Emerging Implications of LKB1 Liver Kinase B1 Gene as Tumor Suppressor Gene

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Abstract

Background: The LKB1 tumor suppressor is a serine/threonine kinase that functions as master regulator of cell growth, metabolism, survival and polarity. LKB1 is frequently mutated in human cancers and research spanning the last two decades have begun decoding the cellular pathways deregulated following LKB1 inactivation, Liver kinase B1 (LKB1) is a major activator of the AMP-dependent kinase/mammalian target of rapamycin pathway. The prevalence and the specificity of LKB1 gene mutation in acute myeloid leukemia (AML) have not been well established.

Summary: Work from preclinical studies in cell lines, xeno-grafts and GEMMs predict that metabolic therapies, targeted therapeutics targeting LKB1 vulnerabilities will be most effective in combination. Several recent studies show that loss of Lkb1 in adult mice leads to loss of hematopoietic stem cell (HSC) quiescence, resulting in depletion of the HSC pool and a marked reduction of HSC repopulating potential in vivo. LKB1-deficient HSCs and bone marrow cell exhibit reduced mitochondrial membrane potential and depletion of cellular ATP. These data define an essential role of the LKB1 in restricting HSC entry into the cell cycle and in maintaining energy homeostasis through AMPK-dependent and AMP Kindependent mechanisms. Moreover, several studies showed that the anti-diabetic drug metaformin (an LKB1/AMPK activator) exerted significant anti-leukemia cell activity in AML and T-cell acute lymphoblastic leukemia cells through inhibiting mTOR activity. These studies demonstrated that the LKB1/AMPK tumor-suppressor axis is generally functional in hematopoietic cancer and that pharmacological intervention activating this pathway may represent a new target in anticancer therapy.

Keywords: LKB1 gene, metabolic therapies, Acute myeloid leukemia,

Tob Regul Sci. ™ 2022;8(1): 4203-4223

DOI: doi.org/10.18001/TRS.8.1.315

The LKB1/STK11

Human LKB1 gene, also known as serine/threonine kinase 11 (STK11), a tumor suppressor gene that is mutated or deleted in Peutz-Jeghers syndrome (PJS) and in a variety of cancers. It is inactivated in non-small cell lung cancer (NSCLC), malignant melanoma and cervical cancer**(1).**

Peutz-Jeghers Syndrome (PJS), which is a rare hereditary disease and is characterized by mucocutaneous pigmentation, increased risk of gastrointestinal hamartomatous polyposis as well as benign and malignant tumors **(2).**

Knowledge of the biological roles of LKB1 has rapidly expanded over the past decade. Initial research focused on its roles in cell polarity, cell motility, protein translation and energy metabolism, and advances indicated that LKB1 is also involved in the regulation of other cellular process such as DNA damage check point and various signal transduction pathways**(3).**

In mammals, two splice variants of lkb1 are expressed: LKB1L (long) and LKB1S (short), which are both widely expressed**(Fig.1)(4).**

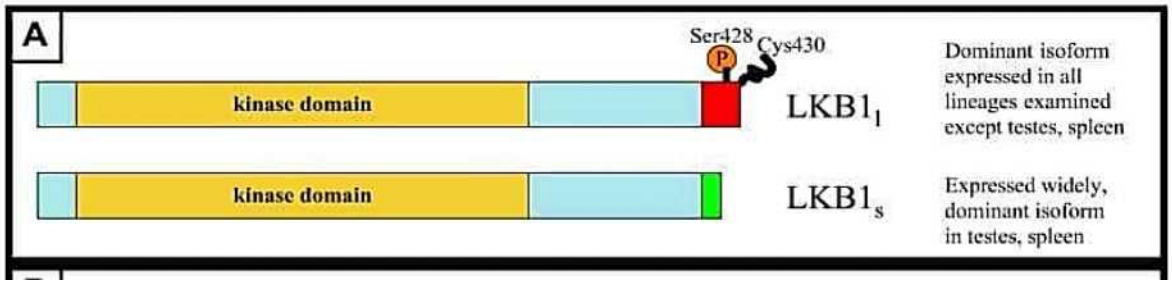

Fig. (1): The two alternative splice forms of LKB1 **(5)**

The two protein variants differ only in their last 63 amino acids; hence LKB1**s** lacks the critical residues S428 and C430. LKB1**s** is essential forspermatogenesis **(4).** Phosphorylation of S399 in LKB1S promotes its nuclear export followed by activation of AMPK and is therefore thought to be functional equivalent to S428in LKB1**L (6)**

Subcellular distribution of LKB1:

LKB1 is mostly localized in the nucleus, with a small portion in the cytoplasm **(7).** However, during the apoptosis of cells, LKB1 is found to translocate into mitochondria **(8).** In addition, LKB1 can be detected on both the plasma membrane and internal membranes in vivo, and this effect is mediated by a functional prenylation motif of LKB1 at the carboxyl-terminus, since mutation of Cys433 to an alanine residue of prenylation motif can block membrane localization of LKB1 **(9).**

Binding Proteins of LKB1:

LKB1 forms complexes which regulate its stability and kinase activity. LKB1 is found to form a heterotrimeric complex with two other proteins, termed STE20-related adaptor (STRAD) and MO25. STRAD, as a pseudo kinase, can induce the transportation of LKB1 from the nucleus to the cytoplasm, and activate LKB1 kinase activity through promoting the active conformation of LKB1. MO25 is a 40 kDa scaffolding protein, can markedly enhance the binding of STRAD to LKB1 and further stimulate its induced kinase activity of LKB1**(10).**

On the other hand, LKB1 can also interact with a chaperone complex made up of heat-shock protein 90 (HSP90) and the CDC37, which can also stabilize LKB1 in the cytoplasm**(11).**

LKB1-HSP90-CDC37 complex is found to function as a repressor of LKB1 kinase activity and disruption of this complex facilitates HSP/HSC70 and E3 ubiquitin ligase carboxyl terminus of HSC70-interacting protein (CHIP)-mediated degradation of LKB1 **(12).**Thus,LKB1-STRAD-MO25 and LKB1-HSP90-CDC37 complexes can both stabilize LKB1 with antagonizing effects on LKB1 activity **(13).**

LKB1 is also reported to interact with other proteins, including LIP1, gene of phosphate and tension homology deleted on chromosome ten (PTEN), p53, protein kinase (PKC), ataxia telangiectasia mutated (ATM) **(13).**

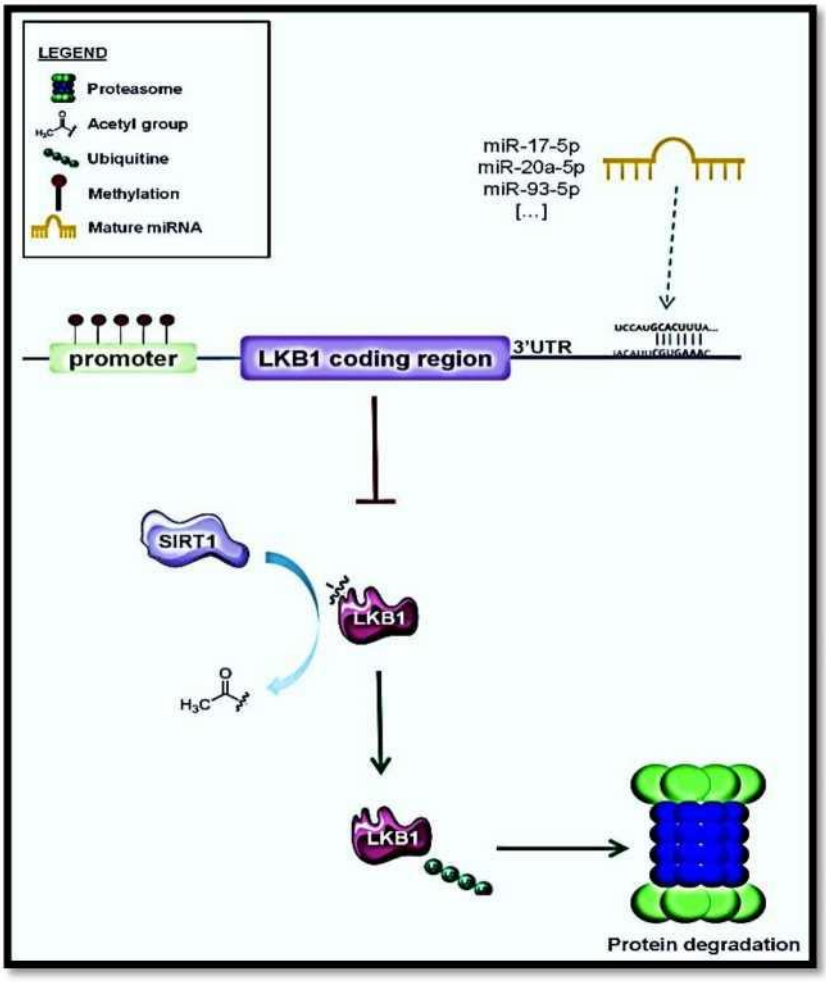

Fig. (2): Epigenetic regulation of LKB1 expression **(14).**

Transcriptional Regulation:

LKB1 gene can be regulated by sex hormones, such as androgen and estrogen. Estrogen is reported to regulate LKB1 gene expression through transcriptional regulation, and 17P— estradiol can down regulate both mRNA and protein level of LKB1 through inhibiting LKB1 promoter

activity(Fig.2)(15).

Testosterone and dihydrotestosterone (DHT) can significantly decrease the mRNA level of LKB1.This effect is mediated by androgen receptor. There is no androgen receptor element (ARE) found in promoter region of LKB1 gene. This indicates that AR may indirectly regulate the LKB1 gene expression (16).

Fibronectin can also regulate LKB1 expression. In non-small cell lung carcinoma (NSCLC) cells, fibronectin is found to inhibit both the mRNA and protein of LKB1 and LKB1-AMP- activated protein kinase (AMPK) signaling (17).

LKB1 downstream pathways:

LKB1 is a master kinase of 13 AMPK-related protein kinases. LKB1 in complex with STRAD and MO25 phosphorylates AMPK and AMPK-like kinases to regulate polarity, adhesion, growth, metabolism and cell survival, including AMPK, NUAK family SNF1-like kinase 1 (NUAK1), sucrose non-fermenting protein-related kinase (SNRK), brain selective kinase1/2 (BRSK1 and BRSK2) or synapses of amphids-deficient kinase (SADK), Saltinducible kinase1/2/3 (SIK1, SIK2 and SIK3), microtubule affinity regulating kinase1/2/3/4 (MARK1, MARK2, MARK3 and MARK4) or partitioning defective gene 1 (Par1) and maternal embryonic leucine zipper kinase (MELK)(Fig.3)(18).

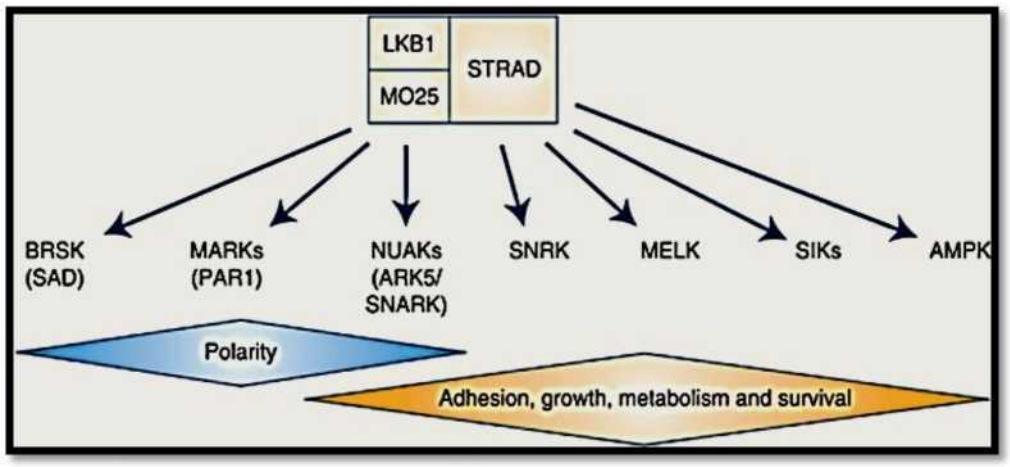

Fig (3):LKB1 regulates an AMPK-like family of kinase (19).

LKB1-AMPK Signaling Pathway:

AMPK is a heterotrimeric protein complex containing a catalytic subunit al/2 and two regulatory subunits, pi/2 and yl/2. It is found that a subunit can directly interact with both P and Y subunits, however, whether P and Y subunits interact with each other remains controversial (20).

The link established between a tumor suppressor (LKB1) with roles in cancer, and a protein kinase (AMPK) that had previously been regarded as a regulator of metabolism (21).

The LKB1-AMPK pathway acts as a tumor suppressor that normally restrains growth and proliferation of cancer cells as well as the associated metabolic changes. Genetic loss of LKB1 due to somatic mutations leads to failure of AMPK activation following metabolic stresses that increase AMP and ADP **(22).**

The pathway can be down-regulated in cancer, through direct effects on AMPK rather than as downstream consequences of LBK1 inactivation. Expression of the AMPK-a2 subunits is reduced in some cases of hepatocellular carcinoma, and this is associated with enhanced tumor cell growth in mouse xenografts, and poorer patient prognosis , while in melanoma cells that carry the V600E mutation in B-Raf, LKB1 appears to be phosphorylated at carboxy-terminal sites, and this is associated with reduced AMPK activation **(23).**

Gene Functions:

1-Cell metabolism: It has been proposed that LKB1 also regulates cellular growth by controlling another tumor suppressor, tuberous sclerosis complex (TSC) via the AMPK- dependent pathway. Under energy starvation conditions, LKB1 phosphorylates and activates AMPK, which directly phosphorylates TSC2, thereby enhancing its ability to switch off the mTORsignaling. In addition, AMPK may also phosphorylate and inactivate one of mTORC1 complex components, Raptor, thereby suppressing synthesis metabolism **(24).**

By inhibiting mTORC1, AMPK not only down-regulates expression of ribosomal proteins, but also reduces expression of HIF-1a and thus expression of the glycolytic enzymes and transporters required for the Warburg effect **(25).**

2-Apoptosis and cell cycle arrest: The role of LKB1 in apoptosis has been indicated by studies showing an absence of apoptosis in polyps from patients with PJS. In this role, LKB1 associates with p53 physically and regulates specific p53-dependent apoptosis pathways. In addition, LKB1 has been reported to interact with and phosphorylate phosphatase and tensin homolog deleted on chromosome ten (PTEN), another tumor suppressor that has lipid phosphatase activity and that inhibits cell proliferation and survival **(1).**

3-Cell polarity:LKB1 induces apical brush border formation in intestinal cells by phosphorylating MST4, which then activates ezrin. LKB1 was found to localize in the primary cilium and basal body, and result in increased AMPK phosphorylation at the basal body and inhibition of the mTOR pathway, which limits cell size **(26).**

4-Mitosis: Banko et al.**(27)** discovered that inactivation of AMPK induced defects in cell mitosis and induced S phase arrest. AMPK regulates the protein phosphatase 1 regulatory subunit 12C (PPP1R12C), which binds to myosin regulatory light chain and 14-3-3 in order to dephosphorylate mitotic proteins for mitotic exit, and is necessary for mitotic progression. Moreover, phosphorylation of AMPKa at Thr-172 was required for the association of AMPK with the centrosome, spindle poles, and mid-body during mitosis.

5-**Maintenance of genome stability:** Some reports showed that AMPK is involved in IR- and ROS-induced DNA damage response. AMPKa2 was recruited to DSBs in an LKB1- dependent manner. LKB1 depletion induced the formation of chromosome breaks and radials. These results suggest that LKB1-AMPK signaling may contribute to DNA damage repair and play a role in the maintenance of genome stability **(28).**

6-Anoikis and inhibition of tumor progression and metastasis: Anoikis is a form of apoptosis that is triggered by poor contact between the cell and the extracellular matrix (ECM). Cancer cells may become resistant to anoikis and consequently display anchorage independent growth. It was found that LKB1 involves in p53-dependent anoikis by regulating salt inducible kinase (SIK1) **(29).**SIK1 is required for LKB1 to promote p53-dependent anoikis and suppress anchorage-independent growth and invasion **(Ng et al., 2012).**

LKB1 is essential for T reg cells stability:

It is a key regulator of glucose and lipid metabolism in T cells and plays a pivotal role in its development and function. LKB1 is a key regulator of lipid metabolism in T reg cells, involved in optimal programming of suppressive activity, immune homeostasis, and tolerance**(30).**

In T reg cells, LKB1 has been reported to stabilize Foxp3 expression by preventing signal transducer and activator of transcription (STAT) 4-mediated methylation of conserved non-coding sequences (CNS2) in the Foxp3 locus **(31)** and also contributed to the activation of b-catenine signaling for the regulation of PD1 and tumor necrosis factor (TNF) receptor **(32).**

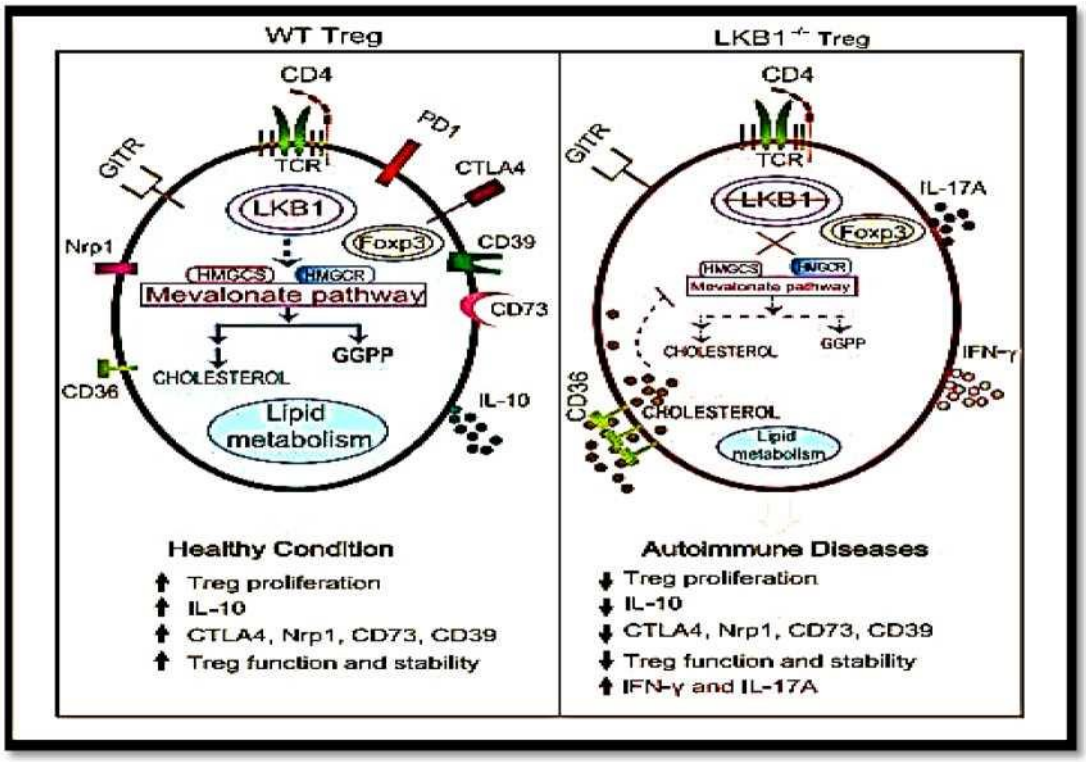

Fig. (4):the LKB1-emediated mevalonate pathway is required for proper function and stability of T reg cells **(33)**

LKB1 regulates cell cycle and energy metabolism in haematopoietic stem cells:

Lkb1 regulates embryogenesis and the metabolism and polarity of differentiated adult cells. Mice deficient for Lkb1 die at midgestation with vascular and neural tube defects **(34).** Using mice in which LKB1 was deleted from hematopoietic tissues, researchers observed that loss of LKB1 leads to a decline in bone marrow cellularity, progressive pancytopenia and animal death due to the increased levels of apoptosis and autophagy in LKB1-deficient HSCs. HSCs transiently increase in number, an effect associated with enhanced proliferation, and then markedly decrease. This suggests that LKB1 is necessary to maintain quiescence specifically in HSCs and its maintenance **(35).**

In addition, it was shown that LKB1-deficient HSCs form fewer colonies than controls in culture; and that LKB1-deficient bone marrow shows a markedly decreased ability to repopulate the hematopoietic system of irradiated mice **(36).** LKB1 deficiency may elevate the metabolic or genotoxic stress, triggering hematopoietic stem cell death and exhausting**(1).**

LKB1 balances proliferation and quiescence in HSCs by regulating mitochondrial function. However, the specific effectors of LKB1 in HSCs have yet to be defined. Whether LKB1 regulates HSC quiescence through the 12 other AMPK-related kinases or through another mechanism remains an important area of investigation **(37).**

The potential mechanisms of LKB1-mediated cancer suppression:`

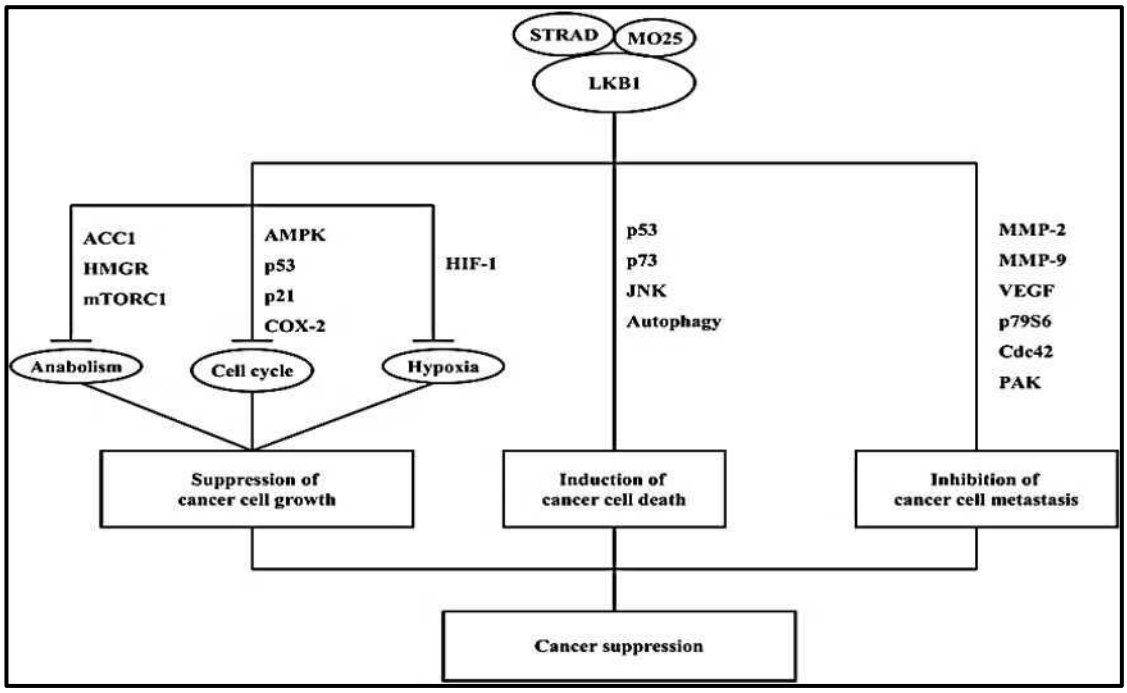

Fig. (5): The potential mechanisms of (LKB1)-mediated cancer suppression **(18).**

1- Suppression of Cancer Cell Growth:

LKB1 has been reported to inhibit the growth of cancer cells, but not normal cells, and this may be partly due to various stresses in cancer cells, such as metabolic stress and hypoxia**(38).**

- LKB1 inhibits anabolism of cancer cells through AMPK activation. LKB1-AMPK signaling can inhibit fatty acid synthesis through suppression of acetyl-CoA carboxylase 1 (ACC1), inhibit sterol synthesis through suppression of 3-hydroxy-3-methylglutaryl- CoA reductase (HMGR) and inhibit protein synthesis through suppression of the mammalian target of rapamycin (mTOR) complex 1 (mTORC1) **(38).**

- On the other hand, LKB1 can induce cell cycle arrest of cancer cells through AMPK signaling. AMPK can also initiate cell cycle arrest through persistent phosphorylation and activation of p53, which leads to accelerated p53-dependent cellular senescence **(39).**

- LKB1 was also reported to physically interact with p53 in the nucleus to stabilize p53, and directly or indirectly phosphorylate p53 Ser^{15} and Ser^{392}, which are required for LKB1-dependent G1 cell cycle arrest **(40).**

Endogenous STRAD knockdown can abrogate G1 cell cycle arrest, which indicates that STRAD plays a crucial role in the tumor suppressor effect of LKB1 **(18).**

2-Induction of Cancer Cell Death:

It is found that in polyps derived from PJS patients, there is a lack of LKB1 staining and reduced numbers of apoptotic cells, and LKB1 is a mediator of p53-dependent cell death. In addition, LKB1 can induce cell apoptosis through activation of JNK pathway. Besides, in osteosarcoma cells, LKB1 is critical for TRAIL and death associated protein 3 (DAP3)- induced cell apoptosis **(41).**

3-Inhibition of Cancer Cell Metastasis:

LKB1 can inhibit cancer cell invasion and metastasis. In study breast cancer cells, overexpression of wild-type LKB1 can significantly inhibit the invasion and metastasis of cancer cells in vitro and in vivo, and this is accompanied with down regulation of matrix metalloproteinase 2 and 9 (MMP-2 and MMP-9) as well as vascular endothelial growth factor (VEGF). LKB1 is essential for adiponectin mediated inhibition of migration and invasion of breast cancer cells, and this effect is dependent on AMPK activation **(42).**

Genetic inactivation and mutations of LKB1:

The Peutz-Jeghers investigation revealed an association between an LKB1 mutation and the development of gastric cancer. The protein expression of LKB1 was lost in gastric cancer tissues, whereas expression of the protein was observed in the normal mucosae adjacent to the tumor **(43).**

LKB1 overexpression led to significant inhibition of tumor cell invasion in breast cancer, reduced tumor growth in the mammary fat pad and microvessel density, and suppressed tumor metastasis to the lung **(44).**

This LKB1 overexpression was associated with the down-regulation of matrix metalloproteinase -2 and 9, vascular endothelial growth factor and basic fibroblast growth factor. In lung cancer, the expression of LKB1 inhibits the invasion capacity of lung cancer cells by suppressing the expression levels of tissue factor and vascular endothelial growth factor **(44).**

In NSCLC, 30% of the patients are reported to be LKB1 inactivated. Reports displayed that 20% primary cervical cancers possess somatically-acquired mutations of LKB1 **(45).**

The LKB1 promoter methylation was also confirmed in colorectal cancer, hepatocellular carcinoma, and astrocytoma. A Korean group found LKB1 promoter methylation in 13% of samples.LKB1 genetic and epigenetic alteration may vary depending on patient ethnicty **(23).**

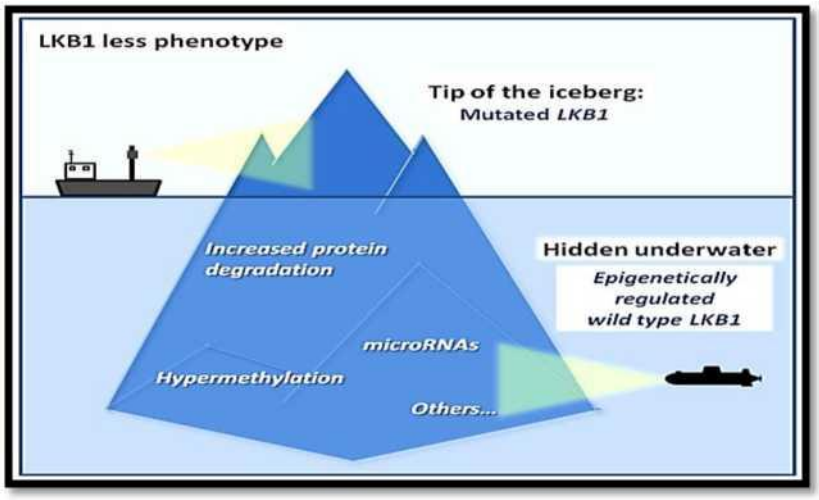

Fig. (6): Schematic representation of the current knowledge on the LKB1less phenotype **(14).**

The tip of the iceberg represents LKB1 mutations. Hidden underwater, the less investigated conditions that led to the LKB1less phenotype in wild-type LKB1 patients (hypermethylation of the promoter, non-coding RNAs, increased protein degradation, etc.) are waiting to be further investigated **(14).**

Phe354Leu mutation of LKB1:

Yang et al., (32) examine mutation of LKB1 in AML and its clinical and pathological implications. A silent mutation (837C>T) of LKB1 was detected in one patient and a pathogenic mutation Phe354Leu which diminishes LKB1 ability to maintain cell polarity was detected in six (7%) patients. The Phe354Leu occurred concurrently with mutations of nucleophosmin 1 (NPM1), fms-related tyrosine kinase 3 (FLT3) and CCAAT/enhancer binding protein alpha (CEBPA), but not with metabolism-related genes, (IDH1) and IDH2**(32).**

This polymorphism occurs in the C-terminal region of LKB1 rather than in the kinase domain(32).

In a study by Forcet et al.(46) the Phe354Leu alteration lessened LKB1-mediated activation of the AMPK and impaired downstream signaling, and diminish LKB1 ability to maintain the polarity of cells.

Other studies reported that LKB1 mutations were relatively rare in patients with cancer who did not have Peutz-Jeghers syndrome, except for non-small cell lung cancers (NSCLCs) (47).

Role of LKB1 Mutations in the Interactions with Tumor Microenvironment (TME):

LKB1 alterations have been reported to be marker of tumor resistance to immune checkpoint blockade. Interestingly, LKB1 loss has been related to a specific immune microenvironment, characterized by production of pro-inflammatory cytokines, a decrease in tumor-infiltrating lymphocytes and PD-L1 expression on tumor cells, and increase in neutrophils recruitment. Moreover, Kitajima and colleagues demonstrated that LKB1 loss leads to the suppression of stimulator of interferon genes (STING), whose activation is critical for anticancer immune response (6).

Interestingly, recent data show that LKB1 alterations in cancer cells affect the release and the content of EVs extracellular vesicles (49). EV uptake induced a specific "reprogramming" of the fibroblasts towards a pro-tumorigenic phenotyp. So the metabolic changes resulting from LKB1 loss may also affect TME cell function.

Further investigation on the effect of LKB1 loss on EVs formation and cargo may uncover new EVs-mediated molecular mechanisms underlying the aggressiveness and malignancy of LKB1less tumors (14).

STK11 mutations are associated with an "immune cold" tumor microenvironment characterized by low or no PD-L1, low T-cell densities, high levels of granulocyte colony stimulating factor and IL-8 family cytokines, high density of neutrophil-like cells, and production of myeloid cell-recruiting chemokines such as IL-6 (48).

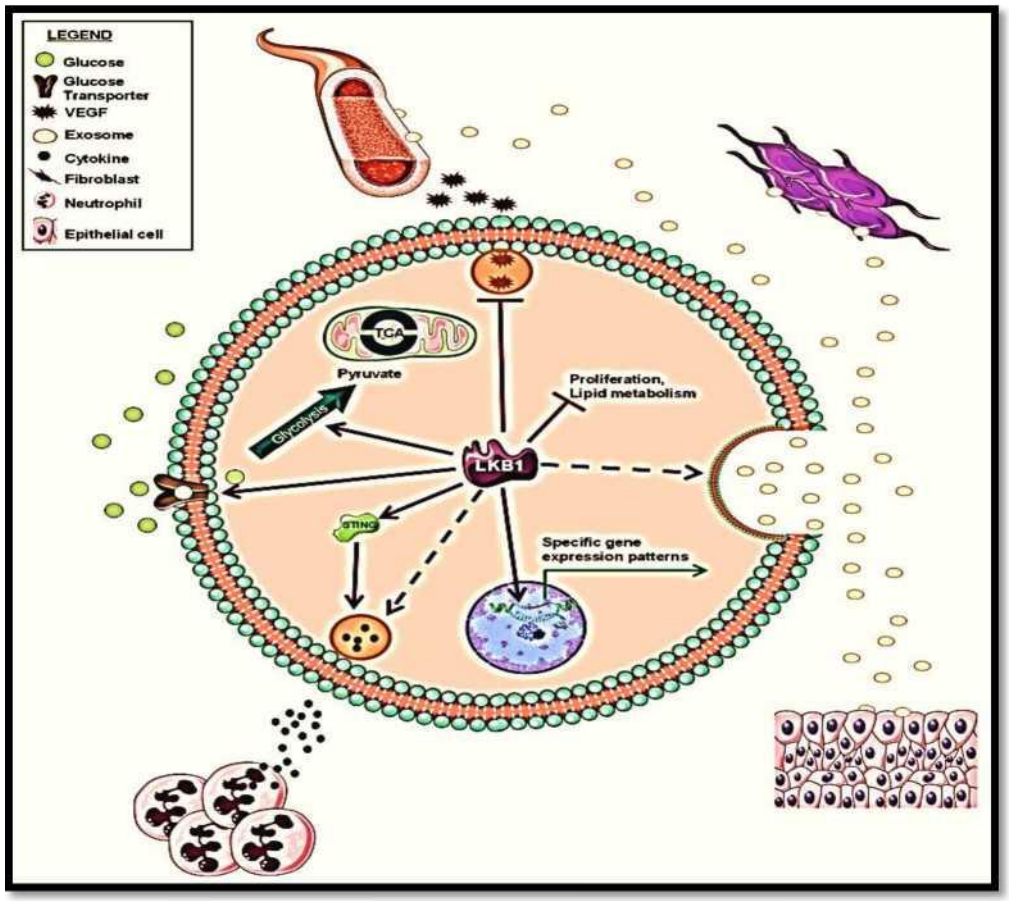

Fig (7): Schematic presentation of LKB1 biological functions within tumor cells and in the interactions with tumor microenvironment. Solid and dashed arrows indicate a positive regulation. Red "T" arrows indicate a negative regulation **(14).**

The LKB1/AMPK signaling pathway has tumor suppressor activity in acute myeloid leukemia:

In acute myeloid leukemia (AML), the oncogenic deregulation of mRNA translation markedly contributes to the malignant phenotype. LKB1 is encoded by the tumor-suppressor gene, STK11, which harbors germ-line mutations in the inherited cancer predisposition and somatic mutations in sporadic cancers **(50)**

LKB1 enhances AMPK activity through the phosphorylation of the a-subunit at T172. AMPK is allosterically activated by the accumulation of AMP molecules, due to metabolic stresses. AMPK is therefore the main cellular energy sensor, acting as a central negative regulator of metabolic pathways, such as fatty acid oxidation and glucose consumption **(Fig.8)(51).**

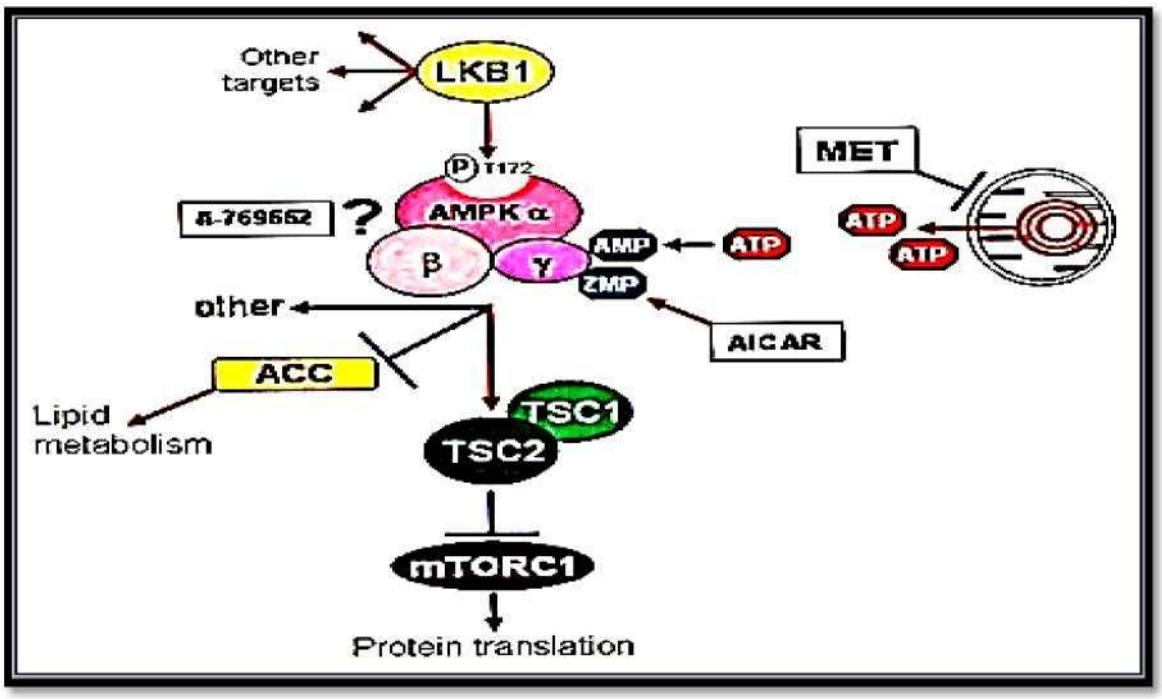

Fig. (8): The LKB1/AMPK/mTOR signaling pathway **(52).**

The LKB1/AMPK pathway also regulates the protein synthesis rate through the control of the serine/threonine kinase mammalian target of rapamycin (mTOR), a process that is consistently deregulated in AML cells **(53).**

AMPK regulates protein translation through its effects on mTOR and pre-rRNA synthesis. The activation of AMPK suppresses mTOR activity,thus interfering with translation initiation. AMPK also phosphorylates TIF-IA to prevent the assembly of pre-rRNA transcription initiation complex, thus prevent synthesis of ribosome which is required for protein translation **(Fig .9) (3).**

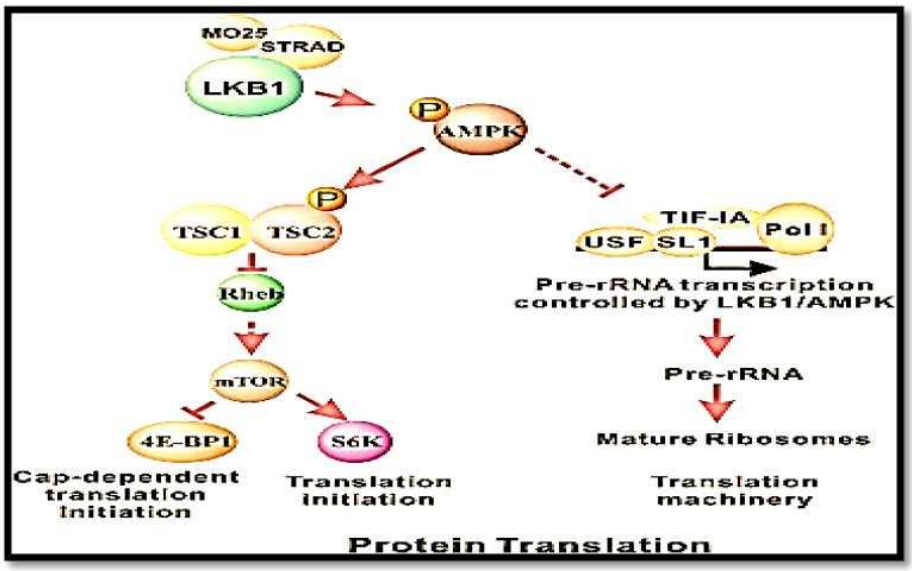

Fig. (9): AMPK regulates protein translation **(3).**

On the other hand, the rapamycin insensitive mTORC2 complex, defined by the interaction between mTOR and rictor (rapamycin-insensitive companion of mTOR), controls the activity of the oncogenic kinase, Akt, but has not been linked to the control of protein translation**(54).**

Deregulated mTORC1 activity within cancer cells increases the synthesis of many oncogenic proteins regulated at the translation initiation level, through the phosphorylation of the physiologic translation repressor 4E-BP1 at multiple residues **(55).**

Negative regulation of LKB1/AMPK pathway by ERK in acute myeloid leukemia:

It is well known that the constitutive activation of the ERK pathway is one of the hallmarks of numerous types of hematologic malignant cells; particularly acute myeloid leukemia (AML) cells **(56,57).**

Therapeutic modulation targeting the LKB1-AMPK Pathway:

A-Activation of LKB1-AMPK Pathway by Biguanides:

The biguanide metformin attracted considerable attention as a potential anticancer drug once the connection between LKB1 and AMPK was discovered. Diabetic patients taking biguanides might have a lower incidence of cancer because of the role of the LKB1-AMPK pathway as a checkpoint inhibitor of cell growth and suppression of mTORC1 and other growth pathways **(58).**

Metformin and the related drug phenformin have been shown to inhibit complex I of the mitochondria, resulting in increased intracellular AMP and ADP levels **(Fig.10)**, which allosterically activates AMPK. The membrane permeable Amino Imidazole C Arboxamide Ribonucleotide (AICAR) is phosphorylated inside the cell and the resulting product, ZMP, acts as an AMP mimetic. The A-769662compound is the only known small molecule that directly binds AMPK, inducing its activity (as shown in fig.8) **(59).** Tumor cells lacking functional LKB1 are acutely sensitive to metabolic stress, resulting in rapid apoptosis, likely a consequence of their inability to sense energy stress **(Fig.10) (5).**

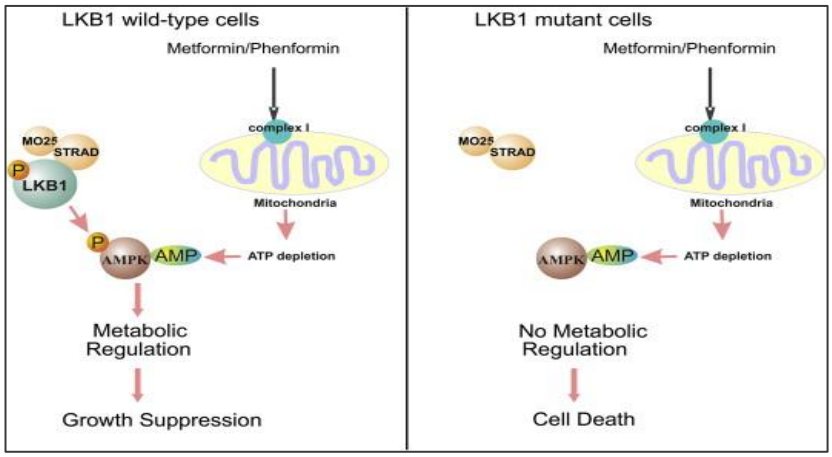

Fig. (10): The effects of AMPK activators in LKB1-wild type and mutant cells**(3)**

Some metabolic pathways controlled by AMPK could have importance in AML biology. Metformin inactivates ACC which may contribute to the antitumor activity of this molecule**(53).**

Metformin also associated with enhanced cisplatin induced in vitro proapoptotic and in vivo antitumor effects specifically in KRAS/LKB1 comutated tumors **(60).**

B-Targeting the Downstream Effectors of LKB1 Pathway:

1-**Inhibition of mTOR:**

LKB1 inactivation promotes mTORC1 signaling, mTOR inhibitors have been extensively tested as a therapeutic approach to target LKB1 mutated tumors. LKB1 inactivation in endometrial cancers resulted in high responsiveness to mTOR inhibitors (**61).**

2-Inhibition of ACC Activity:

ACC was required to maintain de novo FA synthesis needed for growth and viability of NSCLC cells, and its pharmacological inhibition results in robust inhibition of tumor growth**(62).**

3-Role of LKB1 in response to Therapy-Induced Oxidative Stress:

LKB1-AMPK pathway is involved in the maintenance of redox homeostasis by contrasting ROS production and promoting ROS scavenging (**63).**

Studies reported that LKB1 deficiency associated with increased response to several ROS-inducing drugs commonly used in the clinic, arsenic trioxide, paclitaxel, and doxorubicin, LKB1 status could predict tumor response to several chemotherapeutic regimens. Moreover, decrease in reduced glutathione levels following exogenous oxidative stress and are more sensitive to cisplatin and Y-irradiation. LKB1-defective NSCLC cells exposed to exogenous oxidative stress lose their mitochondrial membrane potential and undergo mitochondrial fragmentation (**64).**

C-Deoxy thymidilate kinase (DTYMK) inhibitor:

It catalyses the conversion of deoxy thymidine monophosphate (dTMP) to deoxy thymidine diphosphate (dTDP) and plays a fundamental role in nucleotide synthesis. Liu and colleagues**(22)** demonstrated that LKB1 loss is associated with deficits in nucleotide metabolism. DTYMK inhibition in LKB1-mutated NSCLC cells leads to dUTP misincorporation in DNA, thus blocking replication. Hypersensitivity of LKB1-mutant tumors to antifolates can be speculated.

D-PRAP inhibitors:

Given the role of LKB1 in the maintenance of genomic integrity through the regulation of homologous recombination, its inactivation sensitizes cancer cells to PARP inhibitors; PARP-1 is involved in the repair of single-strand. Ablation of PARP leads to the conversion of single-strand breaks to double-strand breaks during DNA replication, inducing cell death in homologous recombination-defective LKB1-mutated cancer cells.

LKB1/AMPK affecting drugs trials in Hematological Malignancies:

The activation of AMPK by metformin dramatically induced apoptosis in several human leukemia cells. Using metformin, the A-769662 or a dominant activated form of AMPKY, fully inhibited mTORC1 activity. As a result, oncogenic protein synthesis was markedly reduced **(52).**

Robert and colleagues **(65)** showed that AICAR kills CML cells through an AMPKindependent autophagy process. The activation of AMPK has also been shown to effectively eradicate BCR-ABL containing chronic myelogenous leukemia cells ,Importantly, 5- Aminoimidazole-4-carboxamide ribonucleotide (AICAR), a potent activator of AMPK, inhibits the survival of chronic myelogenous leukemia cells with the T315I mutant form of the BCR-ABL fusion protein, which is usually resistant to tyrosine kinase inhibitors.

Sengupta and colleagues **(66)** showed that AICAR has anti-proliferative and pro-apoptotic effects in vitro against a set of ALL cell lines.

Santidrian et al **(67)** demonstrated an alternative pathway for apoptosis induction by AICAR in B-CLL.AICAR induced the mitochondrial pathway of apoptosis, regardless of p53 **(67).**

Campas and colleagues **(68)** reported the use of AICAR in primary samples from follicular lymphoma, mantle cell lymphoma observed increased AMPK phosphorylation along with cell cycle arrest and growth inhibition.

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