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Review

The role of LKB1 and AMPK in cellular responses to stress and damage

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ABSTRACT

The LKB1 and AMPK proteins participate in an energy sensing cascade that responds to depletion of ATP, serving as a master regulator of metabolism that inhibits anabolic processes and stimulates catabolic processes. However in recent years, LKB1 and AMPK have been implicated in a variety of other cellular processes, both cytoplasmic and nuclear, such as control of cell polarity and regulation of gene transcription. In this review, we summarize the most recent discoveries regarding participation of LKB1 and AMPK in signaling pathways that respond to cellular stress and damage, and the relevance of this signaling for disease and therapy.

1. Introduction

The LKB1–AMPK pathway has until fairly recently been thought of as primarily an energy sensing pathway engaged by cells in response to low energy levels. More recently, LKB1 and AMPK have been linked to many other fundamental cellular processes including regulation of cell proliferation, cell polarity, migration, transcription and cellular stress and damage responses, the focus of this mini-review.

2. Discovery and characterization of LKB1 and AMPK

Liver kinase B1 (LKB1) was discovered only 12 years ago, as a serine–threonine kinase that is mutated in Peutz-Jeghers Syndrome [1]. Peutz-Jeghers Syndrome is a rare autosomal dominant hamartoma syndrome, predisposing patients to multiple benign and malignant tumors including gastrointestinal, pancreatic and lung tumors [2]. Initially, studies to identify the function of LKB1 were difficult, as its sequence gave few clues as to its activity. Overall it had little similarity to other protein kinases. However, studies in cell lines including HeLa S3 and G361 melanoma cells, which lack endogenous LKB1, provided insights into LKB1's role as a tumor suppressor, including early evidence of its role in stress and damage responses.

2.1. LKB1 regulation of p53 activity

LKB1 physically associates with p53 upon DNA damage (UVC radiation), and localizes to the p21 promoter region in tandem with p53 [3]. Additional interactions with this damage-response tumor suppressor occur at the level of post-translational modification of p53, with both LKB1 and AMPK acting as kinases for p53 at the serine (Ser) 15 phosphorylation site; LKB1 also phosphorylates p53 at Ser 392, which increases p53 protein stability [3,4].

2.2. LKB1 signaling to AMPK

More recently, LKB1 has been appreciated to be a signaling protein, integrating cellular energy sensing with growth and proliferation, functioning both in the nucleus and the cytoplasm. It is ubiquitously expressed throughout the body, and functions as a heterotrimer with STRAD (sterile-20-related adaptor) and MO25 (mouse protein-25) in cells [5]. LKB1 is post-translationally modified by kinases in several signaling pathways including cAMP–PKA, the ERK–RSK pathway and ATM, a DNA damage sensor, as reviewed in [6]. Despite the fact that it is constitutively active in cells [7], it has also been noted that LKB1 catalytic activity is enhanced when STRAD and MO25 are present in the complex [5].

AMP-activated protein kinase (AMPK) is one of the best characterized substrates of LKB1. AMPK is a heterotrimer that consists of a catalytic subunit, AMPKα, and 2 regulatory subunits, AMPKβ and AMPKγ. There are 2 distinct isoforms of the AMPKα subunit designated AMPKα1 and AMPKα2, which differ in their tissue

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specificity, subcellular localization and mechanisms of activation. While AMPKα1 is widely expressed, AMPKα2 is restricted mainly to skeletal muscle, cardiac muscle and liver cells, where it is highly expressed [8]. On a cellular level under basal conditions, AMPKα1 appears mainly cytoplasmic whereas AMPKα2 is primarily localized to the nucleus. The yeast AMPK homolog, Snf1, is also predominantly located in the nucleus [9]. It is currently unclear how the AMPK complex containing α2 localizes to the nucleus, since no classical nuclear localization sequence has been found, and the ~63 kDa protein is probably too large to passively diffuse through the nuclear pore.

2.3. How is AMPK activated?

At present, a number of stimuli are known to activate AMPK, some of which are related to energy sensing (such as glucose deprivation), as well as others that are not (such as hyperosmotic stress). When cellular energy levels are depleted, and the AMP:ATP ratio rises, AMPK is activated via allosteric binding of AMP to the AMPKγ subunit, which has been proposed to induce a conformational change in the complex, improving the ability of AMPKα-subunit to serve as a substrate for upstream kinases [6]. The primary activation site on the catalytic component AMPKα is threonine (Thr) 172, and while both α1 and α2 can be phosphorylated at this site by the same upstream kinases, α2 subunit phosphorylation is more AMP-dependent [9].

LKB1 has now been shown to be one of the primary kinases that phosphorylates AMPK. AMPK activation by agonists such as 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), metformin or phenformin or in response to stress is absent in LKB1-deficient cells [5], identifying LKB1 as an obligatory AMPK kinase in this setting. Activation of AMPK by AICAR occurs via mono-phosphorylation of AICAR to its active form, ZMP which can mimic the effect of AMP on AMPK, i.e., allosteric activation and enhancement of phosphorylation by upstream kinase(s) [10].

In addition to LKB1, the calmodulin-dependent protein kinases CaMKKα and CaMKKβ also function as kinases for Thr 172 of AMPK [11–13]. This activation pathway is calcium-responsive, does not require an increase in AMP, and is thought to be particularly important for endocrine hormone regulation, for example adiponectin activation of AMPK in vascular endothelial cells [14]. CaM-kinases are most highly expressed in neural tissue, where they play a role in activating AMPK in response to neuronal depolarization caused by K^+-increases and Ca^{2+} influx [11].

2.4. AMPK signaling to mTORC1 and growth suppression

How does activation of LKB1 and AMPK result in growth suppression? One of the key targets of these kinases is the TSC2–mTORC1 signaling node. The tuberous sclerosis complex 2 (TSC2) tumor suppressor is directly phosphorylated by AMPK at Thr 1227 and Ser 1345, which enhances its GTP-ase activity towards Rheb, inactivating this small GTPase to decrease mTORC1 signaling [15]. In addition to this TSC2-dependent pathway, AMPK has been shown to directly phosphorylate raptor (a component of the mTORC1 complex) to inhibit mTORC1 signaling [16]. The reciprocal relationship between AMPK activity and mTORC1 activity allows the cell to coordinate energy requiring anabolic processes with energy availability – under conditions of energy deprivation, activation of AMPK can limit energy-consuming processes such as protein synthesis via repression of mTORC1.

In addition to protein synthesis and cell growth, mTORC1 is also a key negative regulator of autophagy, the catabolic process of breaking down cell components via the fusion of autophagosomes with lysosomes, degrading the contents autophagosomes for recycling by the cell [17]. Activation of AMPK induces autophagy by negatively regulating mTORC1 via TSC2 or raptor, and thereby assists the cell in generating building blocks for energy production and other cellular processes. Recently, ULK1 has been identified as a bona fide AMPK substrate when cells undergo nutrient deprivation, providing a molecular link between this energy-sensing pathway and regulation of autophagy [18,19].

In contrast to energy stress, DNA damage, which activates p53 via ATM/ATR phosphorylation, can both promote and inhibit autophagy. In the nucleus, p53 functions as a transcription factor, where it regulates the expression of many genes, including several that participate in autophagy [20]. In the cytoplasm, p53 can inhibit AMPK and autophagy, indicating that the relationship between p53 and AMPK in regulation of autophagy is very complex and likely context dependent [21].

A second pathway of AMPK-mediated regulation of autophagy may be via the eEF-2 kinase (also known as Ca^{2+}/calmodulin-dependent kinase III) pathway. AMPK directly phosphorylates and activates eEF-2 kinase, which inhibits protein synthesis at the elongation stage [22]. The precise role that eEF-2 plays in autophagy is still not known, however it was shown that knockdown of eEF-2 inhibited autophagy in glioblastoma cells, whereas overexpression increased autophagy. Finally, another less studied AMPK-kinase, TAK1 (transforming growth factor β-activated kinase 1) was shown to be induced in response to the apoptosis-inducing agent TRAIL, resulting in a cytoprotective autophagy response, which is dependent upon AMPKα1 [23]. It is clear from these studies that AMPK plays important roles in signaling to regulate autophagy in response to diverse stress stimuli.

2.5. Other AMPK-related proteins

Analysis of the human kinome reveals a family of AMPK-related proteins that contain activation loops that are homologous to AMPK, suggesting they could also be targets of LKB1. This family of proteins is now known to contain at least 12 validated LKB1 substrates (not including AMPK) and 8 non-LKB1 substrates [6,24]. One of these AMPK-family members, SNARK, was discovered as a gene induced in response to UV damage in rodent keratinocytes; SNARK also expressed in a number of human tissues [25,26]. Upon additional characterization, a broad range of stressors (including glucose and glutamine deprivation, hydrogen peroxide, hyperosmotic stress, ATP synthesis inhibitors such as oligomycin and arsenite) were capable of activating SNARK kinase activity in a cell-type specific manner. These results suggest that other AMPK-family members also may function as stress responsive proteins.

It is unclear whether AMP directly activates all AMPK-related proteins, although SNARK is activated by AICAR or glucose deprivation in cells [25]. It is likely there are some cell-type differences in activation mechanisms, since in contrast to the first report on SNARK (NUAK2) activation in neonatal rat keratinocytes and baby hamster kidney cells [25], later studies showed that in mouse embryo fibroblasts (MEFs), AICAR and phenformin were unable to activate SNARK [24]. While AMPK-related proteins are less well studied than AMPK, it is already clear that they vary in their substrate specificity, although not all these substrates have been characterized. Future research on AMPK-related proteins will likely expand our knowledge about how they are regulated, including identification of additional upstream kinases and regulatory phosphorylation sites, their physiological and pathological functions, and identify additional substrates.

3. Involvement in DNA damage response pathways

The kinase ATM lies upstream of LKB1 in damage response signaling. ATM, the gene mutated in the disease ataxia-telangiectasia,

is a critical early damage response protein that plays multiple important roles in sensing and responding to different types of damage. In the nucleus, it is well appreciated that ATM is activated by DNA double-strand breaks, leading to induction of cell cycle checkpoints, DNA repair and if necessary, apoptosis if the damage is too severe to be repaired. However, ATM has also been localized to the cytoplasm, where it can be activated in the absence of DNA damage [27,28]. Dario Alessi's group in 2002 identified a series of phosphorylation sites on LKB1, none of which affected LKB1's kinase activity towards p53, one of which, threonine 366 (Thr 366), lay within an ATM consensus phosphorylation motif. They went on to show that ionizing radiation (which activates ATM) led to phosphorylation of LKB1 at this site [29]. The function of this phosphorylation site remained somewhat elusive since mutation of this site to alanine had little affect on cell growth when transfected into LKB1-deficient cells. However until recently, the impact of phosphorylation at Thr 366 on downstream signaling to AMPK or mTORC1 had not been studied further.

We have shown that a damage response signaling pathway exists from ATM to LKB1 that regulates AMPK in the cytoplasm [30]. In response to oxidative damage, cytoplasmic ATM is rapidly activated, phosphorylating LKB1 in the cytoplasm, activating AMPK, TSC2 and suppressing mTORC1. As a consequence of this mTORC1 suppression, autophagy is induced, suggesting this pathway may function as a mechanism to maintain redox homeostasis.

ATM has also been shown to directly phosphorylate AMPKα independently of LKB1 on T172 in response to DNA damage (etoposide), which has been linked to mitochondrial biogenesis in a variety of mammalian cell types [31]. While not definitively shown in this paper, the authors proposed that in response to DNA damage, AMPK was activated both by a decrease in ATP (increase in AMP:ATP ratio) as well as by ATM phosphorylation. Induction of mitochondrial biogenesis in response to DNA damage by a variety of agents including chemotherapeutics and radiation (which can damage mitochondria and release ROS, inducing oxidative damage), coupled with autophagy of damaged mitochondria, may be a mechanism for limiting cellular damage while ensuring sufficient energy to carry out the important repair pathways necessary for survival. Apart from the DNA damage response, AMPK has also been linked to mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation or endurance training exercises [32]. The mechanisms responsible involve master regulators of mitochondrial biogenesis including the peroxisome proliferator activator receptor γ coactivator 1α, and calcium/calmodulin-dependent protein kinase IV [32].

A second DNA damage-induced pathway for AMPK activation involves p53 [33]. Two recently-identified p53 transcriptional targets, sestrin 1 and sestrin 2 were shown to mediate AMPK activation and mTORC1 suppression by DNA damage (specifically camptothecin in vitro and the hepatocarcinogen diethylnitrosamine in vivo). While the precise mechanism of AMPK activation by sestrins was not analyzed, this pathway was p53-dependent, and resulted in the formation of large protein complexes containing sestrins, AMPK, TSC2, and TSC1, suggesting that the sestrins may function as adaptor proteins to bring together these proteins, promoting AMPK activation of TSC2/1 and mTORC1 repression.

4. Regulation of cell death pathways

4.1. Interactions with p53 family members

Apart from autophagy, AMPK activation in response to DNA damage may regulate apoptosis or other cell death pathways. For example, the chemotherapeutic drug cisplatin, which damages DNA by forming intrastrand crosslinks, has been shown to activate AMPK in several tumor types including colon cancer, gastric cancer and glioma [34,35]. In one study, AMPK activation by cisplatin correlated with ROS generation, and resulted in enhanced survival. In this setting, as would be predicted, inhibition of AMPK sensitized the cells to cisplatin-induced apoptosis, which was preceded by a dramatic decrease in ATP levels [34]. This pathway was dependent on p53, and involved hyper-phosphorylation of p53 at Ser 15 by ERK, which occurred in the presence of AMPK inhibition. Interestingly, when a panel of different tumor lines were treated with a relatively high dose of cisplatin, highly chemosensitive cells such as HeLa, which are LKB1-null, were refractory to AMPK activation, whereas more resistant cells exhibited AMPK activation, suggesting a role for LKB1 signaling to AMPK in a survival pathway. Thus in the context of cytotoxic therapy, it is possible that inhibition of LKB1/AMPK in p53-proficient cells may reduce survival and improve cell killing.

In addition to p53, other members of this transcription factor family are phosphorylated by AMPK in response to DNA damage. For example, AMPKα interacts with both p73α and p63α, with multiple functional consequences, including altering subcellular localization of AMPKα subunits [36]. Under normal conditions, endogenous AMPKα1 is mainly cytoplasmic, whereas AMPKα2 is nuclear [9,37,38]. However, when exogenous p73α is overexpressed, both AMPKα1 and AMPKα2 localize to the nucleus, where AMPK:p73α complexes act as transcriptional co-repressors. This was directly shown by overexpressing AMPKα1 or AMPKα2, and measuring luciferase activity at p53-family responsive promoters such as the *Bax* promoter. Using these luciferase reporters as readouts of transcriptional activity, Lee et al. showed that increasing expression of AMPKα1 or AMPKα2 resulted in inhibition of p73α-mediated repression of gene expression. Interestingly, the kinase activity of AMPK was not required for this activity. Further evidence of in vivo relevance of this observation was obtained using ChIP assays of the endogenous p21 promoter, which could immunoprecipitated with antibodies directed against AMPKα2. Importantly, this AMPK-mediated p73α transcriptional repression was shown to enhance survival in response to DNA damage, with cells overexpressing AMPK2α resistant to cisplatin-induced apoptosis [36].

4.2. Other nuclear functions of AMPK

Histone H2B has also been identified recently as an AMPKα2 stress-response substrate [39]. When AMPK is activated by a variety of DNA damaging agents and stressors such as 2-deoxyglucose, histone H2B is phosphorylated at Ser 36, inducing transcription of a large number of stress-inducible genes. This pathway for direct modification of chromatin by AMPK allows this kinase to participate in a global transcriptional response in addition to directly phosphorylating specific transcription factors, which may allow cells to fine-tune gene expression to survive damage and stress.

4.3. Regulation of p27 stability and localization

Both cell death (apoptosis) and survival (autophagy) are regulated by AMPK in response to the stress of glucose deprivation. One of the key regulators of this "life or death" decision downstream of AMPK is p27, a cyclin-dependent kinase inhibitor (CKI) and a direct substrate for AMPK in response to serum starvation or 2-deoxyglucose treatment [40]. AMPK phosphorylation of p27 at the carboxy-terminal threonine (Thr 198 in humans) results in increased protein stability and combined with cytoplasmic localization of this CKI, promotes autophagy. However, in cells depleted of p27, glucose deprivation induces apoptosis, demonstrating that p27-dependent autophagy functions downstream of AMPK as a survival mechanism under conditions of energetic stress. AMPK also phosphorylates p27 at its nuclear localization signal (NLS),

causing p27 to be sequestered in the cytoplasm away from its nuclear substrates (such as cdk2), inhibiting its function as a CKI [41]. This finding has potential implications for cancer therapy, since many targeted therapies such as anti-angiogenic agents, or even traditional cytotoxic agents induce stress responses that can activate AMPK, potentially inducing this p27-dependent autophagic survival mechanism.

5. Tissue-specific damage response roles for LKB1 and AMPK

5.1. LKB1–AMPK signaling in cardiac function

Studies from LKB1 and AMPK knockout mice, and the discovery of human disease-causing mutations in AMPK, have pointed to an important role for these signaling kinases in cardiac protection. The LKB1 cardiac-myocyte specific knockout mouse develops a cardiac hypertrophic phenotype by 12 weeks of age, and eventually succumbs to cardiac failure by 6 months [42]. As expected, hearts from LKB1-knockout mice have elevated mTORC1 signaling leading to increased protein synthesis, likely contributing to hypertrophy. In contrast to LKB1-knockout models, mice lacking AMPKα1 or AMPKα2, do not have a baseline cardiac hypertrophy phenotype, however upon stress from pressure overload, AMPKα2 knockout mice can develop left ventricular hypertrophy [43].

One of the key roles that AMPK plays in cardiac muscle is in the response to hypoxia/ischemia. Since the heart is a highly metabolic organ that requires significant energy for continuous function, the ability to temporarily deal with stress from hypoxia is essential to maintain function. When blood supply to the heart is compromised, AMPK is activated, stimulating fatty acid oxidation, glucose uptake and glycolysis to generate ATP needed for continued function. Consistent with the key role of AMPK in this process, mice expressing a kinase-dead form of AMPK are more susceptible to damage from ischemia and exhibit delayed recovery during reperfusion [44].

Hypoxia, such as induced by ischemia, signals to mTORC1 via both AMPK-dependent and independent pathways. It has recently been shown that activation of AMPK by hypoxia is independent of energy levels, but involves generation of ROS [45]. While severe hypoxia or anoxia (0% O_2) does inhibit mitochondrial respiration and induce AMPK activation by decreasing energy levels, acute hypoxia (defined as 1–2% O_2) does not significantly alter the AMP:ATP ratio, suggesting other mechanisms are involved in activating AMPK. Another pathway that is engaged in response to hypoxia to inhibit mTORC1 signaling involves upregulation of Redd1 (regulated in DNA damage-1) and activation of TSC2 [46,47].

5.2. Role of AMPK signaling in the brain

Similar to the heart, the brain has a high basal metabolic rate, utilizing more than 50% of the total glucose in the body. It is exquisitely sensitive to changes in the supply of glucose or oxygen, both of which can activate AMPK. AMPK activation in the brain has been linked to survival in response to different types of stress including glucose depletion, oxidative stress generated by amyloid β peptide and glutamate [48]. While the precise mechanisms for this protection have not been identified, some potential AMPK targets such as HMG-CoA and Raf1 (an upstream kinase of the MAPK pathway) have been hypothesized to be involved. It is thought that the CaMKK pathway for AMPK activation is particularly relevant in this tissue due to the frequent cycling of Ca^{2+} levels when K^+ depolarization occurs [11]. If so, it suggest that targeting this pathway pharmacologically could provide a novel therapeutic strategy for treating chronic neurodegenerative conditions.

Recently, in an experimental model of cholesterol-induced neurotoxicity, AMPK was shown to play a role in reversing some of the pathological changes induced by a high-cholesterol diet, including elevation of ROS, inflammation and amyloid β deposition [49]. Quercetin, a naturally occurring flavonoid found at high levels in green and black teas was shown to decrease ROS and other markers of tissue damage in these mice, in part via activating AMPK, which resulted in cognitive improvement. At the molecular level, quercetin activated AMPK via decreasing the expression of the PP2C phosphatase. In cells with elevated ROS (such as ATM- or TSC2-deficient cells), we showed that rapamycin, a drug that inhibits mTORC1, decreased ROS to baseline levels [30]. It is likely that quercetin acts in a similar mechanism, since AMPK activation results in repression of mTORC1 activity. Further work is necessary to determine whether this pathway is the main factor responsible for the reversal of disease symptoms. In addition, prior to moving forward with clinical trials in humans to treat Alzheimer's with drugs that activate AMPK, it will be important to understand more fully the role of AMPK signaling in response to these drugs, as it also has been reported recently that metformin increases amyloid β deposition, opening the possibility that in elderly patients AMPK activation could have harmful effects in the brain [50].

5.3. DNA-damage induced LKB1 activation in B cells

LKB1 activation in response to DNA damage may play a specialized role in the immune system. After V(D)J recombination in the bone marrow, B-cell differentiation continues in the lymph node to generate the 10^9 clones of B-cells found in humans recognizing different antigens. In the germinal centers, the B cells rapidly proliferate and hypermutate the variable region of the immunoglobulin gene, in a class switch recombination process requiring activation-induced cytidine deaminase (AID). This AID activity generates double-strand breaks, which activate the DNA damage response, and likely contributes to lymphomagenesis. A recent report by Sherman et al. showed that AID induced ATM activation and LKB1 phosphorylation [51]. LKB1 phosphorylation of the CREB transcriptional coactivator (CRTC2) resulted in cytoplasmic

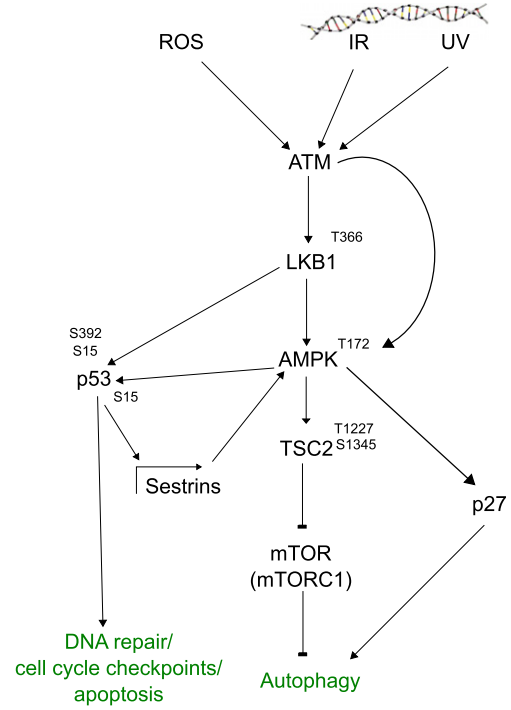

Fig. 1. Schematic showing activation of LKB1 and AMPK by diverse stresses and the phosphorylation sites that are targeted by the upstream kinase.

sequestration and inhibition of its ability to block B cell proliferation and promote B cell differentiation. Interestingly, CRTC2 has also been shown to be phosphorylated by the AMPK-family members MARK2 and SIK2 in pancreatic beta cells, highlighting this pathway as an important mediator of LKB1/AMPK activity [52].

6. Summary

Taken together, it is apparent that LKB1 and AMPK, initially identified as participating in energy sensing in the cell, also play diverse roles in cellular responses to many types of stress. As illustrated in Fig. 1, the LKB1–AMPK pathway plays critical roles in regulating important life-and-death processes including apoptosis and autophagy, which may function in a tissue-specific manner, such as cardioprotection from damage in the heart. As we develop a more detailed understanding of how these kinases function in normal physiology, and are their role in pathophysiology and disease, it is likely that their utility as therapeutic targets will increase. Thus, both LKB1 and its target AMPK, hold promise as therapeutic targets to treat, and possibly prevent, many diseases including neurodegeneration, cardiovascular disease and cancer.

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