Chapter 3
AMPK Regulation of Cell Growth, Apoptosis, Autophagy, and Bioenergetics

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The original version of this chapter was revised. An erratum to this chapter can be found at DOI 10.1007/978-3-319-43589-3_21.

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M.D. Cordero, B. Viollet (eds.), *AMP-activated Protein Kinase*, Experientia Supplementum 107, DOI 10.1007/978-3-319-43589-3_3

Abstract In eukaryotic cells, AMP-activated protein kinase (AMPK) generally promotes catabolic pathways that produce ATP and at the same time inhibits anabolic pathways involved in different processes that consume ATP. As an energy sensor, AMPK is involved in the main cellular functions implicated in cell fate, such as cell growth and autophagy.

Recently, AMPK has been connected with apoptosis regulation, although the molecular mechanism by which AMPK induces and/or inhibits cell death is not clear.

This chapter reviews the essential role of AMPK in signaling pathways that respond to cellular stress and damage, highlighting the complex and reciprocal regulation between AMPK and their targets and effectors. The therapeutic implications of the role of AMPK in different pathologies such as diabetes, cancer, or mitochondrial dysfunctions are still controversial, and it is necessary to further investigate the molecular mechanisms underlying AMPK activation.

Keywords AMPK • Cell growth • Autophagy • Apoptosis • Bioenergetics

3.1 Introduction

Every cell uses ATP as the main source of energy; therefore, the ATP levels must be matched by the energy demand needed to carry on different functions. In order to maintain the equilibrium between nutrient supply and energy demand, the eukaryotic cell uses an ATP sensor coupled to different pathways that are initiated when a decrease in ATP levels is detected. AMPK exerts this function in the eukaryotic cells; therefore, it is considered a master regulator of cell metabolism (Viollet and Andreelli 2011).

Activation of AMPK occurs in response to stress circumstances such as low glucose, hypoxia, ischemia, heat shock, increased reactive oxygen species (ROS), and exercise (Luo et al. 2005), although AMPK can be activated in response to several pharmacological agents as well. Under these situations, the intracellular AMP + ADP/ATP ratio is increased, and then AMPK is phosphorylated in its kinase domain to become a catalytically competent protein. By sensing changes in adenine nucleotide ratios, AMPK is activated by different stresses that diminish cellular energy levels and/or increase cellular ATP consumption. Once activated, AMPK is able to stimulate catabolic processes, ATP-generating pathways, and inhibits

anabolic processes such as synthesis of lipids, carbohydrates, and proteins in order to assure cell survival.

The involvement of AMPK in many regulatory pathways makes it considered as a master metabolic switch. In fact, in mammals, AMPK has an essential role in coordinating survival, growth, and metabolism under conditions of low energy. Therefore, AMPK regulates in a coordinate way catabolism, cell growth, autophagy, and apoptosis, since all these cell functions cannot be balanced independently.

The AMPK signaling network covers numerous genes involved in survival and cell growth. Among them, mammalian Target Of Rapamycin (mTOR) regulation is the axis of numerous cell processes since it plays an essential role in metabolism regulation, autophagy, cell growth, and apoptosis (Xu et al. 2012). This relation is complex but essential to understand the dialogue and the interplay between autophagy and apoptosis, which has been gaining importance in the last years and is closely related with cell growth and survival.

3.2 AMPK Regulation of Cell Growth

AMPK is situated in the center of an energy-sensing cascade that is activated by ATP depletion. AMPK promotes catabolic pathways that produce ATP, and at the same time AMPK inhibits anabolic pathways involved in cell growth and other processes that consume ATP (Grahame Hardie 2014).

Moreover, AMPK is part of a tumor suppressor network that regulates cell growth and proliferation in stress situations; in fact, it is assumed that AMPK is responsible for the tumor suppressor effects of LKB1 (Liver Kinase B1) (Hardie 2011). Consistent with these functions, AMPK acts as a cell growth inhibitor in different stress situations, such as glucose deprivation, energy depletion, tumor proliferation, or DNA damage.

How does activation of AMPK result in cell growth suppression? Cell growth requires balanced nutrient availability and energy production. Therefore, protein, rRNA, and lipid synthesis are needed, in addition to replication of DNA (Vander Heiden et al. 2009). In this sense, AMPK prevents cell growth by inhibiting protein, rRNA, and lipid synthesis, and it also induces cell cycle arrest in G1, preventing DNA replication (Table 3.1).

3.2.1 AMPK Inhibits Protein and rRNA Synthesis

AMPK is considered an antigrowth molecule, among other reasons, due to its relationship with the tumor suppressor gene Tuberous Sclerosis Complex 2 (TSC2), also known as Tuberin. AMPK directly phosphorylates TSC2 on T1227 and S1345 to enhance its Rheb-GAP activity, which causes an inhibition

Table 3.1 Inhibitory effects of AMPK on cell growth

Pathway	Targets	Consequence
Protein synthesis	TSC2/Raptor → mTORC1	↓ Translation initiation
	eEF2 kinase	↓ Translation elongation
rRNA synthesis	TSC2/Raptor → mTORC1	↓ RNA Pol I-mediated transcription
	TIF-1A	
Regulation of cell cycle	MDMX/p53	Cell cycle arrest
		↑ P53 stabilization and activation
Lipid synthesis	ACC1/FAS/SREBP-1c/HMG-CoA reductase/GPAT	↓ Substrate for fatty acid synthesis ↓ Lipogenesis and lipid accumulation ↓ Cholesterol synthesis ↓ Glycerolipid metabolism

of mammalian Target Of Rapamycin Complex 1 (mTORC1) kinase activity (Inoki et al. 2003). Moreover, AMPK is able to directly inhibit mTORC1 by phosphorylating raptor, the mTORC1-binding partner (Gwinn et al. 2008). The phosphorylation of two conserved serines (Ser722 and Ser792) of raptor by AMPK blocks the ability of the mTORC1 kinase complex to phosphorylate its substrates.

By inhibiting mTORC1, AMPK prevents two major biosynthetic pathways required for cell growth, such as protein and rRNA synthesis. The objective of the relationship between AMPK and mTORC1 is adjusting energy required by anabolic process to energy availability (Alexander and Walker 2011).

mTORC1 regulates two key proteins which are enhancers of protein synthesis: p70S6K (p70S6 Kinase) and 4EBP1 (eIF-4E-binding protein 1) (Zoncu et al. 2011). p70S6K is a positive regulator of translation initiation and elongation. When it is phosphorylated by mTORC1, p70S6K phosphorylates multiple substrates including eukaryotic translation initiation factor 4B (eIF4B) (Holz et al. 2005). In contrast, 4EBP1 inhibits mRNA translation. When phosphorylated by mTORC1, it dissociates from eIF4E, allowing the latest to recruit eukaryotic translation initiation factor 4 gamma (eIF4G) to the 5′ end of most mRNAs (Ma and Blenis 2009).

AMPK inhibits protein synthesis in an mTOR-independent manner by promoting phosphorylation of eukaryotic elongation factor 2 (eEF2). eEF2 phosphorylation regulates the peptide chain elongation (Browne and Proud 2002). Phosphorylation of eEF2, which inhibits its activity, is catalyzed by a specific calcium/calmodulin-dependent protein kinase termed eEF2 kinase. AMPK inactivates eEF2 by directly phosphorylating eEF2 kinase (Browne et al. 2004).

By this way, AMPK and mTOR provide the link between cellular energy status and protein synthesis, a major consumer of metabolic energy.

Related to rRNA synthesis, mTORC1 regulates RNA Pol I-mediated transcription by enhancing the activity and localization of TIF-IA, a transcription factor for RNA polymerase-1 that senses nutrient availability (Mayer et al. 2004).

AMPK also inhibits rRNA synthesis in an mTOR-independent manner: it directly phosphorylates TIF-1A at Ser635, and by this way the assembly of functional transcription initiation complexes becomes impaired (Hoppe et al. 2009).

By the mechanisms described above, AMPK allows the adaptation of protein and rRNA synthesis (and finally cell growth) to changes in cellular energy supply.

3.2.2 AMPK Induces Cell Cycle Arrest

Besides protein and rRNA synthesis, cell growth needs DNA replication. AMPK uses different mechanisms to induce cell cycle arrest. First, AMPK is able to phosphorylate MDMX, which regulates p53 proteasomal turnover since it is a component of the E3 ubiquitin ligase complex (He et al. 2014). AMPK-mediated phosphorylation of MDMX on Ser342 promotes the association between MDMX and 14-3-3. This inhibits p53 ubiquitylation, preventing its turnover by the proteasome. Moreover, inhibition of p53 ubiquitylation induces cell cycle arrest by promoting stabilization and activation of p53.

Second, AMPK directly phosphorylates p53 on Ser15 to stabilize it and initiate AMPK-dependent cell cycle arrest (Jones et al. 2005). In stress conditions, such as glucose deprivation, cell cycle arrest promoted by p53 allows cell to survive. Upon glucose restoration, cells reenter the cell cycle. Interestingly, p53 activation also regulates AMPK. It has been demonstrated that the products of Sestrin1 and Sestrin2, which are two p53 target genes, activate AMPK (Budanov and Karin 2008). Furthermore, p53 inhibits mTORC1 by increasing expression of genes that negatively regulate its function, such as insulin-like growth factor-binding protein 3 (igf-bp3), PTEN (phosphatase and tensin homolog), and TSC2 (Buckbinder et al. 1995; Feng et al. 2007). Thus, the stabilization of p53 by AMPK not only induces cell cycle arrest but also indirectly promotes protein and rRNA synthesis inhibition.

At this point, we can consider that the connection between AMPK, mTORC1, and p53 balances the growth-inhibiting response to cellular stress.

3.2.3 AMPK Inhibits Lipid Synthesis

During cell division, cells must double their lipid content. Among different lipid classes, fatty acids are particularly important for cell growth, since cells need them for membrane generation, protein modification, and bioenergetic requirements. Therefore, fatty acid synthesis plays an essential role in cell growth.

It has been described that AMPK inhibits fatty acid synthesis by different mechanisms. First, AMPK inhibits lipogenesis by phosphorylating and inactivating the acetyl-CoA carboxylase ACC1 on Ser79 (Davies et al. 1992; Hardie and Pan 2002). ACC1 catalyzes the synthesis of malonyl-CoA, the substrate for fatty acid

synthesis. Moreover, AMPK also downregulates the expression of fatty acid synthase (FAS), which is also important in fatty acid synthesis (Foretz et al. 1998).

Second, AMPK downregulates the expression of enzymes involved in fatty acid synthesis at the transcriptional level by phosphorylating the transcription factor Sterol regulatory element-binding protein-1c (SREBP-1c). SBREP activity controls fatty acid and sterol synthesis; therefore, activation of SREBP and expression of SREBP target genes are required for efficient cell growth in mammalian cells (Porstmann et al. 2008). AMPK phosphorylates SREBP-1c on Ser372 to prevent its translocation to the nucleus, leading to inhibition of lipogenesis and lipid accumulation (Li et al. 2011).

Besides, AMPK phosphorylates and inactivates other enzymes involved in lipid synthesis, such as HMG-CoA reductase, essential for cholesterol synthesis (Henin et al. 1995), or glycerol-3-phosphate acyltransferase (GPAT), which participates in glycerolipid and glycerophospholipid metabolism (Muoio et al. 1999).

3.3 AMPK Regulation of Autophagy

As we have exposed above, AMPK mainly stimulates catabolic pathways and prevents anabolic pathways, and it exerts its function via direct phosphorylation of different downstream enzymes or indirect regulation of transcription factors activities (Hardie 2007). Autophagy is one of the most important processes in cell catabolism, since cytosol and organelles are sequestered within double-membrane vesicles (pre-autophagosomes) that deliver the contents to the lysosome/vacuole (autolysosome) for hydrolytic degradation and recycling of the resulting macromolecules. Moreover, autophagy is considered a survival mechanism of the cell in stress situations (Goldman et al. 2010); hence, an appropriate regulation of autophagy is essential for cellular homeostasis, since excessive self-digestion can be harmful (Mizushima 2007).

Autophagy can be a nonselective or selective process because autophagy proteins and receptors of the autophagosome can interact specifically with the cytoplasmic component that needs to be eliminated (Johansen and Lamark 2011). Whereas autophagy usually refers to the nonselective elimination of any component of the cytoplasm, different terms have been coined to define the selective degradation of different organelles such as mitochondria (mitophagy) (Lemasters 2005), peroxisomes (pexophagy) (Till et al. 2012), or even lipids (lipophagy) (Liu and Czaja 2013).

Autophagy is generally activated by starvation and nutrient deprivation to generate metabolic intermediates to maintain ATP production. In fact, the autophagy checkpoint is a major mechanism for the maintenance of intracellular homeostasis that can be upregulated by nutrient deprivation and/or organelle damage. Therefore, autophagy is controlled by several metabolites, including the ATP/ADP ratio and the availability of acetyl-CoA, which affect the activity of various acetyltransferases (Green et al. 2014).

Since AMPK was primarily characterized as a kinase allosterically activated by AMP (Yeh et al. 1980), it was logical to hypothesize that AMPK would have a critical role in autophagy regulation.

Energy levels manifested in the form of ADP/ATP ratio trigger autophagy by activating AMPK. AMP enhances kinase activity of AMPK by binding to its γ subunits. On the contrary, ATP inhibits the allosteric activation of AMPK by AMP (Hardie 2007). Moreover, oxidative stress, which manifests in the form of high levels of ROS or oxidizing agents, activates AMPK by different pathways, some of them in an AMP-independent manner (Hwang et al. 2004; Emerling et al. 2009; Mungai et al. 2011).

In addition to energy levels, it has been hypothesized that other stimuli trigger autophagy by AMPK activation, under normal conditions indeed. Intracellular calcium (Ca^{2+}) levels seem to be crucial in AMPK activity, since Calmodulin-Dependent Kinase Kinase β (CaMKKβ) is suggested as an upstream kinase that activates AMPK (Hawley et al. 2005; Woods et al. 2005). It has been observed that different Ca^{2+} mobilizing agents, such as vitamin D compounds, induce autophagy by CaMKKβ and AMPK phosphorylation and activation. Moreover, inhibition of CaMKKβ by siRNA causes the inactivation of AMPK and an attenuation of autophagy (Høyer-Hansen et al. 2007).

Therefore, although AMPK activation is necessary to trigger autophagy, it is not known if it is sufficient or there are other molecular pathways which induce the change between basal autophagy and a huge autophagy in response to a cellular stress.

3.3.1 AMPK Regulation of Nonselective Autophagy

Independently of the stimuli, AMPK activation can induce the autophagic process through two different mechanisms: inhibition of mTOR and direct phosphorylation of ULK1 (Unc-51 Like Kinase 1, a mammalian orthologue of Atg1).

mTOR complex is a main regulator of diverse intracellular pathways that controls growth, proliferation, and survival. It is an effector in the PI3K/AKT pathway, and it carries out its action by two different complexes called mTORC1 and mTORC2. mTORC1 promotes protein synthesis, lipid biogenesis, cell growth, and anabolism and inhibits cellular catabolism by preventing autophagy. Instead, mTORC2 regulates cell survival, cell proliferation, and metabolism.

Although mTOR name comes from its sensitivity to rapamycin, rapamycin inhibits autophagy by affecting solely mTORC1, which is formed by the serine–threonine kinase mTOR, mLST8, PRAS40, and raptor, a regulatory protein which recruits downstream substrates such as 4EBP1 and ribosomal S6 kinase. mTORC1 inhibits autophagy by blocking the autophagy initiator complex activity, which is formed by ULK1, Atg13, Atg101, and FIP200 (Hosokawa et al. 2009; Jung et al. 2009). ULK1 is the essential protein for autophagy initiation in mammalian cells (Kuroyanagi et al. 1998; Chan et al. 2007; Wong et al. 2013).

The autophagy initiator complex is assembled independently of mTOR activity or nutrient conditions. However, under fed conditions, mTORC1 phosphorylates ULK1 in Ser638 and Ser758 and inhibits its kinase activity, preventing autophagy initiation (Shang and Wang 2011). Atg13 is also a substrate of mTOR kinase, and its phosphorylation has negative effects on ULK1 activity (Hosokawa et al. 2009). Moreover, ULK1 phosphorylation by mTOR disrupts its interaction with AMPK, preventing autophagy. Under starvation conditions, mTORC1-dependent phosphorylation of ULK1 is removed and ULK1 autophosphorylates and phosphorylates Atg13 and FIP200, initiating autophagy.

Since activation of mTORC1 is the principal mechanism to inhibit autophagy in mammalian cells, one of the mechanisms of AMPK-dependent induction of autophagy is inhibition of mTORC1 activity. AMPK is able to inhibit mTORC1 in a direct way by phosphorylating raptor (Gwinn et al. 2008) and in an indirect way by phosphorylating Tuberous Sclerosis Complex 2 (TSC2 or Tuberin) (Inoki et al. 2003).

When energy levels are low or there is a starvation situation, AMPK is phosphorylated and activated AMPK is able to bind and phosphorylate raptor, the mTORC1-binding partner (Gwinn et al. 2008). Gwinn et al. firstly revealed by bioinformatics techniques that raptor could be a direct substrate of AMPK since it presents two serine residues (Ser722 and Ser792) which match with the AMPK consensus motif. Secondly, the bioinformatics data were corroborated by biological evidences in vivo. Under energy stress conditions (low ATP levels), activated AMPK phosphorylates raptor in the predicted serine residues and this phosphorylation causes mTORC1 inhibition, so the autophagy initiator ULK1 can start the autophagic cascade. The mechanism by which raptor phosphorylation inhibits mTORC1 kinase function is a common mechanism for phosphorylation-based inactivation of target proteins. Phosphorylation of Ser722 and Ser792 induces 14-3-3 binding to raptor. 14-3-3 binding do not cause a disruption of mTORC1 complex but an inhibition of mTORC1 kinase activity.

Moreover, activated AMPK phosphorylates TSC2, an upstream inhibitor of mTORC1, since it inactivates GTPase Rheb, an activator of mTORC1 (Inoki et al. 2002). TSC2 forms a functional complex with TSC1 to inhibit two key regulators of translation: S6K and 4EBP1, which are phosphorylated by mTOR. It has been observed that under energy stress or starvation conditions, AMPK directly phosphorylates TSC2 on T1227 and S1345 to enhance its Rheb-GAP activity, which causes an inhibition of mTOR kinase activity (Inoki et al. 2003).

Therefore, energy stress results in AMPK phosphorylation and subsequently AMPK activation, which phosphorylates the mTORC1 inhibitor TSC2 and the mTORC1 regulatory subunit Raptor to inhibit mTORC1 and regulate catabolism, principally by initiating autophagy (Hardie 2008; Shaw 2009).

More recently, it has been observed that AMPK can also activate autophagy by direct phosphorylation of ULK1 (Guan et al. 2011; Egan et al. 2011b). However, there are some important discrepancies between the two investigations. The principal one is that the key phosphorylation sites identified by the different groups are not the same. Moreover, recently it was published that ULK1 forms a stable

complex with AMPK (Behrends et al. 2010). Guan et al. described that this complex is disrupted when mTOR is activated and phosphorylates ULK1, but Egan et al. did not observe this mechanism. Therefore, more investigation about regulation of autophagy by AMPK is needed to resolve these differences.

Furthermore, in recent times it has been suggested that phosphorylation of ULK1 by AMPK stabilizes a negative regulation of autophagy initiation (Löffler et al. 2011). ULK1 is able to phosphorylate the three subunits of AMPK, and this effect reduces the phosphorylation of AMPKα Thr172, preventing AMPK kinase activity. By this way, ULK1 participates in the elimination of the initial autophagy signal, in addition to initiate the autophagic cascade.

Thus, we can conclude that AMPK uses a dual mechanism to activate autophagy by inactivating mTOR and also directly activating ULK1 by direct phosphorylation. Interestingly, the patterns of activation of AMPK and mTOR are opposite, regulating by this way the activation of autophagy depending on the nutrient conditions (Egan et al. 2011a; Alers et al. 2012) (Fig. 3.1).

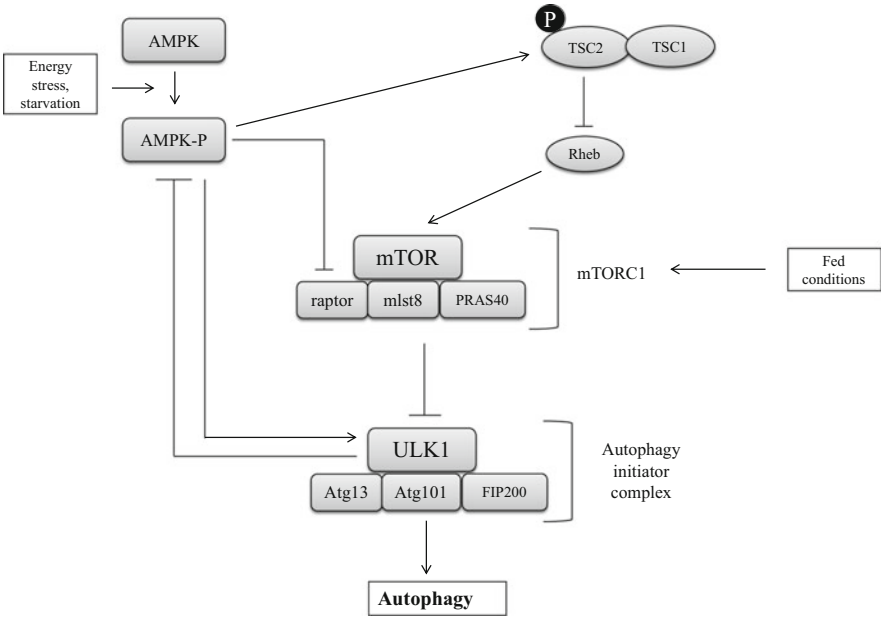

Fig. 3.1 Dual regulation of autophagy by mTOR and AMPK

3.3.2 *AMPK Activation of Selective Autophagy of Mitochondria*

Besides activating macroautophagy, AMPK promotes a selective form of autophagy called mitophagy, by which dysfunctional mitochondria are engulfed in autophagosomes and transported to lysosomes where they are degraded to recycle their components. In mammals, loss of AMPK resulted in aberrant accumulation of the autophagy adaptor p62 and defective mitophagy (Egan et al. 2011b). p62 binds to specific cargo targeted for autophagy-mediated degradation. Especially, p62 is bound to dysfunctional mitochondria targeted for mitophagy and is involved in mitochondrial aggregation and clearance (Geisler et al. 2010). Moreover, number of mitochondria per cell is significantly increased in AMPK-deficient cells, suggesting that AMPK plays an important role in mitochondria turnover.

Moreover, although the best-known function of the autophagy-initiating factor ULK1 is the autophagy induction (Mizushima 2010; Wirth et al. 2013), recent studies suggest that ULK1 has a more selective function in mitophagy (Kundu et al. 2008; Itakura et al. 2012). Recently, it has been described that ULK1 translocates to dysfunctional mitochondria in response to hypoxia or FCCP. Translocated ULK1 phosphorylates FUNDC1 (a mitophagy receptor located in external mitochondrial membrane) allowing its interaction with LC3 and inducing mitochondria degradation by autophagy (Wu et al. 2014).

In relation with this novel role of ULK1, it has been demonstrated that specific phosphorylation of ULK1 at Ser555 by activated AMPK is crucial for ULK translocation to the mitochondria (Tian et al. 2015). Therefore, AMPK-mediated phosphorylation of ULK1 is essential for mitophagy to initiate under stress conditions such as hypoxia.

For these reasons, AMPK is considered essential for the regulation of selective autophagy, besides its role as a macroautophagy inductor.

3.4 AMPK Regulation of Apoptosis

Although the best-known functions of AMPK are maintaining ATP homeostasis and regulating metabolism, AMPK has recently been proposed as a regulator of cell apoptosis or survival under stress conditions.

However, it is not clear whether AMPK is a proapoptotic or pro-survival molecule. In fact, AMPK participates in cell death or survival depending on the kind of stress, the cell type, and the duration of the activation of the signaling cascade. All these features make the role of AMPK in apoptosis controversial and complex.

3.4.1 AMPK as a Proapoptotic Molecule

Several metabolic checkpoints convert metabolic changes (signals), which are detected by specific systems (sensors), into stimuli which regulate the function of components of the cell death-regulatory machinery (effectors).

AMPK activation has been shown to mediate its proapoptosis effects primarily through modulation of several downstream signaling events by regulating JNK (c-Jun N-terminal protein kinase) and p53, inhibiting mTORC1 or directly enhancing the activation of proapoptotic proteins, among other different pathways (Fig. 3.2).

3.4.1.1 AMPK Induces Apoptosis by Activating JNK Pathway

The first demonstration of a proapoptotic effect of AMPK was observed in liver rat cells. It was observed that AMPK activation by high concentrations of AICAR triggers apoptosis in liver cells by activating JNK and Caspase 3 (Meisse et al. 2002). JNK pathway regulates proteins involved in apoptosis such as p53, c-myc, and members of the Bcl-2 family.

AICAR activation of apoptosis mediated by JNK and caspase 3 has been observed in different cell types. Kefas et al. observed that prolonged stimulation of AMPK induced apoptosis of insulin-producing cells (MIN6) of mouse. It is interesting that apoptosis induction was proportional to concentration of AICAR and time of exposure. Prolonged exposure to low glucose concentration induced apoptosis by AMPK activation too (Kefas et al. 2003a). The same response was observed in pancreatic beta cells (Kefas et al. 2003b).

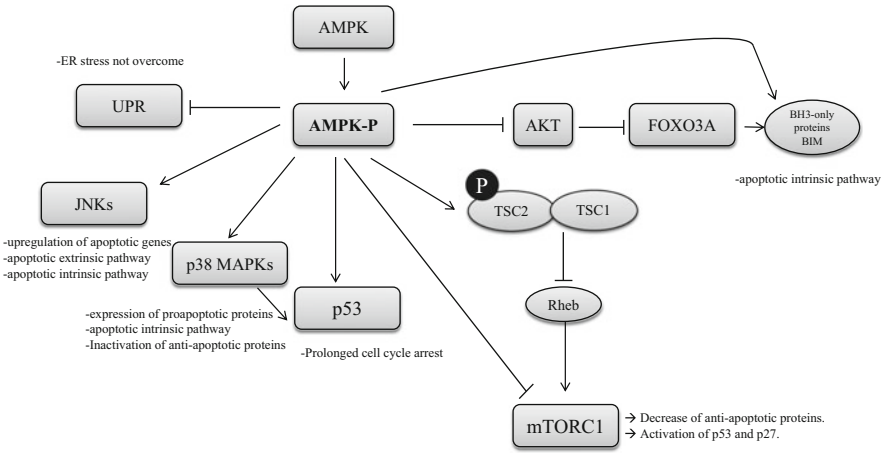

Fig. 3.2 Different pathways by which AMPK can induce apoptosis

3.4.1.2 AMPK Induces Apoptosis by Regulating and Stabilizing p53

Apart from its involvement in JNK activation, it has been described how AICAR induced p53 activation by AMPK stimulation. One of the first evidences that AMPK could be involved in regulating p53 function was observed in human hepatocellular carcinoma cell lines (HepG2) (Imamura et al. 2001). Later, it has been described how AMPK relates glucose availability to the p53 pathway, a master regulator of survival and proliferation (Jones et al. 2005). Prolonged cell cycle arrest induced by p53 may be followed by apoptosis activation. Glucose deprivation also induces phosphorylation of AMPK and subsequent activation of p53 and bax, a proapoptotic protein, promoting thymocytes and osteosarcoma cells to undergo apoptosis (Okoshi et al. 2008). Moreover, AICAR also induces apoptosis in other cancer cells such as glioblastomas due to AMPK-dependent inhibition of mTORC1 (Guo et al. 2009). Therefore, the same compound can activate AMPK and induce apoptosis in different types of cells by various mechanisms.

AMPK also contributes to UV- and H_2O_2-induced apoptosis in human skin keratinocytes (Cao et al. 2008). Chiefly, on UV- or H_2O_2-treated cells, AMPK induces apoptosis by inhibiting mTORC1 and positively regulating p53 and p38 MAPK, which mediates apoptosis elicited by both energy imbalance and pro-oxidant conditions.

3.4.1.3 AMPK Induces Apoptosis by Inhibiting mTORC1

One of the most important metabolic checkpoints that control cell death is the AMPK–mTORC1 pathway, which is based on the short half-life of antiapoptotic proteins such as FLIPL and MCL-1. In this sense, mTORC1 inhibition by AMPK activation causes the inhibition of protein translation; therefore, the abundance of short-lived antiapoptotic proteins such as MCL1 decreases and the cell is sensitized to mitochondrial apoptosis (Meynet et al. 2013).

Moreover, since mTOR inhibits apoptosis by the mediation of p53 and p27 (Faivre et al. 2006), AMPK-induced inhibition of mTORC1 could sensitize cells to undergo apoptosis too.

3.4.1.4 AMPK Directly Upregulates Proapoptotic Proteins Such as BH3-Only Proteins

For instance, it has been shown that prolonged AMPK activation can result in the direct induction of apoptotic excitotoxicity injury in neocortical neurons. This effect is dependent on Bim (proapoptotic BH3-only protein essential for the initiation of cell death). AMPK couples energy depletion to Bim mRNA induction and the subsequent activation of the Bcl-2-regulated apoptotic pathway in neurons (Concannon et al. 2010).

Moreover, AMPK activates the proapoptotic BH3-only proteins either through an indirect mechanism, by the AKT–FOXO3A pathway, because it has been suggested that FOXO3A is able to induce the translocation of Bim to mitochondria (Weisová et al. 2011).

3.4.1.5 AMPK Can Induce Apoptosis by Inhibiting the Unfolded Protein Response Pathway

Apart from inhibition of mTORC1, p53 stabilization, and JNK pathway activation, recently it has been proposed a new mechanism to explain AMPK-induced apoptosis. Metformin-induced activation of AMPK triggers apoptosis in acute lymphoblastic leukemia (ALL) cells by inhibiting the Unfolded Protein Response (UPR) pathway (Leclerc et al. 2013), which is triggered in response to the accumulation of unfolded/misfolded proteins in the ER lumen. First, metformin induces ER stress by accumulation of unfolded/misfolded proteins in the ER as a consequence of ATP depletion. At the same time, metformin activates AMPK, which acts as a negative regulator of the UPR, preventing cells from effectively engaging the UPR to overcome ER stress, leading to apoptotic death by inositol-requiring enzyme 1α (IRE1α), C/EBP homologous protein (CHOP), caspases, and JNK activation.

3.4.1.6 AMPK Involvement in Apoptosis Is Controversial

Recently, AICAR-induced apoptosis has been controversial since it has been shown that AICAR can induce an apoptosis in an AMPK- and p53-independent mechanism (Santidrián et al. 2010; Gonzalez-Girones et al. 2013). It has been shown that AICAR induced apoptosis in chronic lymphoid leukemia (CLL) through the mitochondrial apoptotic pathway, by inducing the modulation of RNA levels of Bcl-2, Bim, and Noxa, independently of AMPK and p53.

In the same way, AMPK sustained activation causes apoptosis induction in beta cells due to enhanced production of mitochondria-derived oxygen radicals which induce the activation of the intrinsic mitochondrial apoptosis pathway (Cai et al. 2007). Besides this mechanism, it is suggested an induction of the intrinsic apoptosis pathway by sustained AMPK activation, since dysregulation of BH3 members of the Bcl-2 family can be observed.

Other AMPK activators such as metformin have been demonstrated to induce apoptosis by different mechanisms that could be dependent or independent on AMPK. Metformin induces apoptosis in different cancer cell types (Leclerc et al. 2013; Queiroz et al. 2014), but it is important to notice that metformin has other targets apart from AMPK, for example, it functions as an inhibitor of complex I of the electron transport chain (Batandier et al. 2006). Moreover, the AMPK inhibitor, compound C, has been shown to compromise cell survival by AMPK-dependent and AMPK-independent mechanisms, since inhibition of AMPK is not sufficient to induce apoptosis in some cell types (Vucicevic et al. 2009).

Therefore, role of AMPK in AICAR/metformin-mediated apoptosis remains unclear and more investigations are needed to understand the real function and importance of AMPK in apoptosis induction (Bonini and Gantner 2013). What is clear is that AMPK is necessary (although in some cases is not sufficient) to promote apoptosis in different cell types.

3.4.1.7 Involvement of AMPK in Anticancer Drug Effects

Besides metformin, other anticancer drugs have been demonstrated to activate AMPK-dependent cell apoptosis pathway, although the molecular mechanisms involved in apoptosis initiation are different between the diverse compounds.

Vincristine, a drug that binds to tubulin and causes microtubule depolymerization, induces apoptosis in cells undergoing mitosis by AMPK activation. In B16 melanoma cells, high levels of ROS and LKB1 and AMPK activation have been observed, which induce both p53 activation and stabilization and mTORC1 inhibition, which are necessary mechanisms to mediate melanoma cell apoptosis (Chen et al. 2011).

Taxol (paclitaxel) is another drug that also affects microtubules but by a different mechanism. Taxol stabilizes the microtubule polymer and protects it from disassembly. Due to its action, chromosomes are unable to achieve a metaphase spindle configuration. It has been observed that taxol activates AMPK and downstream ACC in ovarian cancer cells and AMPK activation is implicated in the apoptosis induction mechanism (Sun et al. 2011). Apoptosis induction could be caused by ROS augmentation, mTORC1 inhibition, and activation of JNK pathway.

Temozolomide (TMZ) is another anticancer drug that exerts its action by a different mechanism, due to its ability to alkylate/methylate DNA. This methylation damages the DNA and has been demonstrated to induce apoptosis in primary cultured human glioblastoma cell lines. TMZ-mediated apoptosis is induced by AMPK activation, among other mechanisms. AMPK phosphorylation and activation in turn stabilize and activate p53 and induce the upregulation of p21, Noxa, and Bax. Activation of AMPK by TMZ also inhibits mTORC1 signaling and promotes antiapoptosis protein Bcl-2 downregulation (Zhang et al. 2010).

However, other drugs that induce DNA damage responses, such as doxorubicin (intercalating agent), induce AMPK activation in a ROS-dependent manner, which in turn contributes to apoptotic cell death by mTORC1 inhibition (Ji et al. 2010). It is interesting that inhibition of ROS production and AMPK activation by antioxidants such as *N*-acetylcysteine (NAC) or manganese (III) tetrakis (4-benzoic acid) porphyrins (MnTBAP) inhibits doxorubicin-induced AMPK activation and cell death. Therefore, the high level of ROS is the activating signal for AMPK in this situation.

Therefore, there are so many signals that are able to activate AMPK under stress situations for cells to undergo apoptosis.

3.4.2 AMPK as a Pro-survival Molecule

AMPK-mediated apoptosis has been reported in numerous cell types as we have exposed above. Conversely, it is recognized that activation of this kinase leads to metabolic alterations that can prevent ATP depletion in certain cell types, resulting in improved cell survival under different stress situations and protection from apoptosis. In fact, in conditions like starvation, energy deprivation, and oxidative stress, AMPK activation is required for cell survival. The molecular targets and effectors of the AMPK-mediated cell survival are diverse and different depending on the stress which induces the ATP depletion situation (Fig. 3.3).

3.4.2.1 AMPK Induces Cell Survival in Oxidative Stress Conditions

In oxidative stress conditions, for example, cell exposure to H_2O_2, AMPK is required for cell survival and inhibits apoptosis in osteoblasts (She et al. 2014). AMPK activation inhibits H_2O_2-induced oxidative stress through inhibiting NADPH depletion. Moreover, H_2O_2 is demonstrated to induce autophagy in MG63 cells. AMPK-dependent ULK1 activation and mTORC1 inactivation involve autophagy activation, which exerts a pro-survival effect instead of apoptosis induction. Therefore, activation of AMPK by H_2O_2 is pro-survival in osteoblasts through two mechanisms: activation of autophagy and inhibition of NADPH depletion.

It is important to observe that in the presence of the same condition (e.g., oxidative stress), AMPK can act as a pro-survival or proapoptotic molecule. It depends on the type of cell, the time of exposure, and the duration of the AMPK

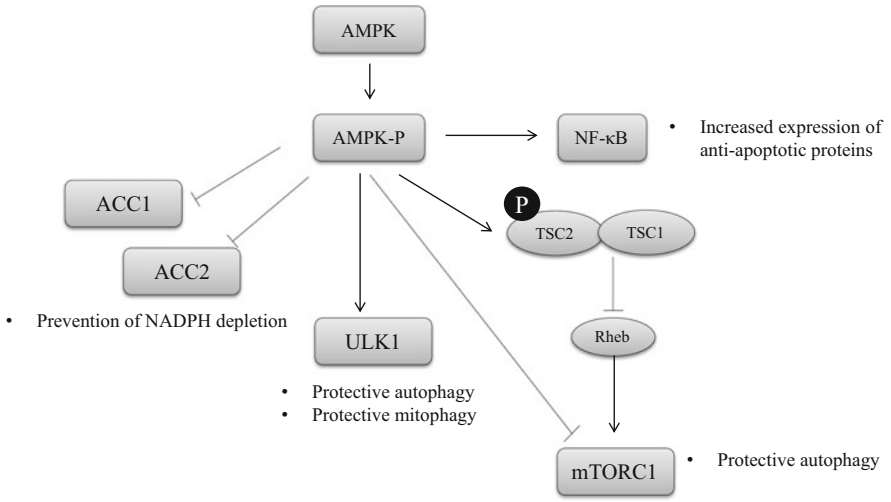

Fig. 3.3 Different pathways by which AMPK can prevent apoptosis

activation, among other features. Therefore, AMPK regulation of cell survival and death is very complex, and it is important to study every case separately.

3.4.2.2 AMPK Induces Cell Survival in Starvation Conditions

TSC2 and its phosphorylation by AMPK protect cells from glucose deprivation-induced apoptosis. As we have mentioned above, one of the major cellular functions of TSC1/TSC2 is to prevent protein translation by inhibiting the phosphorylation of S6K and 4EBP1. AMPK directly phosphorylates TSC2 on T1227 and S1345. Although TSC2 phosphorylation by AMPK plays an essential role in inhibiting cell growth, it is important for regulating cell survival under glucose starvation conditions too. Energy starvation activates TSC2, which then prevents cell growth and promotes cell survival. Activation of TSC2 by AMPK-dependent phosphorylation prepares cells for an unfavorable growth environment and results in protection from cell death. In fact, a mutant version of TSC2, TSC2-3A, which cannot be phosphorylated by AMPK, has demonstrated to be incapable to protect cells from glucose deprivation-induced apoptosis (Inoki et al. 2003).

3.4.2.3 AMPK Protects from Energy Stress-Induced Apoptosis

AMPK activation under an energy stress situation extends cell survival by redox regulation. In glucose limitation conditions, NADPH generation by the pentose phosphate pathway (PPP) is reduced, and this situation generates oxidative stress, since NADPH is needed for the regeneration of reduced glutathione (GSH), which is used by glutathione peroxidase (GPX) to eliminate H_2O_2. However, AMPK is able to induce alternative routes to maintain NADPH and inhibit cell death. By this way, AMPK plays an essential role in NADPH maintenance, which is critical for cancer cell survival under energy stress conditions. AMPK maintains NADPH levels by inhibiting the acetyl-CoA carboxylases ACC1 and ACC2. These effects induce a decrease in NADPH consumption (inhibition of fatty acid synthesis) and an increase in NADPH generation (induction of fatty acid oxidation) (Jeon et al. 2012).

Recently, it has been shown that AMPK promotes cell survival and suppresses apoptosis by directly increasing the expression of antiapoptotic proteins (Blc2 and Survivin) via NF-κB activation in endothelial cells exposed to hypoxia and glucose deprivation (Liu et al. 2010).

NF-κB has been proposed to induce the expression of different genes whose products can enhance cell survival and protect cells from apoptosis (Mitsiades 2002; Angileri et al. 2008). Nevertheless, the underlying mechanisms by which AMPK activation induces NF-κB-mediated cell survival remain poorly defined.

3.5 AMPK Regulation of Bioenergetics

Bioenergetics is the study of the transformation of energy in living organisms and the different cellular processes that can lead to production and usage of energy in different forms such as ATP.

AMPK has an essential role in bioenergetics regulation because it is a cellular energy sensor activated by conditions of metabolic stress characterized by an increase in the AMP/ATP ratio. AMPK activation restores cellular bioenergetics by inhibiting anabolic pathways that consume ATP and by activating specific catabolic reactions that generate ATP (Hardie 2011; Hindupur et al. 2015). Under stress conditions, the objective of AMPK activation is the conservation of cellular energy in order to avoid bioenergetic catastrophe (Faubert et al. 2015).

AMPK activation changes the way used by the cells to obtain energy in the form of ATP molecules. Predominantly, AMPK upregulates the production of ATP by glycolysis and fatty acid oxidation, for this reason, AMPK is crucial for the metabolic adaptation of cells which present some dysfunctions of the OXPHOS (Oxidative Phosphorylation) system (Wu and Wei 2012; Garrido-Maraver et al. 2015).

Activated AMPK switches off anabolic pathways such as fatty acid, glycogen, RNA, and protein synthesis to avoid ATP waste. However, in this section, we are focusing on the different catabolic pathways that are upregulated upon AMPK activation, in order to maintain energetic homeostasis (Fig. 3.4) (Hardie 2014), such as glucose uptake, fatty acid uptake, glycolysis, and fatty acid oxidation.

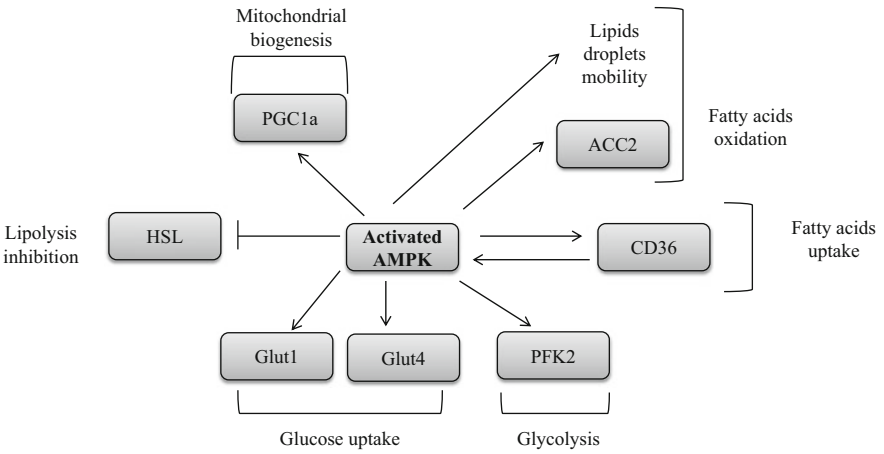

Fig. 3.4 Different catabolic pathways activated by AMPK

3.5.1 Activation of Glucose Uptake by AMPK

AMPK activation induces the upregulation of two glucose transporter: GLUT1 and GLUT4. GLUT1 is responsible for the low level of basal glucose uptake required to maintain respiration in cells, so AMPK is involved in basal glucose uptake maintenance (GLUT1 upregulation). Moreover, AMPK is responsible for insulin-dependent glucose uptake in adipose tissues and striated muscle, due to its role in GLUT4 upregulation.

3.5.1.1 GLUT1 Upregulation by AMPK

First studies in glucose uptake regulation by AMPK demonstrated that AMPK activation increased the Vmax of GLUT1-dependent glucose uptake (Barnes et al. 2002). More recently, a possible explanation for these results has been found. It has been suggested that AMPK activation increases the levels of GLUT1 mRNA due to the AMPK-dependent phosphorylation of Thioredoxin-interacting protein (TXNIP) (Wu et al. 2013).

TXNIP is induced in response to glucose elevation and suppresses glucose uptake directly, by binding to GLUT1 and inducing GLUT1 internalization, as well as indirectly, by reducing the level of GLUT1 mRNA. AMPK phosphorylation of TXNIP induces its rapid degradation by proteasome system. Therefore, TXNIP is released from GLUT1, and there is an increase in GLUT1 mRNA levels and function.

3.5.1.2 GLUT4 Upregulation by AMPK

First studies in glucose uptake regulation showed that AMPK activation induced the GLUT4 translocation from an intracellular location to the plasma membranes (Kurth-Kraczek et al. 1999). Recently, it has been demonstrated that AMPK-mediated translocation of GLUT4 is due in part to phosphorylation of the Rab GAP protein TBC1D1 by AMPK (Pehmøller et al. 2009). TBC1D1 is a component of the insulin-signaling cascade downstream of Akt that regulates GLUT4 translocation. Phosphorylation of TBC1D1 by AMPK promotes its 14-3-3 binding, which could increase basal and insulin-stimulated GLUT4 translocation.

Moreover, AMPK activation promotes upregulation of GLUT4 by a second mechanism because AMPK induces the transcription of the GLUT4 gene, due to phosphorylation of histone deacetylase-5 (HDAC5), a transcriptional repressor of GLUT4 (McGee et al. 2008). AMPK-mediated phosphorylation of HDAC5 reduces HDAC5 association with the GLUT4 promoter, increasing the GLUT4 mRNA levels.

3.5.2 AMPK Activation of Fatty Acids Uptake

Long chain fatty acid (LCFA) uptake is mostly dependent on the translocase CD36 (Koonen et al. 2005; Habets et al. 2007). AMPK activation stimulates LCFA uptake inducing the translocation of CD36 to the sarcolemma, which is essential for AMPK-induced fatty acid (FA) metabolism (Luiken et al. 2003; Bonen et al. 2007; Habets et al. 2009). Recently, it has been proposed that CD36 also interacts with AMPK to coordinate FA uptake. When exogenous FA levels are low, CD36 maintains AMPK inactive by indirectly promoting LKB1 transport to the nucleus. When exogenous FA levels increase, CD36-induced LKB1 transport to the nucleus is disrupted, and the cytosolic enrichment in LKB1 promotes AMPK activation and FA uptake (Samovski et al. 2015).

3.5.3 AMPK Activation of Glycolysis

Under cellular stress conditions (glucose deprivation, hypoxia, or oxidative stress), AMPK signaling drives glycolytic flux to maintain energy homeostasis and cellular ATP levels through glucose catabolism. AMPK promotes glycolysis via phosphorylation and activation of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase (PFKFB). PFKFB is a bifunctional enzyme with different tissue-dependent isoforms. The phosphofructo-2-kinase (PFK-2) domain functions to promote glycolysis by increasing levels of fructose-2,6-bisphosphate through phosphorylation of fructose-6-phosphate, and the fructose-2,6-biphosphatase (FB) domain catalyzes the reverse reaction producing fructose-6-phosphate.

In fact, AMPK is responsible for the increased glycolytic flux present after an ischemia in the heart, since it phosphorylates PFK-2 at Ser466 (Marsin et al. 2000). AMPK also induces glycolysis under hypoxia conditions (Marsin et al. 2002). It is important to remark that oxidative stress also promotes AMPK to increase glycolytic flux (Wu and Wei 2012).

3.5.4 AMPK Activation of Fatty Acid Oxidation

AMPK plays an important role in the regulation of FA oxidation because AMPK activation induces a change in different steps involved in FA oxidation pathway. First, as we commented above, AMPK controls fatty acid transport across the cell membrane by inducing CD-36 translocation.

Second, activated AMPK phosphorylates and inactivates the ACC2 isoform of acetyl-CoA carboxylase, which catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, the limiting step in fatty acid synthesis. Malonyl-CoA is also an

inhibitor of fatty acid absorption into mitochondria (Merrill et al. 1997). Therefore, AMPK activation increases the rate of fatty acid oxidation.

Third, very recently, it has been shown that AMPK phosphorylation promotes lipid droplets (LDs) mobility, dispersion, and consumption by affecting the microtubules network, increasing the rate of FA oxidation (Herms et al. 2015). By this way, AMPK promotes FAs from LDs to be channeled preferentially to different metabolic fates depending on the cellular energetic status: in the absence of glucose, FAs are directed to beta oxidation in mitochondria.

3.5.5 Induction of Mitochondrial Biogenesis by AMPK

Besides promoting different catabolic pathways, AMPK activates mitochondrial biogenesis to maintain the overall ATP-generating capacity of cells and avoid cell death. The most important regulator of mitochondrial biogenesis is PGC1α (Hardie et al. 2012). It has been observed that PGC1α is directly phosphorylated and transactivated by AMPK (Jäger et al. 2007). PGC1α can also be activated by Sirt-1-mediated deacetylation, which is induced by AMPK activation too (Cantó et al. 2009). PGC1α upregulates the activity of transcription factors involved in mitochondrial biogenesis, such as NRF-1, which in turn modulates the expression of other factors such as mtTFA, important for the regulation of mtDNA replication and transcription.

3.5.6 Inhibition of Lipolysis by AMPK

In response to glucose deprivation, AMPK inhibits lipolysis in adipocytes. One of the most important enzymes involved in lipolysis, hormone-sensitive lipase (HSL) is phosphorylated by AMPK on a key serine residue that antagonizes phosphorylation of HSL by cyclic AMP-dependent protein kinase (PKA) (Garton and Yeaman 1990), causing suppression of lipolysis. Although it is a catabolic pathway, its inhibition by AMPK is considered as a way to maintain cell energy homeostasis because an excessive accumulation of fatty acids promotes the synthesis of triglycerides, an ATP-consuming process (Garton 1989; Daval 2005).

3.6 Conclusion

AMPK is a critical regulator of energy homeostasis at both the cellular and whole-body levels. However, as a result of the wide range of pathways and signaling in which AMPK is involved, diverse contradictions arise about the role of AMPK in cell death and survival. For example, AMPK has different roles in apoptosis

regulation depending on the duration of stimulation, dosage of induction, the type of cell, and the type of stress that induce its activation. Thus, AMPK phosphorylation induces apoptosis in mouse astrocytoma but may protect normal brain cells from apoptosis under similar energy stress condition (Mukherjee et al. 2008). Therefore, for now, it is necessary to study every case separately because we do not have all the knowledge about AMPK pathways, signaling, and targets.

Understanding the complex role of AMPK in regulation of ATP balance is important because disturbances in energy homoeostasis underlie a number of diseases in humans, e.g., cancer, mitochondrial dysfunctions, diabetes, etc. In this sense, discovering new targets of AMPK is a research field which is gaining importance in the last years. For example, the involvement of AMPK in the insulin/IGF1 and MAPK–ERK pathways to control cell growth is being extensively studied.

As more targets of AMPK are known, we can discover which effectors are responsible for the beneficial effects of AMPK activation seen in diverse diseases. It is accepted that AMPK is emerged as a potential therapeutic target in different human diseases, but it is necessary to investigate about the molecular mechanisms underlying its therapeutic function.

Moreover, it is logical to think that AMPK regulation of cell growth, bioenergetics, and cell fate (death or survival) is closely related with the aging process regulation. Some investigations suggest that aging is linked to a loss of sensibility of AMPK—it loses its capacity to sense changes in AMP/ATP ratio—which induce a generalized energy imbalance situation. Then, the complete understanding of AMPK-mediated regulation of cell metabolism might lead to improve the quality of life in the elderly and to fight different diseases which have not curative treatments yet.

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