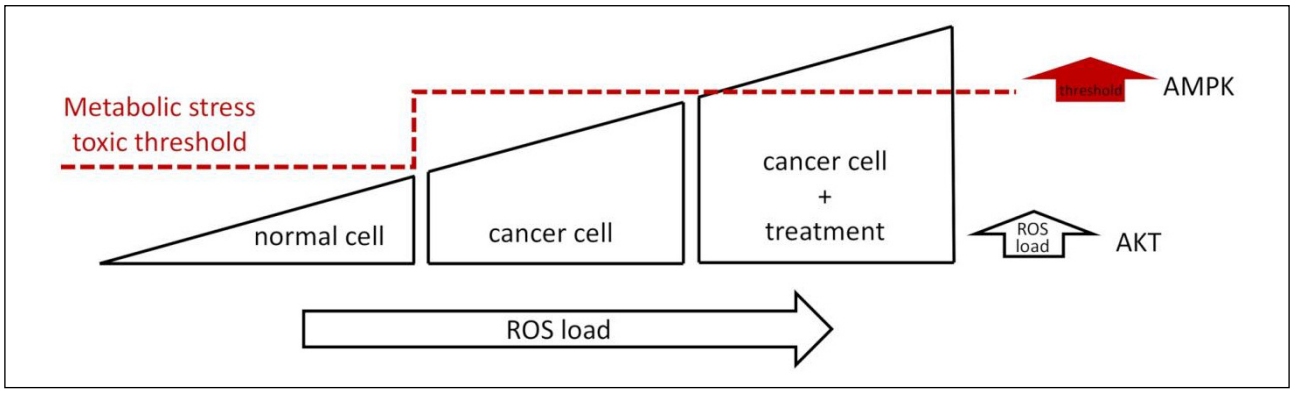

Fig. 4. AMPK moderates cancer cell resistance to ROS load. The transformation of normal cell into malignant one is associated with elevating of ROS level. AMPK promotes autophagy and limits oxygen consumption, thus may enhance cancer cell resistance to ROS. In contrast, AKT increases ROS production.

several antioxidant enzymes such as superoxide dismutase (SOD), catalase and sestrin. In this way the tolerance to ROS mediated oxidative stress is enhanced upon the activation of FOXO. Moreover, FOXO induces the expression of autophagy-related genes and increases the production of fatty acid and amino acids (40). AMPK can maintain FOXO activity by facilitating its nuclear localization, sustaining transcriptional activity and, finally, by phosphorylation of the protein. By contrast, the phosphorylation of FOXO by AKT inhibits the transcriptional functions of FOXO by driving its translocation from nucleus to cytoplasm (41). Moreover, AKT may promote FOXO ubiquitination and subsequent degradation of the protein (42, 43).

In conclusion, the survival of cancer cell in limited glucose delivery conditions is a result of the dominant role of AMPK activation over AKT signaling. Downregulation of AKT leads to the inhibition of mTOR activity, while FOXO becomes activated. Thus, the limitation in further ROS production occurs and at the same time the tolerance to oxidative stress induced by ROS is elevated. Therefore, one of the important mechanism triggered by the AMPK/AKT cross-talk may be the development of apoptosis resistance.

CANCER CELLS RESISTANCE TO ANOIKIS AS A CONSEQUENCE OF AMPK MODERATED REACTIVE OXYGEN SPECIES INSUSCEPTIBILITY

ROS overproduction in normal cells results in severe damage and cell death. During cancer progression, the cells may develop mechanisms to fight against enhanced ROS accumulation. The induction of antioxidant mechanisms, endogenous antioxidant enzymes and molecules (*e.g.*, glutathione, coenzyme Q, ferritin, and bilirubin) facilitates the resistance to apoptosis (44). Especially anoikis, a type of apoptosis related to the loss of attachment to extracellular matrix (ECM), is obligatory for cancer cell to migrate from its niche and survive in the vascular/lymphatic circulation. As a consequence of anoikis resistance, tumor cells are able to metastasize to distant organs. Normal cells, when detaching from ECM, are exposed to heavy ROS load, which results in anoikis. On the contrary to this, the constant increase of ROS generation in cancer cells may make these cells independent of attachment signals. ROS activate pro-survival pathways of Src and epidermal growth factor receptor (EGFR), that triggers degradation of pro-apoptotic molecules (45). In tumor cells, the disturbance caused by detachment from ECM is alleviated through autophagy induction and global inhibition of protein synthesis. These processes are elicited by AMPK (46). Along with AMPK, PI3K-independent activation of AKT by tyrosine kinase receptor signaling may promote glucose uptake and upregulate anti-apoptotic pathways facilitating cell survival during ECM (47). In anoikis resistant cells, the activation of AMPK and AKT is associated with permanent mTOR inhibition, which, in turn, appears to be of essential importance to elevated ROS load management, redox homeostasis and avoiding cell death (48, 49).

METABOLIC REPROGRAMMING OF CANCER CELLS PROMOTES METABOLIC FLEXIBILITY UNDER NUTRIENTS DEPRIVATION: THE REGULATORY EFFECTS OF AMP-ACTIVATED PROTEIN KINASE AND ITS UPSTREAM KINASE LKB1

Within intensively proliferating tumor cell, the reprogrammed metabolism plays crucial role to meet high energetic demands. Many cancer cells favor aerobic glycolysis over mitochondrial metabolism, but under glucose deficiency they may adapt to use the same energy substrates as non-tumor cells, such as lactate, pyruvate, glutamine, fatty acids, yet, at much higher rates. However, glucose is the prime substrate to generate ATP within cancer cell (50, 51). Additionally, the import of nutrients into the cancer cell is also enhanced in order to sustain survival, growth and proliferation. The intracellular abundance of glucose leads to the activation of glycolysis and fuels pentose phosphate pathway (PPP), which results in elevated NADPH production for synthesis. It was well established that cancer cell lines and solid tumors display enhanced glycolysis comparing to the normal counterparts. Although glycolysis generates less ATP per molecule than mitochondrial oxidative phosphorylation (OXPHOS), the net rate of ATP synthesis *via* glycolysis may be faster than with OXPHOS. The reliance on glucose supply is linked to the aggressiveness of malignant cells independently of cancer origin (51). Otto Warburg observed the increased conversion of glucose to lactate in the presence of oxygen in tumors (the phenomenon called ‘the Warburg effect’). Since then, the aerobic glycolysis has been demonstrated in a variety of malignant cells (52, 53). Although aerobic glycolysis is thought to be the most common metabolic phenotype of cancer cells, it is not a universal feature of tumors (2). On the contrary to Warburg’s conclusions, growing evidence indicates that cancer cells do not solely depend on glycolysis, but rather may switch between glycolysis and mitochondrial-based energy production *via* OXPHOS appropriately to current demands (54, 55). Oxidative phosphorylation may represent a significant contribution to ATP

generation and intermediates supply for synthesis within malignant cell (57, 58). Moreover, recent studies have shown that in conditions of metabolic stress some tumors may prioritize OXPHOS or even completely depend on oxidative phosphorylation. The mitochondrial-based metabolism plays the essential role in the maintenance of survival and growth of cells. For example, leukemic cells are heavily dependent on mitochondrial ATP production (58). Rodriguez-Enriquez *et al*. reported that OXPHOS flux was active in cervical carcinoma cells. In breast carcinoma even over 90 percent of contribution to cellular ATP supply was generated *via* this pathway (59). Numerous studies have reported that biguanides such as metformin and phenformin exert anticancer effect targeting mitochondrial metabolism of tumor cell. The prime molecular target for metformin is complex I of electron transport chain (ETC) (60). Metformin treatment increases intracellular AMP level and induces AMPK activity as a consequence of its effects on mitochondrial metabolism, as shown by Hardie (61). Our recent study have demonstrated that metformin decreased ATP/ADP ratio in cervical cancer C4-I cells and co-treatment of the cells with caffeic acid inhibited ATP synthesis, activated AMPK signaling and suppressed survival of cells (*Fig. 5*) (62).

Considering the metabolic flexibility of cancer cells, another important implication is that glycolysis generates less ROS than OXPHOS. As angiogenesis induced by malignant cells is a chaotic process, tumors often are poorly perfused and have limited glucose availability but, at the same time, enough oxygen to generate mitochondrial ATP. In such conditions, glucose deficiency becomes the main cause of metabolic stress in cancer cells which use glucose as a main substrate for energy production. Under glucose deprivation, cancer cells overproduce ROS and the mitochondrial respiration is one of the major sources of oxidative stress in aerobic environment. During the process of electron transfer in mitochondria, some electrons may leak from ETC, which lead to the formation of ROS (at physiological oxygen levels 1 – 4% of oxygen may be reduced to free radical form) (63, 64). Due to the compromised metabolism, tumor cells may adopt to disrupted redox state much easier than normal cells. As a result, cancer cells avoid ROS-induced apoptosis or anoikis as opposed to non-tumor cells (65). Indeed, the experimental data suggest that cancer cell lines are able to evade death by balancing between glycolysis and OXPHOS to sustain ROS at the adequate level (66). On the other hand, in case of tumors where OXPHOS is proved to be essential for cell survival, the generation of excessive oxidative stress *via* this pathway may become an important therapeutic target (56).

In some cases mitochondrial oxidative phosphorylation may confer metabolic advantage to tumor cells, but at the same time an addiction to OXPHOS makes neoplasms more sensitive to drugs that impair ATP generation. Therefore, it was suggested in order to improve the efficacy of pharmacological anti-tumor approach, that glycolysis should be suppressed simultaneously with OXPHOS. The recent studies using human breast cancer stem cells have shown that metformin may coordinately deplete intermediates of both, mitochondrial tricarboxylic acid (TCA) cycle and glycolysis. Numerous epidemiological observations confirmed that metformin exerted chemopreventive effects in humans and reduced the risk of prostate, breast, pancreas and colon tumors and this effect was attributed mostly to AMPK activation and its effects on cell bioenergetics and biosynthetic pathways (67, 68). What is more, metabolic action of metformin may depend on the stage of tumorigenesis and is stronger at the initial phases of cellular transformation (69). In early tumor stage, the activation of AMPK may effectively restrain the reprogrammed metabolism of tumor cell and elicit cell death. However, a large body of evidence indicated, that when tumor has arisen the role of AMPK becomes more complex. The overexpression of AMPK was reported in several tumor cell lines at the advanced stage of cellular transformation, such as astrocytic tumors (70) and lung cancers (71, 72). This finding is with compliance with other results, that expression of AMPK in III and IV grades of human gastric cancers is increased when compared to grades I and II (73, 74). It was demonstrated that the suppression of overexpressed AMPK may affect survival of metastatic cells, for example the knock-out of AMPK catalytic subunits in prostate cancer cells lead to cell death, as reported by Park *et al*. (75).

Within tumor cells with malignant phenotype, glucose deficiency restrains ATP generation and leads to ROS overproduction and accumulation, which in turn activates AMPK (*Fig. 3*) (64). As mentioned earlier, the triggering of oxidative pathways by AMPK under metabolic stress lead to utilize alternative substrates for ATP synthesis. In such conditions, once activated AMPK modifies metabolic reprogramming of tumors to persist glucose deprivation by promoting catabolism (glucose uptake, fatty acid oxidation, autophagy), with concomitant suppression of cellular anabolism (protein, glycogen and fatty acid synthesis). It was shown in several malignant cell lines, that the activation of APMK corresponds with the increased tolerance to glucose deficiency (74). Additionally, by activating p38/peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) pathway, AMPK may enhance mitochondrial biogenesis (76). The increased mitochondrial burden under metabolic stress facilitates ROS production, but AMPK may combat ROS accumulation firstly by increasing glycolysis/PPP and then by balancing autophagy and OXPHOS. The shift to oxidative phenotype restrains cell proliferation (50). As mentioned, AMPK opposes the biochemical effects exerted by AKT, thus cytoprotective program triggered by activated AMPK for upregulating ATP synthesis and inhibiting energy expenditure pathways may suppress growth, but, at the same time, under glucose deprivation, it results rather in cancer cell survival than death (2, 3). Additionally, as mention earlier, this adaptive metabolic benefit may be facilitated by the downstream effectors of AMPK, especially by activation of FOXO and inhibition of mTOC1. The abundant glucose supply clearly results in the inhibition of AMPK and the activation of AKT, which in turn promotes cancer cell growth, division and metastasis (*Fig. 3*). Moreover, AKT stimulates oncogenic pathways that drive uncontrolled proliferation of tumor cells (47). In metastatic cells, AMPK acts as master regulator which couples cellular bioenergetics to growth signals under metabolic stress and plays the essential role in the adaptation of cancer cells to changeable environmental conditions. However, growing evidence suggests that simple manipulations of AMPK activity may not be sufficient for effective restraining of tumor. Recently, the approaches targeting the entire AMPK/AKT/mTOR axis instead single regulatory proteins has been proposed (77). However, the exact role of AMPK intracellular signaling network in particular cancer stage should be elucidated and carefully considered at first.

As discussed earlier, the tumor-suppressive activity of AMPK signaling was especially attributed to its essential upstream kinase LKB1 (78). However, during tumorigenesis mutations may occur in gene encoding LKB1, which lead to the impairment of LKB1/AMPK axis (79). The loss of such important tumor suppressor function might be considered as a stimulus promoting cancer growth. However, at the same time LKB1-deficient cells become more vulnerable to the disruption of the cellular energy homeostasis comparing to non-mutated cells. Such tumor cells can be effectively eliminated by drugs eliciting metabolic stress (2). Parker *et al*. investigated the metabolic effects of pharmacological agents that induce energy stress in non-small cell lung cancer cells (80). LKB1-proficient lung cancer cells were less sensitive to drugs compared to LKB1-deficient cells. Following exposition to the treatment, the latter were not able to

effectively balance their metabolism. The absence of functional LKB1/AMPK axis may account for high sensitivity of prostate cancer cells to the energy disruption and ATP depletion, as shown by Loubiere *et al*. In lung cancer cell line, it has been demonstrated that treatment of cells with metformin induced energy deficiency which restrained cell proliferation (81). In compliance and as mentioned above, our recent study showed that caffeic acid decreased ATP level with following activation of AMPK in cervical tumor cells lacking a functional LKB1 protein and co-treatment of cells with metformin further reduced ATP, disrupted energy balance and elicited massive cell death (82, 83).

TARGETING METABOLIC REPROGRAMMING OF CANCER CELLS *VIA* REGULATORY PROTEINS OF CARBOHYDRATE AND LIPID METABOLIC PATHWAYS

Cancer cells rely not only on glucose but also glutamine-derived carbon supply in order to meet bioenergetic and biosynthetic demands. An increased consumption of glutamine occurs in many cancer cells (84). Within mitochondria, glutaminase (GLS) converts glutamine to glutamate. Glutamate is then converted to α-ketoglutarate (αKG), an intermediate that supports anaplerotic reactions of the tricarboxylic acid (TCA) cycle for synthesis of macromolecules. Glutamate may be also converted directly into glutathione (GSH) by the action of glutathione cysteine ligase (GCL), thereby improving antioxidant protection of cancer cell (2). In tumors, low glucose level stimulates TCA cycle *via* glutamine metabolism to generate adequate amount of ATP and intermediates for biosynthesis. Therefore, the restriction of TCA cycle supply *via* suppressing GLS was proposed as a promising target for anti-cancer approach (62, 63).

It was recently demonstrated that the exposition of human cervical cancer cells to dichloroacetate (DCA) reduced glycolysis by activating pyruvate dehydrogenase (PDH) complex (85, 86). PDH complex provides a link between glycolysis and TCA cycle. The activation of the PDH enables pyruvate to enter mitochondria instead of its reduction to lactate in cytosolic matrix (87). It was demonstrated that DCA treatment activated PDH which resulted in overproduction of ROS in cervical tumor cells (86, 88) as well as glioblastoma cells (50), which in turn caused massive cell death. The cells exposed to the drug were not able to combat oxidative stress by generation of NADPH at a sufficient level. Our recent study showed that caffeic acid may be another potent PDK inhibitor, since in cervical cancer cell lines the drug restored PDH 'bottleneck' and

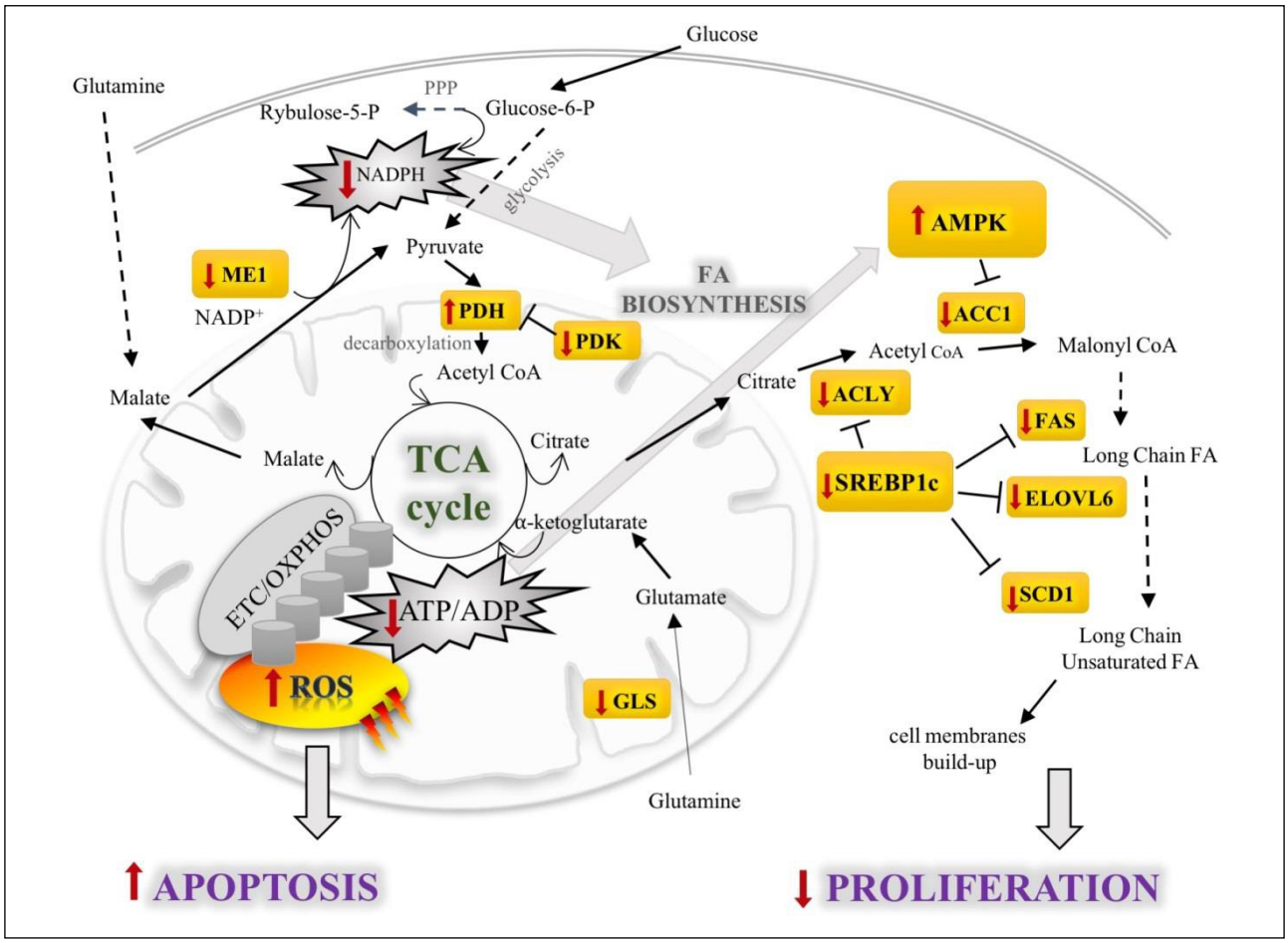

Fig. 5. The effects of metformin and caffeic acid on TCA cycle supply and lipid *de novo* synthesis in human cervical cancer cells. Schematic diagram shows the main actions of the compounds on mitochondrial oxidative metabolism: decarboxylation of pyruvate to acetyl-CoA (*via* PDH activation), TCA cycle supply with glutamine (*via* GLS downregulation), regeneration of pyruvate and NADPH pool for FA *de novo* synthesis *via* ME1 inhibition, enhancement of ROS formation in mitochondria, inhibition of FA biosynthesis by downregulation of transcription factor SREBP1c and its downstream enzymes (ACLY, FAS, ELOVL6 and SCD1) (62, 82, 83). The main actions of the drugs on regulatory proteins and processes were marked with arrows (↑activation, ↓ inhibition).

enhanced fueling of TCA cycle with pyruvate. The treatment lead to intolerable oxidative stress followed by cell death *via* apoptosis (82). We demonstrated that also metformin may exert metabolic stress *via* regulation of PDH activity and TCA cycle anaplerosis in cervical tumors (*Fig. 5*) (83).

The alleviation of the free radical detoxification is a prime target to restrain tumor progression in various anti-cancer strategies, such as radiotherapy and chemotherapy (64). The impairment of ROS balance may increase the sensitivity of cancer cells to therapies targeting oxidative stress. The metabolic adaptations of malignant cell may fight against ROS, but at the same time the requirement for reductive power (NADPH) for biosynthesis rises enormously. AMPK activation may play cytoprotective role by preventing NADPH depletion *via* regulating a variety of AMPK downstream targets, such as acetyl CoA carboxylase 1 (ACC1) and 6-phosphofructo-2-kinase (PFK-2) (89). As suggested by Jeon *et al*., the downregulation of pathways consuming NADPH following AMPK activation may help cancer cells to combat oxidative stress and to some extent support metabolic adaptation to survive (90). However, the distant results of AMPK activation on tumor metabolism *via* inhibiting *de novo* lipogenesis may be more complex. The inhibitory action of AMPK on lipogenesis might be a limiting factor for cells' proliferation, as suggested by Weinberg and Chandel (85). This mechanism was demonstrated at early progression stage of cancer (1). The suppression of lipid biosynthesis may spare NADPH in short term, but paradoxically it can also limit the generation of membrane phospholipids, lipid-derived precursors and other regulatory molecules as a result. Once activated AMPK inhibits lipid biosynthesis *via* downregulation of sterol regulatory element binding protein 1c (SREBP-1c) and its downstream effectors (91). The overexpression of SREBP-1c and its downstream enzymes regulating lipid biosynthesis (ATP citrate lyase, ACLY; fatty acid synthase, FAS; stearoyl-CoA desaturase 1, SCD1) have been correlated with the increased tumor invasiveness (92, 93). Emerging data indicate, that enzymes controlling lipid *de novo* synthesis may induce EMT process and drive migration and invasion of tumor cells, since fatty acids are crucial for membrane build-up and function of metastatic cell (92). What is more, the enhanced synthesis of unsaturated fatty acids has been recognized as essential for sustaining of high fluidity of metastatic cell's membrane. Therefore, this process was pointed out as potent target for novel therapeutic interventions (92, 94, 95). Our studies also demonstrated, that activation of AMPK following double treatment of human cervical cancer cells with metformin and caffeic acid caused the impairment of lipid biosynthesis and resulted in suppressed cell proliferation (62, 82). We showed that co-treatment caused the downregulation of SREBP-1c transcription factor and its effectors ATP citrate lyase (ACLY), fatty acid synthase (FAS), fatty acyl-CoA elongase 6 (ELOVL6) and stearoyl-CoA desaturase-1 (SCD1) (83). However, Jeon and other authors suggest that FAS inhibition following induction of AMPK activity may promote late stages of carcinogenesis. In such a way tumor cells that had undergone malignant transformation may become more resistant to anti-cancer treatment as a result (1). Griss *et al*. reported that some cancer cells may also use alternative pathways, resistant to metformin, to generate lipids (96). Since the cellular effect of lipid metabolism modulation by AMPK remains unclear, there is a considerable interest in evaluating the possible effects of AMPK regulation. AMPK agonists, by suppressing the formation of lipids, may restrain invasiveness and the metastatic potency of tumors and exert beneficial effects for cancer prevention. On the other hand, in the context of lipid metabolism, the suppression of AMPK may be applicable to treat malignant tumor. Again, the proper therapeutic intervention should recognize and precisely address the stage of carcinogenesis.

ONCOGENES AND SUPPRESSOR GENES INFLUENCE METABOLIC PHENOTYPE OF CANCER CELLS REGULATED BY AMP-ACTIVATED PROTEIN KINASE

Within tumor cell, AMPK network and tumor suppressor p53 are mutually coordinated (56). AMPK can suppress the progress of cell cycle, which limits cancer cell proliferation by acting *via* stabilization of p53 protein and the cyclin-dependent kinase inhibitors $p21^{WAF1}$ and $p27^{KIP1}$ (97). It was shown in some cancer cells that AMPK activation by glucose deprivation or by its chemical activator 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) leads to upregulation of p53 and anti-tumor effect (20). p53-regulated proteins PA26 (Sestrin 1) and Hi95 (Sestrin 2) are implicated in the activation of AMPK with concomitant inhibition of mTOR. Within the cell, the stabilization of hypoxia-inducible factor-1α (HIF-1α) protein triggers the expression of genes involved in the establishing of glycolytic phenotype of cancer cells as well as their invasive properties (98). The activation of HIF-1α downstream proteins promotes EMT and increases anoikis resistance (99). Additionally, the overexpression of oncogene c-Myc has been shown in numerous malignant cell lines. The excessive activity of this transcription factor facilitates metabolic flexibility of tumors and their adaptation to the changeable environmental conditions. c-Myc plays fundamental role in growth and proliferation of cancer cell. It was demonstrated that simultaneous c-Myc overexpression and HIF-1α activation cause a switch to glycolytic phenotype *via* inducing of several molecular targets, particularly glucose transporters (GLUT family) and enzymes, such as lactate dehydrogenase A (LDHA) and PDK1 (100). Additionally, c-Myc may activate transcription of proteins involved in glutamine anaplerosis (2). The modulating of HIF-1α/c-Myc network has been proposed as a target for therapeutic anti-tumor intervention, especially in case of glucose-dependent cancers. Within the cell with functioning mitochondrial OXPHOS, AMPK signaling decreases HIF-1α with following attenuation of glycolytic metabolism (98). It was also demonstrated that the activation of AMPK protects against enhanced proliferation of hepatocellular carcinoma cells caused by c-Myc overexpression. AMPK may exert the inhibitory effect on glycolytic enzymes as a result of repressing HIF-1α/c-Myc proteins and PI3K/Akt/mTOR pathway. In such a way AMPK alleviates the potential of tumor cell to adapt its metabolism to the availability of oxygen and nutrients (101).

HIF-1α activation, apart from inducing glycolysis, in the conditions of limited oxygen supply may also regulate glucose and glutamine entry into TCA cycle. It occurs *via* regulation of the activity of mitochondrial complexes PDH and α-ketoglutarate dehydrogenase (α-KGDH), respectively. It was shown that statins, by activation of AMPK, may restrain HIF-1α effect on the enzymes. In compliance, the dephosphorylation of AMPK by compound c completely abrogated the inhibitory effect of the drugs on HIF-1α and TCA cycle supply (102). In our study, using cervical cancer cells HTB-35 cells we demonstrated that metformin inhibited HIF-1α function and expression of its downstream effectors, hexokinase II (HKII) and pyruvate dehydrogenase kinase-1 (PDK-1). Under hypoxic condition metformin by acting *via* HIF-1α and c-Myc restrained glycolytic phenotype of the cells (83).

THE USE OF COMBINATORY TREATMENT INCREASES EFFICIENCY OF ANTI-CANCER APPROACH

Tumor cells easily develop mechanisms of resistance under the selective pressure of anti-cancer treatment and advantageous traits may be further spread *via* clonal evolution. Therefore, in order to increase effectiveness of the treatment the current

molecularly targeted approach aim to circumvent acquired resistance (103, 104). Numerous studies using *in vitro* and *in vivo* models showed that combinatory approach was more effective than single drug treatment. The combination of several drugs targeting different mechanisms may be better strategy to overcome metabolic adaptations of cancer cells. It was found that the combination of targeted drugs may also act more precisely and with better therapeutic index comparing to the effect of a single drug (105). Similarly, several clinical trials using combination of anti-tumor drugs demonstrated promising outcomes (106, 107). What is more, natural compounds, like polyphenols, may support the efficiency of anticancer treatment (108). The use of AMPK activator, metformin together with mTOR agonist, rapamycin, has been proposed for combined intervention, since simultaneous targeting AMPK and mTOR/AKT may re-couple fuel and growth signals in tumor cells and inhibit cell growth. Such a co-therapy was approved for use in humans by the United States Food and Drug Administration (FDA) (109). Moreover, as AKT plays crucial role in multiple intracellular signaling pathways, targeting this kinase may influence cell proliferation and DNA replication ratio (110).

Metformin was tested for combinatory use and many studies demonstrated its potency to enhance the action of chemotherapeutic drugs, such as cisplatin and 5-fluorouracil (111). In compliance, our recent study showed that metformin supported toxicity of cisplatin against HTB-35/SiHa cells. Metformin together with caffeic acid sensitized quiescent tumor cells to the action of chemotherapeutic drug *via* regulation of cell cycle. Co-treatment enhanced therapeutic response, especially the elimination of cancer cells from co-culture with normal cells. At the same time both drugs were non-toxic to normal cells, even at high levels (83).

Although cisplatin and other chemotherapy medications exert anti-tumor action *via* several independent mechanisms, the induction of oxidative stress within tumor cell remains one of the main effects. The resistance to cisplatin often comes from compensatory signaling pathways, which, in turn, let transformed cells counteract accumulation of ROS by upregulating intracellular antioxidant systems to decrease ROS load. Therefore, the combinatory approach using two or more agents targeting specific redox mechanisms is currently considered to be more effective in improving the treatment outcome (112). Several small molecules, including the PDK inhibitor DCA (63,65) and the inhibitor of HK2, 2-deoxy-D-glucose (2-DG) (113, 114), are currently being under evaluation for their therapeutic utility, as single drugs and as co-treatment.

Perspectives in future therapenties

The current anti-cancer therapies have been developed based solely on genetic approaches without proper understanding of the molecular mechanisms involved in cancer progression and metastasis. Targeting metabolic reprogramming of tumor cells provides new opportunity to address these issues and effectively fight cancer. The emerging data indicate, that the regulation of bioenergetics and biosynthetic pathways may be a relevant target for anti-tumor treatment. In order to realize the full potential of single-drug based and combination therapies in this area, we need to better understand the regulation of tumor cell metabolism with respect to its stage and origin. Additionally, more precise characterization of metabolic profiles of tumors would let to target cancer metabolism and improve the poor outcomes of existing anti-cancer therapies.

Numerous studies have shown that AMPK signaling play the crucial role in cell transformation and cancer progression. In early cancers expressing LKB1/AMPK pathway, AMPK may act as a tumor suppressor. On the other hand, its activation may help malignant cell to manage metabolic stress and evade apoptosis under conditions of reduced mitochondrial function. However, tumor cells lacking functional LKB1 become more sensitive to energy stress and thus the drugs that decrease ATP level may selectively cause malignant cell death. These findings may at least in part explain the phenomenon that metformin expresses chemopreventive activity, but also may be used in chemotherapy against some advanced tumors, especially in combination with other drugs. The controversies on the role of AMPK in tumor promotion have aroused mainly as a result of the incomplete understanding of its functioning in cancer cells with regard to the stage of carcinogenesis. Since intracellular AMPK signaling is complex and its cross-talk with other pathways is poorly recognized, the modulating of AMPK upstream proteins and downstream effectors in various cancer cells may lead to the unexpected results. In particular, various tumor cells may express unique signaling networks and epigenetic signatures, thus despite evident common traits, metabolism of these cell may differ dramatically. During cancer progression, AMPK can probably play distinct roles in each cell's subtype, depending on degree of differentiation and ability to metastasize. The processes by which AMPK impacts metastasis and invasion have not been fully recognized yet. The cellular effects of AMPK also depend on specific environmental context, such as the availability of nutrients/oxygen. Therefore, the application of AMPK agonists in cancer treatment must be introduced with caution. More precise recognition of molecular targets of AMPK may help to develop specific and effective therapeutic approaches.

Abbreviations: ACC1, acetyl-CoA carboxylase 1; ACL, ATP citrate lyase; AMPK, 5'-adenosine monophosphate-activated protein kinase; Akt, protein kinase B; AMP, adenosine monophosphate; ATM, ataxia-telangiectasia mutated kinase; ATP, adenosine triphosphate; CaMKK, Ca^{2+}/calmodulin-dependent protein kinase kinase; DEPTOR, DEP domain-containing mTOR-interacting protein; ECM, extracellular matrix; EGF, epidermal growth factor; eIF4E, eukaryotic translation initiation factor 4E; ELOVL6, fatty acyl-CoA elongase 6; EMT, epithelial-mesenchymal transition; ETC, electron transport chain; FA, fatty acids; FAS, fatty acid synthase; FOXO, the forkhead box O family of transcription factors; GLS, glutaminase; GLUT1, glucose transporter 1; HDAC1, histone deacetylase 1; HGF, hepatocyte growth factor; LKB1, liver kinase B1; LPA, lysophoshatidic acid; MAGE-A3/6, melanoma antigen genes A3/6; ME1, malic enzyme 1; MLST8, target of rapamycin complex subunit LST8; mTOR, mammalian target of rapamycin; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form; NADP, nicotinamide adenine dinucleotide phosphate, oxidised form; OXPHOS, oxidative phosphorylation; PDH, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase; PEITC, phenylethyl isothiocyanate; PFK2, phosphofructokinase 2; PP2, protein phosphatase 2; PPP, pentose phosphate pathway; ROS, reactive oxygen species; S6K, ribosomal protein S6 kinase beta-1; SCD1, stearoyl-CoA desaturase-1; SOD, superoxide dismutase; SREBP1c, sterol regulatory element-binding protein 1 transcription factor; TAK1, transforming growth factor β-activated kinase 1; TCA, tricarboxylic acid; TGF-β, transforming growth factor β; TRIM28, tripartite motif-containing protein 28; ULK1, UNC51like kinase 1; Yap, Yes-associated protein.

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