Role of the LKB1/AMPK pathway in tumor invasion and metastasis of cancer cells (Review)

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Abstract. Liver kinase B1 (LKB1), also known as serine/threonine kinase 11 (STK11), is a tumor suppressor that is inactivated in Peutz-Jeghers familial cancer syndrome. LKB1 phosphorylates and activates AMP-activated protein kinase (AMPK), which negatively regulates cancer cell proliferation and metabolism. However, recent evidence demonstrates that the LKB1/AMPK pathway is involved in the process of tumor invasion and migration, which is an important hallmark of carcinoma progression to higher pathological grades of malignancy. This review focuses on the function of the LKB1/AMPK pathway in the invasion and migration of cancer cells and provides an overview of therapeutic strategies aimed at this pathway in malignant tumors.

Contents

1. The function of liver kinase B1

Liver kinase B1 (LKB1), also named serine/threonine kinase 11 (STK11), was first identified in Peutz-Jeghers syndrome (PJS), which is a tumor susceptible syndrome characterized by inactivation of LKB1 (1,2). Somatic mutations of LKB1 have also been found in many malignant tumors such as lung cancer (3), breast cancer (4) and colon cancer (5). When forming a complex with STE20-related adaptor protein (STRAD), LKB1 translocates from the nucleus to the cytoplasm to exert biological functions (6). Mouse protein-25 (MO25), a scaffold protein, interacts with the carboxyl terminus of STRAD and stabilizes the LKB1-STRAD complex, leading to LKB1 activation (7).

It is well known that LKB1 controls cell polarity and maintains intracellular energy balance (8,9). LKB1 functions as a tumor suppressor by phosphorylating and activating AMP-activated protein kinase (AMPK) (8,9). LKB1/AMPK signaling regulates the formation of cytoskeletal microtubules and the expression of cell polarity proteins, including prostate apoptosis response-4 (PAR-4), to maintain cell polarity (10). LKB1 deficiency disturbs the polarity of mammary epithelial cells, resulting in cell disorder and an increased invasion and migration ability of epithelial cells (11). Moreover, LKB1 inhibits tumor initiation and progression by arresting the cell cycle in the G1 phase and by promoting cell apoptosis (12,13). Therefore, dysregulation of LKB1 is not only the underlying pathological cause of PJS, but it is also related to the initiation and progression of various types of tumors via multiple mechanisms (Fig. 1).

2. The function of AMPK

AMPK is a heterotrimer formed by a catalytic subunit (α) and two regulatory subunits (β and γ) (14). AMPK can be phosphorylated and activated by LKB1 and calcium dependent protein kinase kinase-β (CaMKK-β) (14-16). AMPK can also be activated by small molecules, including 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), metformin and analogues of these proteins (17,18).

AMPK is a tumor suppressor and sensor of cellular energy status. It plays an important role in the control of cellular metabolism, proliferation and autophagy (19). As an energy sensor, AMPK is activated when the intracellular AMP/ATP ratio rises. Activated AMPK increases the production of ATP and decreases AMP consumption to maintain the balance of the AMP/ATP ratio (20). Rapidly growing cancer cells depend on a sustained energy supply. Tumor cells synthesize ATP by increasing glucose uptake and glycolysis to satisfy metabolic

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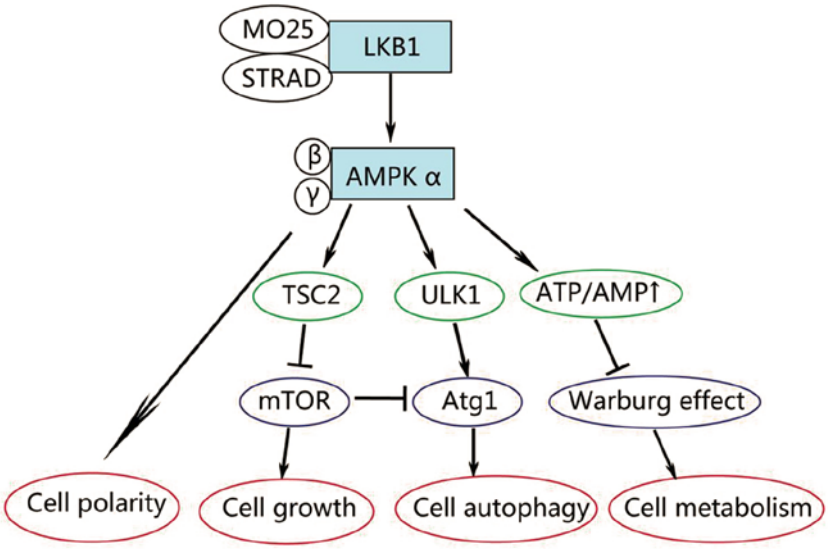

Figure 1. Regulation of cell growth, metabolism and polarity by LKB1/AMPK signaling. MO25 and STRAD form a complex with and activate LKB1, a major upstream kinase of AMPK. AMPK maintains cell polarity and suppresses cell growth and metabolism by negatively regulating the mTOR pathway and Warburg effect. Moreover, AMPK forms a complex with ULK1, leading to initiation of autophagy.

requirements, a phenomenon called the Warburg effect (21). It has been reported that AMPK suppresses the Warburg effect in tumor cells to inhibit tumor energy metabolism (22). Furthermore, dysregulation of AMPK is closely related to tumor growth, neovascularization, invasion and migration of cancer cells (23).

Rattan *et al* reported that AICAR increases protein expression of cyclin-dependent kinase inhibitor 1A (p21), cyclin-dependent kinase inhibitor 1B (p27) and p53 via AMPK activation to suppress cell proliferation in various tumor types (24). In addition, in higher eukaryotes, AMPK directly phosphorylates serine-1387 in tumor suppressor tuberous sclerosis 2 (TSC2) to suppress the mammalian target of rapamycin (mTOR) signaling pathway (25). mTOR is a critical regulator of cell growth and autophagy (26). Autophagy involves degradation of damaged organelles and cytoplasmic components to provide materials and energy for cell survival under stressful conditions, such as starvation (27). mTOR inhibits autophagy through phosphorylating unc-51 like autophagy activating kinase 1 and 2 (ULK1 and ULK2). Stressful conditions, including starvation, hypoxia and energy depletion, lead to suppression of mTOR signaling, which in turn results in activation of autophagy (Fig. 1). Various studies have shown that AMPK stimulates ULK phosphorylation via inhibition of mTOR signaling to promote autophagy (28). Moreover, AMPK can also directly phosphorylate serine-555, threonine-574 and serine-637 on ULK1 to induce mitochondrial autophagy (mitophagy) (29).

3. Signal transduction in tumor invasion and metastasis

Metastasis is the leading cause of morbidity and mortality in patients diagnosed with cancer. In order to acquire metastatic characteristics, cancer cells must go through several stages including epithelial-mesenchymal transition (EMT) (30), degradation of the extracellular matrix (ECM) (31) and angiogenesis (32). At the molecular level, the invasion and migration of tumor cells may cause changes in gene expression such as matrix metalloproteinase-2 (MMP-2) (33), MMP-9 (34), α-smooth muscle actin (α-SMA) (35), and EMT-associated proteins (36).

Recent studies have shown that alterations in signal transduction are hallmarks of tumor progression, which lead to uncontrolled cellular proliferation, invasion and metastasis. EMT is an important process in carcinogenesis by which epithelial cells lose their cell polarity and cell-cell adhesion, and acquire mesenchymal-like properties. The completion of EMT enhances cell motility, migratory capacity and ECM degradation ability, which promotes tumor cell invasion and migration (37,38). The TGF-β signaling pathway plays a crucial role in EMT (39). TGF-β-activated-R-Smad complex translocates from the cytoplasm to the nucleus (40), binds to the promoter of SNAIL1/2, ZEB1/2, triggers EMT and leads to the occurrence of tumor development, invasion and metastasis (41,42). Wiercinska *et al* reported that TGF-β upregulates the expression of MMP-2 and MMP-9 and induces breast cancer cell invasion via a Smad3- and Smad4-dependent manner (43).

TGF-β also promotes cancer cell invasion and migration through non-Smad signaling pathways (Fig. 2). In NMUMG cell lines, the activation of mTOR signaling by TGF-β occurs through activation of phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), ribosomal protein S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E-binding protein 1 (4EBP1) to induce EMT and cell invasion (44). Likewise, in carcinoma cells, TGF-β regulates EMT-associated cytoskeletal changes and protein expression by activating mTOR complex 2 (mTOR2), further inducing EMT and cell invasion (45). Thakur *et al* demonstrated that TGF-β upregulates the expression of Snail1 and increases the motility and invasiveness of human prostate cancer cells by activating the TRAF6/p38MAPK pathway (46).

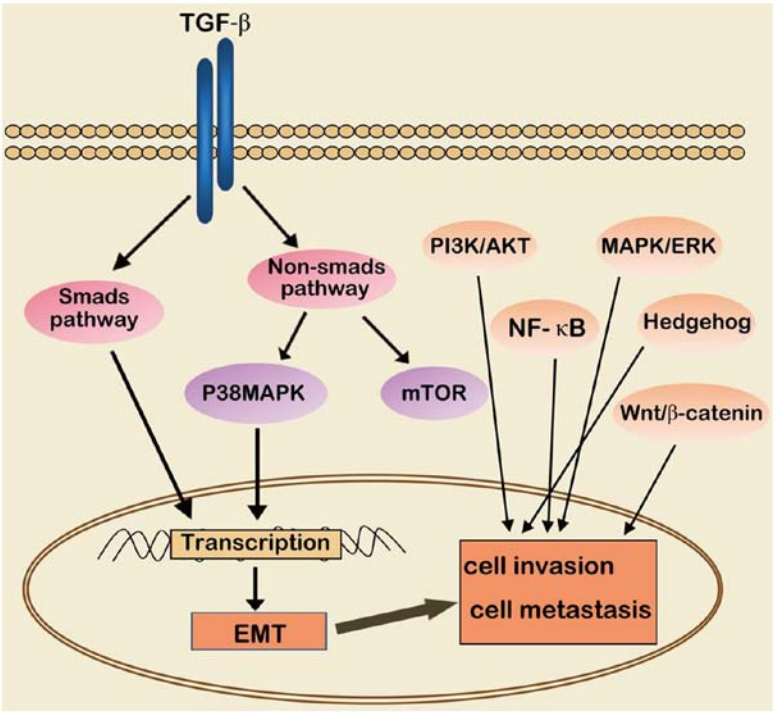

Figure 2. Multiple signaling pathways involved in cancer cell invasion and metastasis. TGF-β induces EMT, invasion and metastasis of cancer cells via Smad and non-Smad pathways. In addition, the PI3K/AKT, NF-κB, Hedgehog, MAPK/ERK, p38MAPK, Wnt/β-catenin signaling pathways are involved in tumor invasion and metastasis.

Apart from TGF-β signaling, other signal pathways including PI3K/AKT (47), NF-κB (48,49), Hedgehog (50), MAPK/ERK (51), p38MAPK (52) and Wnt/β-catenin (53,54) play important roles in tumor progression (Fig. 2). Wu *et al* identified a novel binding site (-626/-617) of NF-κB II in the promoter region of MMP-9 (55). It has been reported that treatment with Hedgehog inhibitors contributed to the motility and invasiveness of rhabdomyosarcoma cell lines (56).

4. Regulation of tumor cell invasion and migration by LKB1/AMPK signaling

The function of LKB1/AMPK in cancer cell invasion and migration. LKB1 is a well-known tumor suppressor and also an upstream kinase of tumor energy metabolism sensor AMPK. SNAIL, an EMT transcriptional factor, is upregulated in LKB1-deficient tumor cells (57). Similarly, in immortalized epithelial cells and lung adenocarcinoma cells depleted of LKB1, ZEB1 was found to be upregulated, accompanied by increased expression of mesenchymal marker proteins, cell viability and invasiveness (58). Double knockout of LKB1 and phosphatase and tensin homolog deleted in chromosome ten (PTEN) in mouse bladder tissues was found to lead to enhanced cell proliferation and tumor initiation, EMT, upregulation of SNAIL, nuclear localization of vimentin, and decreased expression of E-cadherin and tight junction protein zonal occludin-1 (ZO-1) (59). These data indicate that LKB1 plays a key role in the suppression of EMT during tumor progression.

AMPK, a conserved downstream kinase of LKB1, not only inhibits tumor cell proliferation and energy metabolism but also exerts dual regulatory effects on cancer cell invasion. Some studies have proposed that AMPK induces cell migration. Lysophophatidic acid (LPA) can induce the activation of AMPK and promote ovarian cancer metastasis (60). Park *et al* reported that silencing of AMPK abrogated the ability of anthocyanins to inhibit the migratory phenotype of hepatocarcinoma cells. However, more recent studies underscore the ability of AMPK to negatively regulate tumor growth and invasion. Metformin, an AMPK activator, was reported to inhibit EMT and melanoma metastasis in an AMPK/p53-dependent manner (61). Similarly, metformin was found to inhibit MMP-2 and MMP-9 levels, cell proliferation and migration of human umbilical vein endothelial cells (62). Chemokine ligand 3 (CCL-3) can promote MMP-2 expression and the migration of human chondrosarcoma cells, which is abolished by AMPK activation (63). Kim *et al* reported that berberine dramatically increased AMPK phosphorylation but decreased ERK activity and cytochrome *c* oxidase subunit II (COX-2) expression, leading to suppression of human melanoma cell migration (64). These studies suggest that AMPK plays an important role in tumor invasion and migration.

The LKB1/AMPK pathway regulates cancer cell invasion and migration via a variety of signaling pathways. Tumor cell invasion and metastasis is a complex process that results from interaction and crosstalk between multiple pathways. The LKB1/AMPK pathway regulates invasion and metastasis via diversified signaling pathways, such as NF-κB, AKT, forkhead box O3 (Foxo3a), TGF-β and mTOR. It has been reported that AICAR activates AMPK to suppress AKT/FOXO3 signaling, inhibiting EMT and reversing mesen-

chymal characteristics of tumor cells (65). In PC3 and PC3M cells, AICAR and A769662 downregulated mTOR and S6K1 levels, and inhibited migration by activating AMPK (66). Emerging evidence indicates that LKB1/AMPK inhibits tumor invasion and migration through downregulation of the downstream factors of TGF-β signaling, such as MMPs and Snail (67,68). It was reported that AMPK inhibited TGF-β-induced EMT in HK-2 cells (69). In addition, AICAR, metformin and adiponectin were found to activate AMPK, but inhibited TGF-β-induced fibrosis via downregulation of collagen type I α 1 (COL1A) and α-SMA expression in hepatic stellate cells (70). AMPK-mediated proteasomal degradation of p300 is the underlying mechanism of activation (70). Moreover, treatment of breast cancer cells with metformin decreased the functions of TGF-β (71). Conversely, silencing an AMPK downstream effector, lipopolysaccharide-induced TNF-α factor (LITAF), by RNA interference, accelerated tumor growth (72). Furthermore, metformin was shown to arrest cell cycle progression, inhibit proliferation and induce apoptosis of breast cancer cells through suppression of AKT and ERK signaling pathways (73). In contrast, inhibition of AMPK resulted in enhanced cell proliferation and migration of prostate cancer cells, accompanied by an upregulation of S6K1 and insulin-like growth factor 1 (IGF-1) and downregulation of p53 and p21 (74).

AMPK can also indirectly modulate tumor progression through various other signaling pathways. Capsaicin suppressed the invasion and migration of cholangiocarcinoma cells by inhibiting the AMPK-NF-κB pathway, leading to subsequent reduced expression of MMP-9 (75). AMPK/JAK/STATA3 and AMPK/GSK3β/β-catenin pathways were found to be involved in hepatocarcinoma cell and endometrial cancer cell invasion and metastasis (76,77). Novel (nua) kinase family 1 (NUAK1) and ARK5, members of the AMPK family, play important roles in cancer cell migration and invasion by regulating the AKT and NF-κB signaling pathways (78,79).

5. Conclusions

LKB1, an upstream kinase of AMPK, is a tumor suppressor. LKB1/AMPK maintains cell polarity and inhibits cell proliferation and energy metabolism. Moreover, recent studies demonstrated that the LKB1/AMPK pathway plays an important role in the invasion and migration of tumor cells by regulating gene expression and activation of multiple signaling pathways. The TGF-β signaling pathway promotes cancer metastasis while the LKB1/AMPK pathway negatively regulates the progression of cancer. Thus, the regulation of TGF-β signaling may be an important mechanism by which LKB1/AMPK inhibits tumor metastasis. Further investigation of the crosstalk between the LKB1/AMPK pathway and other signaling pathways will accelerate the development and approval of highly targeted cancer drugs.

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