

INVITED MINI-REVIEW

**Adenosine Monophosphate-Activated Kinase (AMPK) and its Key Role in Catabolism:
Structure, Regulation, Biological Activity and Pharmacological Activation.**

Sukriti Krishan, Des R. Richardson, and Sumit Sahni

*Molecular Pharmacology and Pathology Program, Department of Pathology and Bosch Institute,
Blackburn Building (D06), University of Sydney, Sydney, New South Wales, 2006, Australia*

Running Title: AMPK - A Key Regulator of Catabolism.

To whom correspondence should be addressed:

Dr. Des R. Richardson, Phone: +61-2-9036-6548; Fax: +61-2-9036-6549; E-mail:

d.richardson@sydney.edu.au;

Dr. Sumit Sahni, Phone: +61-2-9036-7120; Fax: +61-2-9351-3429; E-mail:

sumit.sahni@sydney.edu.au

Text pages: 47

Tables: 1

Figures: 7

References: 228

Word count:

Abstract: 170

Article: 7696

Abbreviations:

4EBP1- 4E-binding protein 1	HMGCR- hydroxymethylglutaryl-CoA reductase
ACC1- acetyl CoA carboxylase 1	IL-1- interleukin-1
AD- Alzheimer's disease	LDL- low-density lipoprotein
AICAR- 5-aminoimidazole-4-carboxamide riboside	LKB1- liver kinase B1
AICART- aminoimidazole-carboxamide ribonucleotide formyl-transferase	MAPKKK- mitogen activated protein kinase kinase kinase
AIS- auto-inhibitory regulatory sequence	MO25- mouse protein 25
AMPK- adenosine monophosphate activated protein kinase	mTOR- mammalian target of rapamycin
AS160- Akt substrate 160	mTORC1- mammalian target of rapamycin complex 1
α -CTD- <i>C</i> -terminal β/γ - subunit interacting domain	NES- nuclear export domain
α -RIM- regulatory-subunit-interacting module	p38 MAPK- p38 mitogen activated kinase
β -CTD- <i>C</i> -terminal domain of the β -subunit	PD- Parkinson's disease
CaM- calmodulin	PPAR γ - proliferator-activated receptor- γ
CaMK- calmodulin-dependent kinase	Rheb- Ras homolog enriched in brain
CaMKK β - calmodulin-dependent kinase kinase β	S6K- S6 kinase
CBM- carbohydrate binding motif	SGLT- sodium dependent glucose co-transporter
CBS- cystathionine- β -synthase	SREBP1- sterol regulatory element-binding protein-1
CPT1- carnitine palmitoyltransferase 1	STRAD- STE20-related pseudokinase
EMT- epithelial to mesenchymal transition	TAK1- TGF- β activated kinase 1
FASN- fatty acid synthase	TGF- β - transforming growth factor- β
GLUT4- glucose transporter 4	TLR- toll like receptor
HD- Huntington's disease	TSC1/2- tuberous sclerosis complex 1/2
HMGCoA- hydroxymethylglutaryl-CoA	TZDs- thiazolidinediones
	ULK1- unc-51 like kinase 1
	WPW- Wolff-Parkinson White syndrome
	ZMP- AICAR 5'-monophosphate

Abstract

Adenosine monophosphate-activated protein kinase (AMPK) is a cellular energy sensor, which once activated, plays a role in several processes within the cell to restore energy homeostasis. The protein enhances catabolic pathways such as β -oxidation and autophagy to generate ATP, and inhibits anabolic processes that require energy including fatty acid, cholesterol and protein synthesis. Due to its key role in the regulation of critical cellular pathways, deregulation of AMPK is associated with the pathology of many diseases, including cancer, Wolff-Parkinson White syndrome, neurodegenerative disorders, diabetes and the metabolic syndrome. In fact, AMPK is a target of some pharmacological agents implemented in the treatment of diabetes (metformin and thiazolidinediones), as well as other naturally-derived products such as Berberine, used in traditional medicine. Due its critical role in the cell and the pathology of several disorders, research into developing AMPK as a therapeutic target is becoming a burgeoning and exciting field of pharmacological research. A profound understanding of the regulation and activity of AMPK would enhance its development as a promising therapeutic target.

General Introduction

Adenosine monophosphate-activated protein kinase (AMPK) belongs to a family of highly conserved, heterotrimeric, serine-threonine kinases, with orthologs present in all eukaryotes (Kahn et al., 2005). AMPK is an energy sensor that is activated during cellular stress (Hardie, 2007). During low energy conditions, it increases catabolic processes to generate ATP and inhibits anabolic processes that require ATP (Hardie, 2007). The protein is activated through phosphorylation of Thr172 in the α -subunit by three main kinases under different stimuli, namely liver kinase B1 (LKB1) (Woods et al., 2003), calmodulin-mediated kinase kinase β (CaMKK β) (Anderson et al., 2008; Hawley et al., 2005; Hurley et al., 2005; Woods et al., 2005) and transforming growth factor- β (TGF- β) activated kinase 1 (TAK1) (Momcilovic et al., 2006). Along with phosphorylation, AMPK also requires allosteric activation through AMP-binding to the γ -subunit (Suter et al., 2006). Once activated, AMPK stimulates β -oxidation (Hardie, 1989), glucose uptake (Kurth-Kraczek et al., 1999) and autophagy (Egan et al., 2011; Kim et al., 2011), while inhibiting cellular proliferation and the synthesis of fatty acids (Hardie and Pan, 2002), cholesterol (Habegger et al., 2012) and protein (Gwinn et al., 2008). Hence, AMPK is a critical enzyme involved in the regulation and integration of catabolic and anabolic pathways.

Due to its central role in regulating multiple processes within the cell, AMPK deregulation is associated with several pathological conditions, including diabetes and other metabolic diseases (Hardie, 2011), cancer (Faubert et al., 2013), neurodegeneration (Lim et al., 2012; Lopez-Lopez et al., 2007) and cardiac hypertrophy (Gollob et al., 2001a). The key role of AMPK in these conditions makes it a promising therapeutic target and this is discussed in the final section of the review.

Structure and Regulation of AMPK

Structure of AMPK

AMPK belongs to a family of serine-threonine protein kinase complexes that exist as a heterotrimer, composed of a catalytic subunit (AMPK α) and two regulatory subunits (AMPK β and AMPK γ) (Fig. 1A, B) (Carling et al., 2008; Hardie, 2007). The AMPK α - and AMPK β -subunits have two isoforms each (α 1, α 2 and β 1, β 2), and the AMPK γ -subunit exists as three different isoforms (γ 1, γ 2 and γ 3) (Hardie, 2011) (Table 1). These isoforms of AMPK-subunits interact with each other, to form one of 12 possible heterotrimers in different tissues (Hardie, 2007).

AMPK α is the catalytic subunit of the protein (Hardie, 2007). Complexes containing the α 1 isoform were shown to be predominantly expressed in the liver and adipose tissue, whereas the AMPK α 2 complexes were localized to the brain, heart and skeletal muscle (O'Neill, 2013; Steinberg and Kemp, 2009). Furthermore, the AMPK α 1 isoform has been shown to localize intracellularly in the cytosol, while the AMPK α 2 isoform is primarily found in the nucleus (Salt et al., 1998). The *N*-terminal of the protein harbors the kinase domain that extends from residues 1-280, comprising of Thr172 in the activation loop, which is phosphorylated for kinase activity (Carling et al., 1994; Halford et al., 2003; Suter et al., 2006) (Fig. 1B). The *C*-terminal of the protein has the auto-inhibitory regulatory sequence (AIS) of ~70 residues next to the kinase domain (Chen et al., 2009), and a *C*-terminal β/γ -subunit interacting domain (α -CTD) extending from residues 395-550 (Fig. 1B) (Jiang and Carlson, 1997; Xiao et al., 2007). Furthermore, the final 20 residues at the *C*-terminal end were shown to be conserved across diverse species (Brenman and Temple, 2007), and it was recently identified as the nuclear export sequence (NES) (Kazgan et al., 2010) (Fig. 1B).

To date, little is known about the nuclear and cytoplasmic pools of AMPK and how its intracellular distribution is controlled. However, it has been shown that post-translational modifications of the AMPK β 1-subunit affect enzyme activity and cellular distribution of the trimeric protein (Oakhill et

al., 2010; Oakhill et al., 2011; Warden et al., 2001). AMPK β exists in two isoforms, the first isoform is AMPK β 1 which is a 270 amino acid (30 kDa) protein, whereas AMPK β 2 is a protein consisting of 272 amino acids (34 kDa). Both isoforms are 71% identical and differ mostly at the *N*-terminal region (Thornton et al., 1998). Additionally, there have been studies showing that the carbohydrate-binding module (CBM) of the β 2 subunit binds more tightly to oligosaccharides when compared to the β 1 subunit (Koay et al., 2010). One of the main reasons for this was shown to be a single threonine insertion at position 101 in AMPK β 2 after Trp99 (Koay et al., 2010). The latter residue has been shown to be essential for oligosaccharide-binding and glycogen association *in vitro* (McBride et al., 2009; Polekhina et al., 2005), and insertion of Thr101 in the CBM region of β 1 subunit resulted in an increased affinity for oligosaccharides (Koay et al., 2010).

Similar to AMPK α , the expression of AMPK β varies in different tissues (Chen et al., 1999; Thornton et al., 1998; Winder, 2001). AMPK β 1 is predominantly expressed in liver, pancreas, kidney, brown fat and brain, while AMPK β 2 is expressed in cardiac and skeletal muscle (Chen et al., 1999; Thornton et al., 1998; Winder, 2001). Furthermore, studies have demonstrated that each AMPK β isoform has distinct roles in different tissues (Dasgupta et al., 2012; Dzamko et al., 2010). AMPK β 1 was shown to have a role primarily in the liver, with AMPK β 1-null mice demonstrating reduced fasting gluconeogenesis and enhanced hepatic insulin sensitivity (Dzamko et al., 2010). In addition, decreased AMPK β 1 activity led to defective proliferation and differentiation, un-regulated apoptosis and neuronal loss in the brains of these mice (Dasgupta and Milbrandt, 2009).

On the other hand, AMPK β 2-containing complexes play a crucial role in regulating glucose, glycogen and lipid metabolism in skeletal muscle during metabolic stress (Dasgupta et al., 2012; Steinberg et al., 2010). Studies using AMPK β 2-null mice demonstrated that these animals showed hyperglycemia, glucose intolerance and insulin resistance and were unable to maintain muscle ATP levels during exercise (Dasgupta et al., 2012; Steinberg et al., 2010). The AMPK β -subunit contains

the CBM region (residues 75-157) (Jiang and Carlson, 1996, 1997; McBride et al., 2009) and the C-terminal domain of the β - subunit (β -CTD), near the C-terminal of the subunit at approximately residues 186-270 (Iseli et al., 2005) (Fig. 1B).

The CBM region in the β -subunit plays a role in the binding of glycogen to AMPK, and allows the protein to detect changes in glycogen levels in the cell (McBride et al., 2009). Additionally, this region is also involved in the interaction of the α -subunit kinase domain with the regulatory fragment, which consists of the C-terminal domains of the α and β -subunits and all domains of the γ subunit (Momcilovic et al., 2008; Xiao et al., 2011; Xiao et al., 2013). More recently, the CBM region was also shown to be involved in protecting AMPK from cellular phosphatases (Xiao et al., 2013), such as human protein phosphatase-2c α (PP2C α) (Davies et al., 1995). In fact, the CBM region binds to the N-lobe of the kinase domain and is also connected to the regulatory fragment (Xiao et al., 2013). It has been suggested that an AMPK-activator, namely compound 991, promotes the interaction between the kinase and CBM region to protect active AMPK against dephosphorylation (Xiao et al., 2013). Furthermore, replacing ADP/AMP with ATP resulted in the displacement of the “ α -hook” (discussed in detail below; also referred to as regulatory-subunit-interacting motif; α -RIM; residues α 1₃₅₉₋₃₆₅, α 2₃₆₅₋₃₇₁) (Chen et al., 2013; Xiao et al., 2013; Xin et al., 2013). Xiao et al speculated that this alteration leads to the dissociation of the kinase domain and CBM from the regulatory fragment (Xiao et al., 2013). In this form, the kinase is no longer allosterically activated and is susceptible to dephosphorylation, and thus, inactivation (Xiao et al., 2011; Xiao et al., 2013). The second domain, β -CTD, was shown to bind with both the α - and γ -subunits in mammalian cells and acts as an anchoring region (Iseli et al., 2005) (Fig. 1B). Furthermore, the N-terminal has a more variable sequence that regulates N-myristoylation, which is important for membrane-targeting and -binding (Oakhill et al., 2010) (Fig. 1B). This study also showed that this post-translational modification of the protein is crucial for AMPK activation *via* AMP-binding, as loss of the myristoyl group abolished AMP activation and reduced the extent of α -Thr172 phosphorylation (Oakhill et al., 2010).

The AMPK γ -subunit acts as an allosteric activator of the AMPK complex (Hardie, 2011). It contains four tandem repeats of a structural module called the cystathionine- β -synthase (CBS) motif (Fig. 1B) (Bateman, 1997), which is involved in AMP/ADP-binding, a critical step in AMPK activation (Hardie, 2011). A recent study revealed that three of the four AMP-binding sites (*i.e.*, CBS) can be occupied by other nucleoside phosphates, such as ATP, ADP or AMP (Xiao et al., 2011). One of these sites (CBS site 4) was believed to have AMP permanently bound to it (Kemp et al., 2007), but more recent studies have shown that the AMP can be exchanged for an ATP at this site (Chen et al., 2012; Zhu et al., 2011). The two sites, namely CBS sites 1 and 3, that are sometimes referred to as exchangeable sites, bind AMP or ATP, and this allows the protein to respond to alterations in AMP/ATP levels (Oakhill et al., 2012; Xiao et al., 2007; Xiao et al., 2011). However, unlike AMP-binding, ATP-binding does not result in a conformational change of the protein structure (Carling et al., 1989; Xiao et al., 2011). AMP/ADP binds to the weaker of the two exchangeable sites (*i.e.*, CBS site 3) which interacts with the α -hook region (Xiao et al., 2011; Xiao et al., 2013). The Glu368(α_2 -hook) residue has been shown to form salt bridges with Lys170(γ) and possibly Arg70(γ). These latter sites are suggested to mediate the initial signaling distinguishing nucleotide-binding (Xiao et al., 2011; Xiao et al., 2013). This interaction leads to restricted flexibility of the α -linker region (residues 300–370), promoting the interaction of the kinase domain with the regulatory fragment (Xiao et al., 2011; Xin et al., 2013). This conformation protects the activation loop from dephosphorylation by phosphatases (Xiao et al., 2011; Xiao et al., 2013). Alternatively, ATP-binding leads to a steric clash of the regulatory fragment with the α -hook, causing the α -hook to dissociate, and this increases the flexibility of the linker that promotes dissociation of the kinase domain (Xiao et al., 2011; Xiao et al., 2013). In this form, the activation loop is no longer protected by packing against the regulatory fragment and is accessible to attack by phosphatases (Xiao et al., 2011). Thus, the shift between AMP/ADP- or ATP-binding determines the change in conformation between dephosphorylation-sensitive and -insensitive states (Xiao et al., 2011).

Regulation of AMPK Activity

The AMPK protein is activated by two crucial mechanisms. First, Thr172 in the activation loop of the α -subunit is phosphorylated by one of the three main upstream kinases: LKB1 (Woods et al., 2003), CaMKK β (Anderson et al., 2008; Hawley et al., 2005; Hurley et al., 2005; Woods et al., 2005) and TAK1 (Momcilovic et al., 2006) (Fig. 2). Second, AMP-binding to the γ -subunit of AMPK leads to allosteric activation of AMPK (Carling et al., 2008) (Fig. 2).

LKB1 phosphorylates the Thr172 residue on the AMPK α -subunit (Hurley et al., 2005) during low cellular energy conditions and was found to be the key upstream kinase (Woods et al., 2003) (Fig. 2). Alternatively, activation by CaMKK β occurs as a response to increased cytosolic calcium levels (Hawley et al., 2005) (Fig. 2). Furthermore, along with activating AMPK, AMP-binding also protects phosphorylated Thr172 from the activity of phosphatases (Davies et al., 1995) (Fig. 2). The mechanisms through which TAK1 activates AMPK are still uncharacterized (Momcilovic et al., 2006).

Activation of AMPK by LKB1

The key upstream kinase of AMPK, namely LKB1, acts as a tumor suppressor in several cancers (Gan and Li, 2014) and is also mutated (loss-of-function) in Peutz-Jeghers syndrome (Hemminki et al., 1998), with most of the subjects carrying just one of allele of this mutation. LKB1 has several phosphorylation sites which are either phosphorylated by upstream kinases (Collins et al., 2000), such as ataxia telangiectasia mutated kinase (Sapkota et al., 2002b), cAMP-dependent kinase (Collins et al., 2000; Sapkota et al., 2001), p90RSK (Sapkota et al., 2001), and protein kinase C zeta (Song et al., 2008), or by auto-phosphorylation (Sapkota et al., 2002a; Xie et al., 2009).

In addition to phosphorylation, activation of LKB1 requires STE20-related pseudokinase (STRAD) and mouse protein-25 (MO25)-binding to LKB1 to form the LKB1/STRAD/MO25 complex

(Boudeau et al., 2003) (Fig. 2). Formation of this complex stabilizes LKB1, activates its kinase activity (Boudeau et al., 2003), and allows the protein to translocate from the nucleus to the cytoplasm (Baas et al., 2003; Baas et al., 2004). This translocation might have an effect on phosphorylation of nuclear AMPK, but further studies are required to confirm this. When in the cytoplasm, the LKB1/STRAD/MO25 complex phosphorylates proteins of the AMPK family (Hawley et al., 2003) (Fig. 2). It has been proposed that, under physiological conditions, the net phosphorylation at Thr172 is determined by the balance between the rates of phosphorylation and dephosphorylation, and under basal conditions, the rate of dephosphorylation is high, resulting in low net phosphorylation (Hawley et al., 2003). However, under high AMP conditions, AMP-binding to the AMPK γ -subunit causes a conformational change in the protein structure, and inhibits the dephosphorylation of Thr172 on the AMPK α -subunit, allowing it to remain phosphorylated (Davies et al., 1995; Sanders et al., 2007; Suter et al., 2006; Xiao et al., 2013). Furthermore, it has been shown that AMP-binding to AMPK enhanced the LKB1-mediated phosphorylation at Thr172 (Gowans et al., 2013). More recent studies have reported that activation of AMPK by LKB1 may take place on the lysosomal surface, suggesting a role of the late endosome/lysosome in controlling metabolic programs (Zhang et al., 2014).

Activation of AMPK by CaMKK β

CaMKK β is another key upstream kinase of AMPK and it belongs to the Ca²⁺/calmodulin-dependent protein kinase (CaMK) family (Berridge, 2001) (Fig. 2). The two most well characterized substrates of CaMKK β are CaMKI and CaMKIV, but more recently, AMPK was also shown to be a target (Hawley et al., 2005). Unlike LKB1, CaMKK β does not phosphorylate AMPK in response to low ATP levels, but as a reaction to increased cytosolic calcium (Ca²⁺) levels (Green et al., 2011) (Fig. 2). Generally, calcium is known to regulate many processes within the cell by forming a complex with calmodulin (CaM) (Berridge, 2001; Chin and Means, 2000). Studies have shown that even though

CaMKK β possesses autonomous activity, it requires Ca²⁺/CaM-binding to phosphorylate AMPK at Thr172 (Hurley et al., 2005).

Activation of AMPK by TAK1

TAK1 is one of the most recently discovered activators of AMPK and is a member of the mitogen-activated protein kinase kinase kinase (MAPKKK) family (Herrero-Martin et al., 2009) (Fig. 2). Its activity is regulated by various cytokines, including: interleukin-1 (IL-1) (Landstrom, 2010), transforming growth factor- β (TGF- β) (Yamaguchi et al., 1995), and by toll-like receptors (TLR) (Landstrom, 2010), CD40 and B cell receptors (Landstrom, 2010). Once activated, TAK1 then stimulates further downstream kinases, including the p38 mitogen-activated protein kinase (p38 MAPK) (Shim et al., 2005), c-jun N-terminal kinase (JNK) (Shim et al., 2005) and I-kappa B kinase complex (IKK) (Ninomiya-Tsuji et al., 1999; Shim et al., 2005). Interestingly, TAK1 was also shown to directly phosphorylate Thr172 on the AMPK α -subunit (Herrero-Martin et al., 2009) (Fig. 2). Furthermore, mice carrying a cardiac-specific dominant negative mutation for TAK1 showed signs of the Wolff-Parkinson-White syndrome, which were similar to those signs associated with mutations in human AMPK α/γ (Xie et al., 2006).

Despite extensive investigation of the topic, the mechanism behind AMPK activation by TAK1 remains elusive. Some studies have suggested that TAK1 indirectly phosphorylates AMPK *via* activating LKB1 (Inokuchi-Shimizu et al., 2014). However, TAK1 has also been shown to directly activate AMPK (Scholz et al., 2010). Additionally, a recent study suggests AMPK acts as an activator of TAK1, and that these two proteins reciprocally regulate their activation (Kim et al., 2012b).

Once activated, AMPK regulates several different metabolic processes in cells (Hardie, 2011). In most cells, including skeletal and cardiac muscle, liver and adipose tissue, AMPK stimulates fatty

acid oxidation, mitochondrial biogenesis, glucose transporter type 4 (GLUT4) translocation and glucose uptake, while inhibiting protein synthesis, gluconeogenesis and fatty acid and cholesterol synthesis (Ruderman et al., 2013; Srivastava et al., 2012; Steinberg and Kemp, 2009). Insulin secretion from pancreatic β -cells can be inhibited by AMPK (Steinberg and Kemp, 2009) and it has been shown that AMPK can increase food intake *via* activating signaling in the hypothalamus (Minokoshi et al., 2004). AMPK also initiates autophagy, a catabolic process that is activated during starvation or stress (Egan et al., 2011; Kim et al., 2011). Overall, AMPK plays a major role in regulating energy homeostasis through modulation of cellular metabolism (Habegger et al., 2012; Hardie, 1989), protein synthesis and cellular proliferation (Shackelford and Shaw, 2009).

Biological Functions of AMPK

Regulation of Metabolism and Glucose Uptake

AMPK plays a critical role in regulating β -oxidation, fatty acid and cholesterol synthesis (Hardie, 2007, 2011) (Fig. 3). Catabolism of fatty acids *via* β -oxidation results in the generation of acetyl CoA, which then enters the citric acid cycle to generate ATP (Eaton et al., 1996). On the other hand, both fatty acid and cholesterol synthesis are anabolic processes, and hence, require ATP (Horton et al., 2002; Wakil et al., 1983). Fatty acid synthesis is a multi-step energy consuming process that generates fatty acids from acetyl CoA and malonyl CoA (Wakil et al., 1983) (Fig. 3). Similarly, cholesterol is synthesized *in vivo* from acetyl CoA to generate hydroxymethylglutaryl CoA (HMGCoA) (Horton et al., 2002). The HMGCoA is then converted into mevalonate which is the committed step in cholesterol synthetic pathway (Horton et al., 2002) (Fig. 3).

AMPK regulates these processes by phosphorylating and inhibiting the enzymes, acetyl CoA carboxylase 1 (ACC1) (Bianchi et al., 1990; Hardie and Pan, 2002) and hydroxymethylglutaryl CoA reductase (HMGCR) (Habegger et al., 2012) (Fig. 3). The enzymes ACC1/ACC2 and HMGCR are rate-limiting steps in the synthesis of fatty acids and cholesterol, respectively (Bianchi et al., 1990;

Habegger et al., 2012; Hardie and Pan, 2002). Importantly, ACC1/ACC2 is required for the carboxylation of acetyl CoA to malonyl CoA (Bianchi et al., 1990; Thampy, 1989; Wakil et al., 1958). Malonyl CoA subsequently inhibits carnitine palmitoyltransferase 1 (CPT1) (Bremer, 1963), an enzyme essential for enabling activated long chain fatty acids to enter the mitochondrion for metabolism *via* the β -oxidation pathway (Fig. 3). This process eventually results in increased ATP synthesis (Bremer, 1963; Fritz and Yue, 1963). Hence, inhibition of ACC1 by AMPK results in increased activity of CPT1, leading to enhanced β -oxidation and ATP synthesis. Furthermore, inhibition of acetyl CoA carboxylation to malonyl CoA by AMPK, interrupts fatty acid synthesis (Wakil et al., 1983) (Fig. 3). Similarly, upon phosphorylation, HMGCR is inactivated and leads to reduced cholesterol synthesis (Habegger et al., 2012) by preventing the reduction of HMGCoA to mevalonate (Horton et al., 2002) (Fig. 3).

AMPK has also been shown to suppress fatty acid synthesis by inhibiting the activity of sterol regulatory element-binding protein-1 (SREBP1) (Li et al., 2011) (Fig. 3). SREBP1 is a transcription factor which up-regulates the expression of fatty acid synthase (FASN) and ACC1 (Hardie, 1989). Both these latter enzymes are crucial for the *de novo* synthesis of fatty acids, and by inhibiting SREBP1, AMPK inhibits the expression of FASN and ACC1 (Hardie, 1989) (Fig. 3). These effects result in decreased *de novo* fatty acid synthesis which requires ATP hydrolysis (Foretz et al., 1999).

Apart from regulating energy expenditure, studies have shown that AMPK translocates the GLUT4 from intracellular storage vesicles to the plasma membrane, resulting in increased glucose uptake into the cell (Fig. 3) (Kurth-Kraczek et al., 1999). Translocation and fusion of the GLUT 4 vesicles is regulated by the Rab family of G proteins when bound to GTP (active form) (Steinberg and Kemp, 2009). Rabs are inactivated by Rab GTPase-activating proteins (Rab GAPs), such as Akt substrate 160 (AS160; also known as TBC1D4) and TBC1D1 (Cartee and Wojtaszewski, 2007; Roach et al., 2007; Sano et al., 2003; Steinberg and Kemp, 2009). Phosphorylation of AS160 promotes its

association with the 14-3-3 scaffolding protein (Chen et al., 2008; Kramer et al., 2006; Pehmoller et al., 2009; Steinberg and Kemp, 2009), resulting in inhibition of its Rab GAP activity (Fig. 3). Chen *et al* observed regulatory interactions among different phosphorylation sites of TBC1D1. For example, phosphorylation at Ser235 prevented subsequent phosphorylation at Ser237 (Chen et al., 2008). Phosphorylated TBC1D1 was also shown to bind the 14-3-3 scaffolding protein resulting in its inactivation (Chen et al., 2008). Moreover, these authors also showed that AMPK activators such as 5-aminoimidazole-4-carboxamide riboside (AICAR) and phenformin, phosphorylated TBC1D1 (Chen et al., 2008). In contrast, TBC1D4 was phosphorylated in response to insulin, but not AMPK activators (Chen et al., 2008). Hence, inactivation of Rab GTPases by AMPK prevents the hydrolysis of Rab GTP (Steinberg and Kemp, 2009), which then enhances GLUT4 translocation to the plasma membrane, and increases glucose uptake into the cell (Pehmoller et al., 2009).

Collectively, AMPK plays an important role in energy homeostasis *via* increasing catabolic pathways including β -oxidation, and inhibiting anabolic processes such as fatty acid and cholesterol synthesis.

AMPK Regulates Signaling *via* the Mammalian Target of Rapamycin

AMPK plays a role in controlling protein synthesis and cellular growth by inhibiting the mammalian target of rapamycin (mTOR) pathway (Gwinn et al., 2008) (Fig. 4). The mTOR complex 1 (mTORC1) plays a critical role as a nutrient/energy/redox sensor and controls protein synthesis (Kim et al., 2002). The complex, mTORC1, is composed of mTOR1 and the regulatory-associated protein of mTOR (raptor), which phosphorylates eukaryotic translation initiation factor 4E-binding protein (4EBP1) and ribosomal S6 kinase (S6K) (Fig. 4) (Holz et al., 2005; Wullschlegler et al., 2006).

AMPK has been shown to suppress mTORC1 by a dual mechanism, namely: **(1)** by inhibiting PI3K/AKT signaling (Menon et al., 2014; Shaw, 2009), and **(2)** directly inhibiting raptor (Gwinn et al., 2008) (Fig. 4). AKT inhibits the tuberous sclerosis 1/2 (TSC1/2) complex by

phosphorylating TSC2 serine residues. A recent study by Menon *et al* showed that the AKT-mediated phosphorylation of TSC2 does not affect its GTPase-activated protein (GAP) activity (Menon et al., 2014). Rather, this phosphorylation of TSC2 by AKT was shown to stimulate the dissociation of TSC complex from Ras homolog-enriched in brain (Rheb) at the lysosomal surface and the activation of Rheb (Menon et al., 2014). In contrast, when AMPK is activated, it phosphorylates TSC2 at distinct serine sites (*e.g.*, Ser1387) than those targeted by other kinases (Shaw, 2009). This AMPK-mediated phosphorylation of TSC2 results in activation of the TSC1/2 complex, which leads to inactivation of Rheb (Inoki et al., 2003; Shaw, 2009). This inactivation of Rheb results in inhibition of mTORC1 signaling (Inoki et al., 2003). Furthermore, AMPK also directly inhibits mTORC1 by phosphorylating raptor (Gwinn et al., 2008). This phosphorylation allows 14-3-3-binding to raptor, which reduces mTORC1 activity (Gwinn et al., 2008) (Fig. 4).

Inhibition of mTORC1 by AMPK leads to suppression of 4EBP1 and S6K phosphorylation (Holz et al., 2005; Wullschleger et al., 2006). When phosphorylated, 4EBP1 is unable to bind and inhibit the translational initiation factor eIF4E, resulting in an accumulation of free eIF4E and increased protein translation (Gingras et al., 1999; Haghighat et al., 1995) (Fig. 4). Furthermore, S6K up-regulates the translation of proteins such as, HIF-1 α , MYC and cyclin D1 (Guertin and Sabatini, 2007) that are involved in cell cycle progression and cell growth. Hence, suppression of mTORC1 by AMPK results in decreased protein synthesis and cellular proliferation (Fig. 4) (Shackelford and Shaw, 2009). The suppressive effect of AMPK on cell proliferation could lead to its development as an important therapeutic target in proliferative disorders such as cancer and atherosclerosis (Motoshima et al., 2006).

AMPK as an Initiator of Autophagy

AMPK is an important initiator of autophagy, a catabolic process that is activated during starvation or stress (Choi, 2012). Autophagy regulates the digestion of cellular macromolecules and even whole

organelles, by engulfing the organelles and proteins in a double membrane vesicle (autophagosomes), which fuse with the lysosomes to hydrolyze its contents (Choi, 2012; Sahni et al., 2014). Autophagy acts as a mechanism of avoiding cell death, but under some conditions, can lead to autophagic cell death (Choi, 2012).

Once AMPK is activated, it phosphorylates an initiator of autophagy Unc 51-like kinase (ULK1) at certain sites, namely Ser317 and Ser777 (Kim et al., 2011) (Fig. 5). Phosphorylation of ULK1 at these sites is important for initiation of autophagy (Kim et al., 2011). Another study has shown that AMPK also phosphorylates ULK1 at Ser467, Ser555, Thr574, and Ser637 (Egan et al., 2011). Additionally, ULK1 is also phosphorylated by mTORC1 at Ser757, which has an inhibitory effect on the phosphorylation of ULK1 at Ser317 and Ser777, resulting in suppression of the autophagic pathway (Kim et al., 2011) (Fig. 5). As discussed earlier, when active, AMPK suppresses mTORC1 activity, this leads to decreased phosphorylation of ULK1 at Ser757 (Kim et al., 2011). The removal of the inhibitory phosphorylation at Ser757, allows ULK1 to be phosphorylated at Ser317 and Ser777 by AMPK, which results in the activation of autophagy (Kim et al., 2011) (Fig. 5). Thus, AMPK initiates autophagy directly by activating ULK1 and also by removing the inhibitory effect of mTORC1 on ULK1 (Fig. 5). Together, the suppression of mTORC1 and activation of ULK1 by AMPK, leads to inhibition of protein synthesis (an ATP-consuming process) and activation of autophagy in the cell, leading to prolonged survival.

AMPK in Pathological Conditions

Diabetes and Metabolic Diseases

As AMPK is a potent regulator of cellular metabolism, it plays a crucial role in type 2 diabetes and several other metabolic diseases (Hardie, 2011). Previous studies have indicated that an elevation of plasma fatty acid levels could lead to impaired insulin-mediated glucose uptake (Frayn et al., 1993), which requires the translocation of GLUT4 to the plasma membrane (Cushman et al., 1998).

Considering that AMPK is involved in regulating both fatty acid metabolism (Hardie, 1989) and GLUT4 translocation to the membrane (Kurth-Kraczek et al., 1999), reduced activity of AMPK activity would be expected to inhibit fatty acid oxidation and decrease glucose uptake into the cell. Furthermore, AMPK activators have been shown to improve insulin resistance and glucose uptake in rodent models of the metabolic syndrome (Habegger et al., 2012; Song et al., 2002). These activators also prevented the development of diabetes, lipid deposition and degranulation of pancreatic islet β cells (Yu et al., 2004). Furthermore, AMPK is expressed in adipose tissue and is involved in metabolic regulation in these cells (Wang et al., 2014). Hence, due to the correlation between excess adiposity and metabolic diseases (Abate et al., 1995; Premanath et al., 2014) this suggests another possible pathway through which AMPK plays a role in these disorders. These studies illustrate a crucial role of AMPK in metabolic diseases and diabetes, and signify its importance as a molecular target for novel therapeutics

Wolff-Parkinson-White Syndrome

Wolff-Parkinson White (WPW) syndrome is a genetic disorder caused by mutations in the *PRKAG2* gene, which codes for the AMPK γ 2-subunit (Gollob et al., 2001a; Gollob et al., 2001b; Gollob and Roberts, 2002). As discussed earlier, the γ -subunit is essential for the allosteric activation of the protein (Bateman, 1997; Hardie, 2007). Previous studies have demonstrated that *PRKAG2* gene mutations such as His142Arg, Arg302Gln, His383Arg, Thr400Asn, Tyr487His, Asn488Ile, Gln506Lys, Arg531Gly, Ser548Pro, and InsLeu351 occur in the CBS region of the gene and affect the AMP-binding ability of the subunit (Gollob and Roberts, 2002). However, this notion has been challenged by a recent study in Chinese patients that has discovered a new missense mutation, namely Gly100Ser (G100S) in the *PRKAG2* gene, but unlike the other mutations, this one was not in the CBS region (Zhang et al., 2013a). Over-expression of this mutated protein resulted in thicker heart wall, increased glycogen storage and decreased AMPK enzymatic activity (Zhang et al., 2013b). Cardiomyocytes from patients with WPW syndrome exhibit a variable phenotype consisting

of cardiac hypertrophy, pre-excitation, and conduction abnormalities, which are probably caused by the derangement of AMPK's function (Light, 2006). *PRKAG2*-induced cardiac syndrome is believed to be a result of increased glycogen storage (Gollob, 2003). Studies have shown an accumulation of glycogen-like substances in the myocardium, which leads to increased thickness of the myocardium, *i.e.*, cardiac hypertrophy (Arad et al., 2003). Apart from causing cardiac hypertrophy, increased glycogen storage in cardiomyocytes interferes with electrical conductance (Gollob, 2003). This problem leads to the development of a less efficient accessory pathway (Gollob, 2003), which is another electrical conduction system that connects the atrium to the ventricle (Ho, 2008). Furthermore, studies have suggested the increased glucose uptake in these cells leads to an increase in cytosolic glucose-6-phosphate and consequent activation of glycogen synthesis (Luptak et al., 2007). However, the effect of *PRAKG2* mutation on cardiac growth was shown to be independent of glycogen storage (Kim et al., 2014). In contrast, the electrical defects in these cardiomyocytes seem to be secondary consequences of the high glycogen content (Kim et al., 2014).

Initially, WPW was believed to be due to a loss of function mutation (Sidhu et al., 2005). However, studies have shown that the increased glycogen storage was due to a gain of function mutation in the *PRKAG2* gene (Banerjee et al., 2007; Gollob et al., 2001b). Recent evidence from transgenic mouse studies suggest that some *PRKAG2* mutations, such as N488I, lead to increased AMPK activity and accumulation of glycogen in myocytes, contributing to cardiac hypertrophy (Arad et al., 2003). Another mutation located at R302Q within the AMPK γ 2-subunit, was also identified and shown to amplify glycogen storage in cardiac muscle (Gollob et al., 2001b; Gollob, 2003). However, there is conflicting data that shows mice expressing the γ 2 R302Q variant have reduced AMPK activity when measured with the SAMS peptide (Sidhu et al., 2005), a well-established AMPK substrate widely used for determination of AMPK activity (Davies et al., 1989). A similar γ 2 mutation, T400N, when expressed in transgenic mice, resulted in a biphasic effect of γ 2-subunit on cardiac muscle AMPK activity, with increased activity in samples from young mice, but reduced activity later in life

(Banerjee et al., 2007). These conflicting findings suggest that the lower AMPK activity observed in older mice might be due to secondary effects of the mutation acquired later in life (Banerjee et al., 2007). These studies suggest deregulation of AMPK activity caused by *PRKAG2* mutations leads to the development of WPW, but the mechanisms by which these mutations in the *PRKAG2* gene cause WPW syndrome are not clearly defined.

Furthermore, more recent data has shown that knockdown of cardiac sodium-dependent glucose co-transporter (SGLT) in mice with the *PRKAG2* Thr400Asn mutation (TGT400N) attenuates the phenotype consisting of cardiomyopathy (Ramratnam et al., 2014). Transgenic mice with cardiac SGLT1 over-expression (TGSGLT1-ON) replicate phenotypic features of the cardiomyopathy, suggesting role of SGLT1 in the pathophysiology of the *PRKAG2* mutation (Ramratnam et al., 2014).

Neurodegenerative diseases

Several studies have shown AMPK over-activation in neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD) (Chou et al., 2005; Lim et al., 2012; Lopez-Lopez et al., 2007). Additionally, neurodegenerative diseases (*e.g.*, PD) are also associated with mitochondrial dysfunction (Milakovic and Johnson, 2005; Panov et al., 2002; Ruan et al., 2004). Mitochondrial dysfunction can lead to high lactate levels in PD brains (Harms et al., 1997), which can result in activation of AMPK (Chen et al., 2010). A recent study demonstrated that exposure of neuronal cells to lactate caused an AMPK-dependent accumulation of α -synuclein in the cytoplasm (Jiang et al., 2013), a protein associated with PD development.

In HD, it has been demonstrated that the AMPK α 1-subunit abnormally translocates to the nucleus in the striatal neurons expressing the mutant *huntingtin* gene (Ju et al., 2011). This resulted in the suppression of Bcl2 (an anti-apoptotic protein) expression, leading to neurotoxicity (Ju et al., 2011). Furthermore, this study also showed that the pharmacological agent, CGS21680, that ameliorates HD

symptoms, acts in part, by preventing AMPK α 1 translocation to the nucleus (Ju et al., 2011). In the case of AD, the role of AMPK in progression of this disease is still not clear. In human patients it was observed that AMPK is abnormally activated and translocated from the nucleus to the cytoplasm in AD tangles (Vingtdeux et al., 2011). It was demonstrated that AMPK activation, either directly or indirectly, inhibits tau phosphorylation in rat cortical neurons (Greco et al., 2009). Conversely, other studies have demonstrated that AMPK phosphorylates tau at multiple sites and disrupts the binding of tau to microtubules (Thornton et al., 2011; Vingtdeux et al., 2011). From these findings, it is evident that AMPK is activated during Alzheimer's disease. However, it is not clear if it is the primary cause or a cellular response in order to repair the damage, for example through the activation of autophagy.

The Controversial Role of AMPK in Cancer

Current evidence indicates that AMPK has properties of both a tumor suppressor and a tumor promoter (Bonini and Gantner, 2013). The role of AMPK in cancer is discussed in detail in a recent review by Jones and colleagues (Faubert et al., 2014). It has been suggested that AMPK inhibits tumor growth through its regulation of metabolic pathways (Shackelford and Shaw, 2009). As cancer cells generally have high energy expenditure due to their more rapid metabolism, it would seem that AMPK, as a master regulator of energy homeostasis, would play a key role in tumor progression (Faubert et al., 2013). Additionally LKB1, the key upstream kinase of AMPK, has been shown to inhibit cancer proliferation as well as metastasis, possibly through AMPK activation (Gan and Li, 2014).

Metastasis is an important step in the progression of cancer. It involves the detachment of tumor cells from their primary site, and their dissemination to distant sites *via* blood vessels and the lymphatic system (Geiger and Peeper, 2009). Cell polarity can also be observed in migrating cells and is responsible for directional migration (Marcus and Zhou, 2010). Loss of polarity may be

responsible for the epithelial to mesenchymal transition (EMT) and subsequent tumor invasion that plays a key role in metastasis (Thiery, 2003).

It has been noted that AMPK regulates mammalian cell motility and its loss causes directional migration defects (Nakano et al., 2010), suggesting a potential role for AMPK in energy-dependent regulation of cell motility (Fig. 6). Interestingly, AMPK phosphorylates the microtubule plus end protein, CLIP-170, to regulate cell polarity *via* microtubule dynamics (Fig. 6) (Zheng and Cantley, 2007). CLIP-170 directly binds newly polymerized distal ends of growing microtubules and rapidly dissociates from the older microtubule lattice (Dragestein et al., 2008). Microtubules induce cell polarity by transporting protein complexes to the new end of the cell (Sawin and Nurse, 1998). Inhibition of AMPK leads to an accumulation of non-phosphorylated CLIP-170 and disturbed cell polarity (Nakano et al., 2010), suggesting a role of AMPK in maintaining polarity.

A recent study has shown that activation of AMPK reduces metastasis and invasion by downregulating the levels of integrin $\beta 1$ (Park et al., 2012) that is known to participate in cell migration (Lee et al., 2010). Integrins mediate adhesion to the extracellular matrix, but also participate in the regulation of cytoskeletal organization of the cell (Banno and Ginsberg, 2008), making it important for cell migration and invasion. Hence, reducing integrin $\beta 1$ levels would lead to reduced cell migration and invasion. In addition to regulating cell polarity, studies have shown suppression of metastasis by AMPK occurs through its ability to inhibit S6K (Taliaferro-Smith et al., 2009). Activation of AMPK resulted in the inhibition of the mTORC1 pathway (Gwinn et al., 2008), resulting in suppressed expression of proteins required for tumor growth and metastasis, including HIF-1 α , MYC and cyclin D1 (Guertin and Sabatini, 2007).

Interestingly, there have also been studies showing that AMPK elevates tumor growth and metastasis. One investigation suggested that AMPK activation during energy stress prolongs cell survival by

redox regulation (Jeon et al., 2012). Under these conditions, NADPH generation through the pentose phosphate pathway was down-regulated, but AMPK was able to induce alternative metabolic routes to maintain NADPH levels and inhibit cell death (Jeon et al., 2012). Inhibition of ACC1 by AMPK decreases NADPH consumption in fatty acid synthesis and increases NADPH generation by means of fatty acid oxidation (Jeon et al., 2012). Other investigations have suggested that the pro-survival role of AMPK can be attributed to the induction of autophagy and the maintenance of proliferative quiescence (Degenhardt et al., 2006). It has been suggested that AMPK activation, and therefore autophagy, can serve as a protective mechanisms conferring resistance to growth factor deprivation therapies (Chhipa et al., 2011). Overall, these studies suggest that AMPK promotes tumor growth by inhibiting cell death and prolonging cell survival through autophagy under stressful conditions (Chhipa et al., 2011; Degenhardt et al., 2006; Jeon et al., 2012).

From the investigations described above, it is evident that AMPK plays a primary role in regulating tumor growth and metastasis. While there is some controversy regarding the anti-tumor and pro-tumor effects of AMPK, the majority of studies have demonstrated that AMPK plays a crucial role in inhibiting tumor growth and metastasis.

Therapeutic Targeting of the AMPK Signaling Pathway

Due to the diverse roles of AMPK in the cell, as well as its regulation in several pathological conditions, AMPK acts as a promising therapeutic target for treatment of a number of pathological conditions (Hardie, 2013; Jeong et al., 2014; Russo et al., 2013). There are several known physiological, pharmacological, natural and hormone activators of AMPK, with some of these already being used clinically (Corton et al., 1995; Lee et al., 2006; Zhou et al., 2001a). Currently, pharmaceutical agents such as metformin and thiazolidinediones (TZDs) are used for the treatment of type 2 diabetes *via* their ability to activate the AMPK pathway (Owen et al., 2000). These agents are discussed in detail below.

Metformin

Metformin (Fig. 7A) is used as a treatment for type 2 diabetes (Duncan and Seaton, 1962), as well as polycystic ovarian syndrome (Diamanti-Kandarakis et al., 2010) and metabolic syndrome (Bianchi et al., 2007). The efficacy of metformin in these metabolic disorders is attributed to its ability to reduce hepatic gluconeogenesis and improve insulin sensitivity (Viollet et al., 2012). Metformin activates AMPK indirectly by inhibiting complex I of the mitochondrial respiratory chain which promotes a switch from aerobic to anaerobic glycolysis, to increase the cellular AMP/ATP ratio (Hawley et al., 2010; Owen et al., 2000; Zhou et al., 2001b). Interestingly, recent findings by Fullerton *et al* show that phosphorylation of ACC1 by AMPK is required for metformin's effects on lipid metabolism and insulin sensitivity (Fullerton et al., 2013). In addition to its anti-diabetic effects, studies have also shown that metformin possesses anti-tumor properties including reduced cell growth, induction of apoptosis, and reduced migration and invasion in a variety of human cancer cell lines (Liu et al., 2011; Papanas et al., 2010; Rozengurt et al., 2010; Viollet et al., 2012).

Several *in vitro* and *in vivo* studies have shown that one of the potential pathways *via* which metformin mediates its anti-tumor effects is through AMPK activation and reduced mTOR activity (Ben Sahra et al., 2010; Brown et al., 2010; Tosca et al., 2010; Zakikhani et al., 2008). However, metformin also acts through AMPK-independent mechanisms (Foretz et al., 2010; Janjetovic et al., 2011a; Janjetovic et al., 2011b; Miller et al., 2013; Rattan et al., 2011) . Studies have shown that LKB1 is a crucial protein required for the anti-tumor effects of metformin, as cells lacking LKB1 failed to exhibit reduced tumor growth when treated with this agent (Huang et al., 2008; Legro et al., 2008; Rattan et al., 2011).

Currently, numerous phase II-III clinical trials are underway to investigate the potential of treatment with metformin in a variety of tumor-types, including breast cancer (Goodwin et al., 2011; Hadad et al., 2011; Martin-Castillo et al., 2010), endometrial cancer, colorectal cancer and prostate cancer.

However, some of these studies are in the recruitment stage and are yet to determine the potential of metformin as an adjuvant to current cancer therapeutics.

Thiazolidinediones

TZDs (*e.g.*, Rosiglitazone; Fig. 7B) are another class of drugs used in the treatment of diabetes, which primarily target the peroxisome proliferator-activated receptor- γ (PPAR γ) to improve insulin sensitivity (LeBrasseur et al., 2006). The effects of TZDs are partly mediated through AMPK activation (LeBrasseur et al., 2006). These drugs activate AMPK in a similar way to metformin, by inhibiting complex I of the mitochondrial respiratory chain, increasing the AMP/ATP ratio (Brunmair et al., 2004; Hawley et al., 2010). Additionally, TZDs also increase AMPK activation through PPAR γ to stimulate adiponectin secretion from adipose tissue (LeBrasseur et al., 2006). Adiponectin was shown to increase AMPK phosphorylation and regulate glucose levels and stimulate FA oxidation in skeletal muscle (Yoon et al., 2006). Hence, TZDs are effective drugs to treat diabetes through their ability to activate AMPK which subsequently regulates glucose levels in cells and improves insulin sensitivity.

5-Aminoimidazole-4-carboxamide riboside

The compound, 5-aminoimidazole-4-carboxamide riboside (AICAR; Fig. 7C), is a well known activator of AMPK (Corton et al., 1995; Sullivan et al., 1994). In fact, it was the first compound to be identified as an activator of AMPK (Sullivan et al., 1994). Structurally, AICAR is similar to AMP, and upon intracellular metabolism, it is converted to AICAR 5'-monophosphate (ZMP), an analogue of AMP, by adenosine kinase (Corton et al., 1995). Activation of AMPK by ZMP is similar to that by AMP, where ZMP binds to the CBS domains on the γ -subunit of AMPK and leads to allosteric activation of the complex (Corton et al., 1995). Additionally, AICAR treatment either prevented and/or reversed some aspects of metabolic syndrome in animal models, including improved glucose tolerance, better systemic glucose disposal (Song et al., 2002), and reduced hepatic glucose output, as

well as plasma triglyceride and free fatty acid levels (Bergeron et al., 2001). However, AICAR does have some disadvantages that make it unlikely to be used as a therapeutic agent, such as low bioavailability and a short half-life (Coughlan et al., 2014). Due its ability to mimic AMP, it can interfere with several other pathways in the cell that require AMP (Longnus et al., 2003), making AICAR an unpromising lead agent for drug development.

Similar to AICAR, the drugs pemetrexed and sorafenib also increase AMPK activity through inhibition of aminoimidazole-carboxamide ribonucleotide formyl-transferase (AICART) (Bareford et al., 2014; Racanelli et al., 2009). Inhibition of AICART results in increased ZMP and activation of AMPK (Bareford et al., 2014). These agents have demonstrated anti-tumor effects through regulation of AMPK and subsequently autophagy (Bareford et al., 2014). Currently, these drugs are in phase I clinical trials, and it has been suggested that combination treatment with pemetrexed and sorafenib represent a future therapeutic option for the treatment of solid tumors (Bareford et al., 2014).

Berberine

Berberine (Fig. 7D) is a clinically important pharmacological agent derived from the *Berberis* plant species that has exhibited AMPK activation properties (Imanshahidi and Hosseinzadeh, 2008; Tillhon et al., 2012). It is used in traditional Chinese and Korean medicine to treat bacterial and fungal infections as well as type 2 diabetes (Tillhon et al., 2012). Berberine treatment using several rodent models resulted in improved glucose tolerance (Lee et al., 2006), reduced body weight (Lee et al., 2006), increased expression of both the insulin receptor and low-density lipoprotein (LDL) receptor (Kong et al., 2004), lower total and LDL cholesterol levels, and reduced triglyceride levels (Kong et al., 2004). Studies have shown that Berberine is able to lower blood glucose, triglyceride, and cholesterol levels to a similar extent as metformin (Yin et al., 2008). Collectively, these results suggested that Berberine activates the AMPK pathway (Kong et al., 2004; Lee et al., 2006). Similarly

to metformin, Berberine was shown to act upon AMPK through inhibiting complex I of the mitochondrial respiratory chain (Hawley et al., 2010; Turner et al., 2008).

More recent studies have also demonstrated that the anti-tumor and anti-metastatic effects of Berberine are also mediated through activation of the AMPK pathway (Kim et al., 2012a; Park et al., 2012). Another investigation showed that Berberine-induced AMPK activation inhibits the metastatic potential of melanoma cells *via* reduction of ERK activity and COX-2 protein expression (Kim et al., 2012a). The ERK/COX-2 pathway is involved in the regulation cell proliferation, angiogenesis and metastasis in many cancers (Muller-Decker and Furstenberger, 2007).

Salicylates

Salicylates are natural compounds secreted by many plants as a defense against infections, and synthetic derivatives including acetyl salicylate (aspirin; Fig. 7E) or diester salicylate, are used pharmaceutically (Higgs et al., 1987). Studies have shown that salicylate binds to a site in an AMPK cleft between the kinase domain of the α -subunit and the CBM domain of the β -subunit (Hawley et al., 2012). Furthermore, it was only able to activate AMPK complexes containing the $\beta 1$ isoform and requires β -Ser108 phosphorylation (Hawley et al., 2012). However, high-fat-fed wild-type and AMPK $\beta 1$ knockout mice treated with salicylate showed improved glucose tolerance, reduced fasting glucose and insulin levels, suggesting AMPK-independent activity (Hawley et al., 2012). Hence, even though salicylates seem promising as an AMPK-mediated anti-diabetic treatment, further research is required to determine the role of AMPK in these outcomes.

Resveratrol

Resveratrol (Fig. 7F) is a polyphenol found in red wine that has been shown to stimulate AMPK activity in many different cell lines (Dasgupta and Milbrandt, 2007; Park et al., 2007; Um et al., 2010). The mechanism by which resveratrol activates AMPK is thought to be attributed to an

increase in AMP levels due to inhibition of the mitochondrial F₁ ATPase (Gledhill et al., 2007; Hawley et al., 2010). Resveratrol treatment stimulates glucose uptake (Park et al., 2007) and mitochondrial biogenesis (Baur et al., 2006) in muscle cells, and reduces liver lipid accumulation (Zang et al., 2006). However, it is still unclear as to whether these effects are mediated through AMPK activation (Ruderman et al., 2010).

Conclusions

AMPK is an important protein involved in systemic energy homeostasis. It leads to the activation of catabolic pathways such as β -oxidation and autophagy and suppression of anabolic pathways (for example, fatty acid synthesis) in order to provide an alternate source of energy to the cell under stressful conditions. In conclusion, AMPK is a critical cellular regulators involved in both physiological and pathological conditions. Due to the role of AMPK in energy homeostasis, it is implicated in a number of disorders such as diabetes and cardiac hypertrophy, and is a promising therapeutic target. Indeed, the design of AMPK activators for therapeutic applications represents a thriving field for future research.

Acknowledgements

The authors appreciate comments for improvement of the manuscript prior to submission from Dr. Danuta Kalinowski, Dr. Zaklina Kovacevic, Dr. Patric Jansson, Dr. Darius Lane, Dr. Angelica Merlot and Dr. Vera Richardson.

Author Contribution

Wrote or contributed to the writing of the manuscript: Krishan, Richardson, and Sahni.

References

- Abate, N., Garg, A., Peshock, R., Stray-Gundersen, J., and Grundy, S. (1995). Relationships of generalized and regional adiposity to insulin sensitivity in men. *J Clin Invest*, **96**(1), 88.
- Anderson, K. A., Ribar, T. J., Lin, F., Noeldner, P. K., Green, M. F., Muehlbauer, M. J., Witters, L. A., Kemp, B. E., and Means, A. R. (2008). Hypothalamic CaMKK2 contributes to the regulation of energy balance. *Cell Metab*, **7**(5), 377-388.
- Arad, M., Moskowitz, I. P., Patel, V. V., Ahmad, F., Perez-Atayde, A. R., Sawyer, D. B., Walter, M., Li, G. H., Burgon, P. G., Maguire, C. T., Stapleton, D., Schmitt, J. P., Guo, X. X., Pizard, A., Kupersmidt, S., Roden, D. M., Berul, C. I., Seidman, C. E., and Seidman, J. G. (2003). Transgenic mice overexpressing mutant PRKAG2 define the cause of Wolff-Parkinson-White syndrome in glycogen storage cardiomyopathy. *Circulation*, **107**(22), 2850-2856.
- Baas, A. F., Boudeau, J., Sapkota, G. P., Smit, L., Medema, R., Morrice, N. A., Alessi, D. R., and Clevers, H. C. (2003). Activation of the tumour suppressor kinase LKB1 by the STE20-like pseudokinase STRAD. *EMBO J*, **22**(12), 3062-3072.
- Baas, A. F., Kuipers, J., van der Wel, N. N., Batlle, E., Koerten, H. K., Peters, P. J., and Clevers, H. C. (2004). Complete polarization of single intestinal epithelial cells upon activation of LKB1 by STRAD. *Cell*, **116**(3), 457-466.
- Banerjee, S. K., Ramani, R., Saba, S., Rager, J., Tian, R., Mathier, M. A., and Ahmad, F. (2007). A PRKAG2 mutation causes biphasic changes in myocardial AMPK activity and does not protect against ischemia. *Biochem Biophys Res Commun*, **360**(2), 381-387.
- Banno, A., and Ginsberg, M. H. (2008). Integrin activation. *Biochem Soc Trans*, **36**(Pt 2), 229.
- Bareford, M. D., Park, M. A., Yacoub, A., Hamed, H. A., Tang, Y., Cruickshanks, N., Eulitt, P., Hubbard, N., Tye, G., Burow, M. E., Fisher, P. B., Moran, R. G., Nephew, K. P., Grant, S., and Dent, P. (2014). Sorafenib enhances pemetrexed cytotoxicity through an autophagy-dependent mechanism in cancer cells. *Cancer Res*, **71**(14), 4955-4967.
- Bateman, A. (1997). The structure of a domain common to archaeobacteria and the homocystinuria disease protein. *Trends Biochem Sci*, **22**(1), 12-13.
- Baur, J. A., Pearson, K. J., Price, N. L., Jamieson, H. A., Lerin, C., Kalra, A., Prabhu, V. V., Allard, J. S., Lopez-Lluch, G., Lewis, K., Pistell, P. J., Poosala, S., Becker, K. G., Boss, O., Gwinn, D., Wang, M., Ramaswamy, S., Fishbein, K. W., Spencer, R. G., Lakatta, E. G., Le Couteur, D., Shaw, R. J., Navas, P., Puigserver, P., Ingram, D. K., de Cabo, R., and Sinclair, D. A. (2006). Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*, **444**(7117), 337-342.
- Beauchamp, E. M., and Platanias, L. C. (2013). The evolution of the TOR pathway and its role in cancer. *Oncogene*, **32**(34), 3923-3932.
- Ben Sahara, I., Laurent, K., Giuliano, S., Larbret, F., Ponzio, G., Gounon, P., Le Marchand-Brustel, Y., Giorgetti-Peraldi, S., Cormont, M., Bertolotto, C., Deckert, M., Auberger, P., Tanti, J. F., and Bost, F. (2010). Targeting cancer cell metabolism: the combination of metformin and 2-deoxyglucose induces p53-dependent apoptosis in prostate cancer cells. *Cancer Res*, **70**(6), 2465-2475.
- Bergeron, R., Previs, S. F., Cline, G. W., Perret, P., Russell, R. R., 3rd, Young, L. H., and Shulman, G. I. (2001). Effect of 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside infusion on in vivo glucose and lipid metabolism in lean and obese Zucker rats. *Diabetes*, **50**(5), 1076-1082.
- Berridge, M. J. (2001). The versatility and complexity of calcium signalling. *Novartis Found Symp*, 52-67.
- Bianchi, A., Evans, J. L., Iverson, A. J., Nordlund, A.-C., Watts, T. D., and Witters, L. (1990). Identification of an isozymic form of acetyl-CoA carboxylase. *J Biol Chem*, **265**(3), 1502-1509.
- Bianchi, C., Penno, G., Romero, F., Del Prato, S., and Miccoli, R. (2007). Treating the metabolic syndrome. *Expert Rev Cardiovasc Ther*, **5**(3), 491-506.
- Bonini, M. G., and Gantner, B. N. (2013). The multifaceted activities of AMPK in tumor progression--why the "one size fits all" definition does not fit at all? *IUBMB Life*, **65**(11), 889-896.
- Boudeau, J., Baas, A. F., Deak, M., Morrice, N. A., Kieloch, A., Schutkowski, M., Prescott, A. R., Clevers, H. C., and Alessi, D. R. (2003). MO25alpha/beta interact with STRADalpha/beta enhancing their ability to bind, activate and localize LKB1 in the cytoplasm. *EMBO J*, **22**(19), 5102-5114.
- Bremer, J. (1963). Carnitine in Intermediary Metabolism: The biosynthesis of palmitylcarnitine by cell subfractions. *J Biol Chem*, **238**(8), 2774-2779.
- Brenman, J. E., and Temple, B. R. (2007). Opinion: alternative views of AMP-activated protein kinase. *Cell Biochem Biophys*, **47**(3), 321-331.

- Brown, K. A., Hunger, N. I., Docanto, M., and Simpson, E. R. (2010). Metformin inhibits aromatase expression in human breast adipose stromal cells via stimulation of AMP-activated protein kinase. *Breast Cancer Res Treat*, **123**(2), 591-596.
- Brunmair, B., Staniek, K., Gras, F., Scharf, N., Althaym, A., Clara, R., Roden, M., Gnaiger, E., Nohl, H., Waldhausl, W., and Fornsinn, C. (2004). Thiazolidinediones, like metformin, inhibit respiratory complex I: a common mechanism contributing to their antidiabetic actions? *Diabetes*, **53**(4), 1052-1059.
- Carling, D., Clarke, P. R., Zammit, V. A., and Hardie, D. G. (1989). Purification and characterization of the AMP-activated protein kinase. *Eur J Biochem*, **186**(1-2), 129-136.
- Carling, D., Aguan, K., Woods, A., Verhoeven, A., Beri, R. K., Brennan, C. H., Sidebottom, C., Davison, M. D., and Scott, J. (1994). Mammalian AMP-activated protein kinase is homologous to yeast and plant protein kinases involved in the regulation of carbon metabolism. *J Biol Chem*, **269**(15), 11442-11448.
- Carling, D., Sanders, M., and Woods, A. (2008). The regulation of AMP-activated protein kinase by upstream kinases. *Int J Obesity*, **32**, S55-S59.
- Cartee, G. D., and Wojtaszewski, J. F. (2007). Role of Akt substrate of 160 kDa in insulin-stimulated and contraction-stimulated glucose transport. *Appl Physiol Nutr Metab*, **32**(3), 557-566.
- Chen, J. L., Merl, D., Peterson, C. W., Wu, J., Liu, P. Y., Yin, H., Muoio, D. M., Ayer, D. E., West, M., and Chi, J. T. (2010). Lactic acidosis triggers starvation response with paradoxical induction of TXNIP through MondoA. *PLoS Genet*, **6**(9), e1001093.
- Chen, L., Jiao, Z.-H., Zheng, L.-S., Zhang, Y.-Y., Xie, S.-T., Wang, Z.-X., and Wu, J.-W. (2009). Structural insight into the autoinhibition mechanism of AMP-activated protein kinase. *Nature*, **459**(7250), 1146-1149.
- Chen, L., Wang, J., Zhang, Y. Y., Yan, S. F., Neumann, D., Schlattner, U., Wang, Z. X., and Wu, J. W. (2012). AMP-activated protein kinase undergoes nucleotide-dependent conformational changes. *Nat Struct Mol Biol*, **19**(7), 716-718.
- Chen, L., Xin, F. J., Wang, J., Hu, J., Zhang, Y. Y., Wan, S., Cao, L. S., Lu, C., Li, P., Yan, S. F., Neumann, D., Schlattner, U., Xia, B., Wang, Z. X., and Wu, J. W. (2013). Conserved regulatory elements in AMPK. *Nature*, **498**(7453), E8-10.
- Chen, S., Murphy, J., Toth, R., Campbell, D. G., Morrice, N. A., and Mackintosh, C. (2008). Complementary regulation of TBC1D1 and AS160 by growth factors, insulin and AMPK activators. *Biochem J*, **409**(2), 449-459.
- Chen, Z., Heierhorst, J., Mann, R. J., Mitchelhill, K. I., Michell, B. J., Witters, L. A., Lynch, G. S., Kemp, B. E., and Stapleton, D. (1999). Expression of the AMP-activated protein kinase beta1 and beta2 subunits in skeletal muscle. *FEBS Lett*, **460**(2), 343-348.
- Chhipa, R. R., Wu, Y., and Ip, C. (2011). AMPK-mediated autophagy is a survival mechanism in androgen-dependent prostate cancer cells subjected to androgen deprivation and hypoxia. *Cell Signal*, **23**(9), 1466-1472.
- Chin, D., and Means, A. R. (2000). Calmodulin: a prototypical calcium sensor. *Trends Cell Biol*, **10**(8), 322-328.
- Choi, K. S. (2012). Autophagy and cancer. *Exp Mol Med*, **44**(2), 109-120.
- Chou, S. Y., Lee, Y. C., Chen, H. M., Chiang, M. C., Lai, H. L., Chang, H. H., Wu, Y. C., Sun, C. N., Chien, C. L., Lin, Y. S., Wang, S. C., Tung, Y. Y., Chang, C., and Chern, Y. (2005). CGS21680 attenuates symptoms of Huntington's disease in a transgenic mouse model. *J Neurochem*, **93**(2), 310-320.
- Collins, S., Reoma, J., Gamm, D., and Uhler, M. (2000). LKB1, a novel serine/threonine protein kinase and potential tumour suppressor, is phosphorylated by cAMP-dependent protein kinase (PKA) and prenylated in vivo. *Biochem J*, **345**, 673-680.
- Corton, J. M., Gillespie, J. G., Hawley, S. A., and Hardie, D. G. (1995). 5-aminoimidazole-4-carboxamide ribonucleoside. A specific method for activating AMP-activated protein kinase in intact cells? *Eur J Biochem*, **229**(2), 558-565.
- Coughlan, K. A., Valentine, R. J., Ruderman, N. B., and Saha, A. K. (2014). AMPK activation: a therapeutic target for type 2 diabetes? *Diabetes Metab Syndr Obes*, **7**, 241-253.
- Cushman, S., Goodyear, L., Pilch, P., Ralston, E., Galbo, H., Ploug, T., Kristiansen, S. r., and Klip, A. (1998). Molecular mechanisms involved in GLUT4 translocation in muscle during insulin and contraction stimulation. *Adv Exp Med Biol*, **441**, 63-71.
- Dasgupta, B., and Milbrandt, J. (2007). Resveratrol stimulates AMP kinase activity in neurons. *Proc Natl Acad Sci U S A*, **104**(17), 7217-7222.

- Dasgupta, B., and Milbrandt, J. (2009). AMP-activated protein kinase phosphorylates retinoblastoma protein to control mammalian brain development. *Dev Cell*, **16**(2), 256-270.
- Dasgupta, B., Ju, J. S., Sasaki, Y., Liu, X., Jung, S. R., Higashida, K., Lindquist, D., and Milbrandt, J. (2012). The AMPK beta2 subunit is required for energy homeostasis during metabolic stress. *Mol Cell Biol*, **32**(14), 2837-2848.
- Davies, S. P., Carling, D., and Hardie, D. G. (1989). Tissue distribution of the AMP-activated protein kinase, and lack of activation by cyclic-AMP-dependent protein kinase, studied using a specific and sensitive peptide assay. *Eur J Biochem*, **186**(1-2), 123-128.
- Davies, S. P., Helps, N. R., Cohen, P. T., and Hardie, D. G. (1995). 5'-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP-activated protein kinase. Studies using bacterially expressed human protein phosphatase-2C alpha and native bovine protein phosphatase-2AC. *FEBS Lett*, **377**(3), 421-425.
- Degenhardt, K., Mathew, R., Beaudoin, B., Bray, K., Anderson, D., Chen, G., Mukherjee, C., Shi, Y., Gelinas, C. I., Fan, Y., Nelson, D. A., Jin, S., and White, E. (2006). Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. *Cancer Cell*, **10**(1), 51-64.
- Diamanti-Kandarakis, E., Economou, F., Palimeri, S., and Christakou, C. (2010). Metformin in polycystic ovary syndrome. *Ann N Y Acad Sci*, **1205**, 192-198.
- Dragestein, K. A., van Cappellen, W. A., van Haren, J., Tsibidis, G. D., Akhmanova, A., Knoch, T. A., Grosveld, F., and Galjart, N. (2008). Dynamic behavior of GFP-CLIP-170 reveals fast protein turnover on microtubule plus ends. *J Cell Biol*, **180**(4), 729-737.
- Duncan, L. J., and Seaton, D. A. (1962). The treatment of diabetes mellitus with metformin. *Br J Clin Pract*, **16**, 129-132.
- Dzamko, N., van Denderen, B. J., Hevener, A. L., Jorgensen, S. B., Honeyman, J., Galic, S., Chen, Z. P., Watt, M. J., Campbell, D. J., Steinberg, G. R., and Kemp, B. E. (2010). AMPK beta1 deletion reduces appetite, preventing obesity and hepatic insulin resistance. *J Biol Chem*, **285**(1), 115-122.
- Eaton, S., Bartlett, K., and Pourfarzam, M. (1996). Mammalian mitochondrial beta-oxidation. *Biochem J*, **320**, 345-357.
- Egan, D. F., Shackelford, D. B., Mihaylova, M. M., Gelino, S., Kohnz, R. A., Mair, W., Vasquez, D. S., Joshi, A., Gwinn, D. M., Taylor, R., Asara, J. M., Fitzpatrick, J., Dillin, A., Viollet, B., Kundu, M., Hansen, M., and Shaw, R. J. (2011). Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science*, **331**(6016), 456-461.
- Faubert, B., Boily, G., Izreig, S., Griss, T., Samborska, B., Dong, Z., Dupuy, F., Chambers, C., Fuerth, B. J., Viollet, B., Mamer, O. A., Avizonis, D., DeBerardinis, R. J., Siegel, P. M., and Jones, R. G. (2013). AMPK is a negative regulator of the Warburg effect and suppresses tumor growth in vivo. *Cell Metab*, **17**(1), 113-124.
- Faubert, B., Vincent, E. E., Poffenberger, M. C., and Jones, R. G. (2014). The AMP-activated protein kinase (AMPK) and cancer: Many faces of a metabolic regulator. *Cancer Lett*, PMID 24486219
- Foretz, M., Guichard, C., Ferre, P., and Foulle, F. (1999). Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. *Proc Natl Acad Sci U S A*, **96**(22), 12737-12742.
- Foretz, M., Hebrard, S., Leclerc, J., Zarrinpashneh, E., Soty, M., Mithieux, G., Sakamoto, K., Andreelli, F., and Viollet, B. (2010). Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. *J Clin Invest*, **120**(7), 2355-2369.
- Frayn, K. N., Coppack, S. W., Humphreys, S. M., Clark, M. L., and Evans, R. D. (1993). Periprandial regulation of lipid metabolism in insulin-treated diabetes mellitus. *Metabolism*, **42**(4), 504-510.
- Fritz, I. B., and Yue, K. T. (1963). Long-chain carnitine acyltransferase and the role of acylcarnitine derivatives in the catalytic increase of fatty acid oxidation induced by carnitine. *J Lipid Res*, **4**(3), 279-288.
- Fullerton, M. D., Galic, S., Marcinko, K., Sikkema, S., Pulinilkunnil, T., Chen, Z. P., O'Neill, H. M., Ford, R. J., Palanivel, R., O'Brien, M., Hardie, D. G., Macaulay, S. L., Schertzer, J. D., Dyck, J. R., van Denderen, B. J., Kemp, B. E., and Steinberg, G. R. (2013). Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin. *Nat Med*, **19**(12), 1649-1654.
- Gan, R. Y., and Li, H. B. (2014). Recent Progress on Liver Kinase B1 (LKB1): Expression, Regulation, Downstream Signaling and Cancer Suppressive Function. *Int J Mol Sci*, **15**(9), 16698-16718.

- Gao, G., Fernandez, C. S., Stapleton, D., Auster, A. S., Widmer, J., Dyck, J. R., Kemp, B. E., and Witters, L. A. (1996). Non-catalytic beta- and gamma-subunit isoforms of the 5'-AMP-activated protein kinase. *J Biol Chem*, **271**(15), 8675-8681.
- Geiger, T. R., and Peeper, D. S. (2009). Metastasis mechanisms. *Biochim Biophys Acta*, **1796**(2), 293-308.
- Gingras, A.-C., Gygi, S. P., Raught, B., Polakiewicz, R. D., Abraham, R. T., Hoekstra, M. F., Aebersold, R., and Sonenberg, N. (1999). Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. *Genes Dev*, **13**(11), 1422-1437.
- Gledhill, J. R., Montgomery, M. G., Leslie, A. G., and Walker, J. E. (2007). Mechanism of inhibition of bovine F1-ATPase by resveratrol and related polyphenols. *Proc Natl Acad Sci U S A*, **104**(34), 13632-13637.
- Gollob, M. H., Green, M. S., Tang, A. S., Gollob, T., Karibe, A., Ali Hassan, A. S., Ahmad, F., Lozado, R., Shah, G., Fananapazir, L., Bachinski, L. L., and Roberts, R. (2001a). Identification of a gene responsible for familial Wolff-Parkinson-White syndrome. *N Engl J Med*, **344**(24), 1823-1831.
- Gollob, M. H., Seger, J. J., Gollob, T. N., Tapscott, T., Gonzales, O., Bachinski, L., and Roberts, R. (2001b). Novel PRKAG2 mutation responsible for the genetic syndrome of ventricular preexcitation and conduction system disease with childhood onset and absence of cardiac hypertrophy. *Circulation*, **104**(25), 3030-3033.
- Gollob, M. H., and Roberts, R. (2002). AMP-activated protein kinase and familial Wolff-Parkinson-White syndrome: new perspectives on heart development and arrhythmogenesis. *Eur Heart J*, **23**(9), 679-681.
- Gollob, M. H. (2003). Glycogen storage disease as a unifying mechanism of disease in the PRKAG2 cardiac syndrome. *Biochem Soc Trans*, **31**(Pt 1), 228-231.
- Goodwin, P. J., Stambolic, V., Lemieux, J., Chen, B. E., Parulekar, W. R., Gelmon, K. A., Hershman, D. L., Hobday, T. J., Ligibel, J. A., Mayer, I. A., Pritchard, K. I., Whelan, T. J., Rastogi, P., and Shepherd, L. E. (2011). Evaluation of metformin in early breast cancer: a modification of the traditional paradigm for clinical testing of anti-cancer agents. *Breast Cancer Res Treat*, **126**(1), 215-220.
- Gowans, G. J., Hawley, S. A., Ross, F. A., and Hardie, D. G. (2013). AMP is a true physiological regulator of AMP-activated protein kinase by both allosteric activation and enhancing net phosphorylation. *Cell Metab*, **18**(4), 556-566.
- Greco, S. J., Sarkar, S., Johnston, J. M., and Tezapsidis, N. (2009). Leptin regulates tau phosphorylation and amyloid through AMPK in neuronal cells. *Biochem Biophys Res Commun*, **380**(1), 98-104.
- Green, M. F., Anderson, K. A., and Means, A. R. (2011). Characterization of the CaMKKbeta-AMPK signaling complex. *Cell Signal*, **23**(12), 2005-2012.
- Guertin, D. A., and Sabatini, D. M. (2007). Defining the role of mTOR in cancer. *Cancer Cell*, **12**(1), 9-22.
- Gwinn, D. M., Shackelford, D. B., Egan, D. F., Mihaylova, M. M., Mery, A., Vasquez, D. S., Turk, B. E., and Shaw, R. J. (2008). AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell*, **30**(2), 214-226.
- Habegger, K. M., Hoffman, N. J., Ridenour, C. M., Brozinick, J. T., and Elmendorf, J. S. (2012). AMPK enhances insulin-stimulated GLUT4 regulation via lowering membrane cholesterol. *Endocrinology*, **153**(5), 2130-2141.
- Hadad, S., Iwamoto, T., Jordan, L., Purdie, C., Bray, S., Baker, L., Jellema, G., Deharo, S., Hardie, D. G., Pusztai, L., Moulder-Thompson, S., Dewar, J. A., and Thompson, A. M. (2011). Evidence for biological effects of metformin in operable breast cancer: a pre-operative, window-of-opportunity, randomized trial. *Breast Cancer Res Treat*, **128**(3), 783-794.
- Haghighat, A., Mader, S., Pause, A., and Sonenberg, N. (1995). Repression of cap-dependent translation by 4E-binding protein 1: competition with p220 for binding to eukaryotic initiation factor-4E. *EMBO J*, **14**(22), 5701-5709.
- Halford, N. G., Hey, S., Jhurrea, D., Laurie, S., McKibbin, R. S., Paul, M., and Zhang, Y. (2003). Metabolic signalling and carbon partitioning: role of Snf1-related (SnRK1) protein kinase. *J Exp Bot*, **54**(382), 467-475.
- Hardie, D. G. (1989). Regulation of fatty acid synthesis via phosphorylation of acetyl-CoA carboxylase. *Prog Lipid Res*, **28**(2), 117-146.
- Hardie, D. G., and Pan, D. A. (2002). Regulation of fatty acid synthesis and oxidation by the AMP-activated protein kinase. *Biochem Soc Trans*, **30**(Pt 6), 1064-1070.
- Hardie, D. G. (2007). AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat Rev Mol Cell Biol*, **8**(10), 774-785.

- Hardie, D. G. (2011). AMP-activated protein kinase: a cellular energy sensor with a key role in metabolic disorders and in cancer. *Biochem Soc Trans*, **39**(1), 1-13.
- Hardie, D. G. (2013). AMPK: a target for drugs and natural products with effects on both diabetes and cancer. *Diabetes*, **62**(7), 2164-2172.
- Harms, L., Meierkord, H., Timm, G., Pfeiffer, L., and Ludolph, A. C. (1997). Decreased N-acetyl-aspartate/choline ratio and increased lactate in the frontal lobe of patients with Huntington's disease: a proton magnetic resonance spectroscopy study. *J Neurol Neurosurg Psychiatry*, **62**(1), 27-30.
- Hawley, S. A., Boudeau, J., Reid, J. L., Mustard, K. J., Udd, L., Makela, T. P., Alessi, D. R., and Hardie, D. G. (2003). Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. *J Biol*, **2**(4), 28.
- Hawley, S. A., Pan, D. A., Mustard, K. J., Ross, L., Bain, J., Edelman, A. M., Frenguelli, B. G., and Hardie, D. G. (2005). Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metab*, **2**(1), 9-19.
- Hawley, S. A., Ross, F. A., Chevtzoff, C., Green, K. A., Evans, A., Fogarty, S., Towler, M. C., Brown, L. J., Ogunbayo, O. A., Evans, A. M., and Hardie, D. G. (2010). Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. *Cell Metab*, **11**(6), 554-565.
- Hawley, S. A., Fullerton, M. D., Ross, F. A., Schertzer, J. D., Chevtzoff, C., Walker, K. J., Pegg, M. W., Zibrova, D., Green, K. A., Mustard, K. J., Kemp, B. E., Sakamoto, K., Steinberg, G. R., and Hardie, D. G. (2012). The ancient drug salicylate directly activates AMP-activated protein kinase. *Science*, **336**(6083), 918-922.
- Hemminki, A., Markie, D., Tomlinson, I., Avizienyte, E., Roth, S., Loukola, A., Bignell, G., Warren, W., Aminoff, M., Hoglund, P., Jarvinen, H., Kristo, P., Pelin, K., Ridanpaa, M., Salovaara, R., Toro, T., Bodmer, W., Olschwang, S., Olsen, A. S., Stratton, M. R., Chappelle, A. d. I., and Aaltonen, L. A. (1998). A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature*, **391**(6663), 184-187.
- Herrero-Martin, G., Hoyer-Hansen, M., Garcia-Garcia, C., Fumarola, C., Farkas, T., Lopez-Rivas, A., and Jaattela, M. (2009). TAK1 activates AMPK-dependent cytoprotective autophagy in TRAIL-treated epithelial cells. *EMBO J*, **28**(6), 677-685.
- Higgs, G. A., Salmon, J. A., Henderson, B., and Vane, J. R. (1987). Pharmacokinetics of aspirin and salicylate in relation to inhibition of arachidonate cyclooxygenase and antiinflammatory activity. *Proc Natl Acad Sci U S A*, **84**(5), 1417-1420.
- Ho, S. Y. (2008). Accessory atrioventricular pathways: getting to the origins. *Circulation*, **117**(12), 1502-1504.
- Holz, M. K., Ballif, B. A., Gygi, S. P., and Blenis, J. (2005). mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. *Cell*, **123**(4), 569-580.
- Horton, J. D., Goldstein, J. L., and Brown, M. S. (2002). SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest*, **109**(9), 1125-1131.
- Huang, X., Wullschleger, S., Shpiro, N., McGuire, V. A., Sakamoto, K., Woods, Y. L., McBurnie, W., Fleming, S., and Alessi, D. R. (2008). Important role of the LKB1-AMPK pathway in suppressing tumorigenesis in PTEN-deficient mice. *Biochem J*, **412**(2), 211-221.
- Hurley, R. L., Anderson, K. A., Franzone, J. M., Kemp, B. E., Means, A. R., and Witters, L. A. (2005). The Ca²⁺/calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. *J Biol Chem*, **280**(32), 29060-29066.
- Imanshahidi, M., and Hosseinzadeh, H. (2008). Pharmacological and therapeutic effects of *Berberis vulgaris* and its active constituent, berberine. *Phytother Res*, **22**(8), 999-1012.
- Inoki, K., Li, Y., Xu, T., and Guan, K. L. (2003). Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev*, **17**(15), 1829-1834.
- Inokuchi-Shimizu, S., Park, E. J., Roh, Y. S., Yang, L., Zhang, B., Song, J., Liang, S., Pimienta, M., Taniguchi, K., Wu, X., Asahina, K., Lagakos, W., Mackey, M. R., Akira, S., Ellisman, M. H., Sears, D. D., Olefsky, J. M., Karin, M., Brenner, D. A., and Seki, E. (2014). TAK1-mediated autophagy and fatty acid oxidation prevent hepatosteatosis and tumorigenesis. *J Clin Invest*, **124**(8), 3566-3578.
- Iseli, T. J., Walter, M., van Denderen, B. J., Katsis, F., Witters, L. A., Kemp, B. E., Mitchell, B. J., and Stapleton, D. (2005). AMP-activated protein kinase beta subunit tethers alpha and gamma subunits via its C-terminal sequence (186-270). *J Biol Chem*, **280**(14), 13395-13400.
- Janjetovic, K., Harhaji-Trajkovic, L., Misirkic-Marjanovic, M., Vucicevic, L., Stevanovic, D., Zogovic, N., Sumarac-Dumanovic, M., Micic, D., and Trajkovic, V. (2011a). In vitro and in vivo anti-melanoma action of metformin. *Eur J Pharmacol*, **668**(3), 373-382.

- Janjetovic, K., Vucicevic, L., Misirkic, M., Vilimanovich, U., Tovilovic, G., Zogovic, N., Nikolic, Z., Jovanovic, S., Bumbasirevic, V., Trajkovic, V., and Harhaji-Trajkovic, L. (2011b). Metformin reduces cisplatin-mediated apoptotic death of cancer cells through AMPK-independent activation of Akt. *Eur J Pharmacol*, **651**(1-3), 41-50.
- Jeon, S. M., Chandel, N. S., and Hay, N. (2012). AMPK regulates NADPH homeostasis to promote tumour cell survival during energy stress. *Nature*, **485**(7400), 661-665.
- Jeong, K. J., Kim, G. W., and Chung, S. H. (2014). AMP-activated protein kinase: An emerging target for ginseng. *J Ginseng Res*, **38**(2), 83-88.
- Jiang, P., Gan, M., Ebrahim, A. S., Castanedes-Casey, M., Dickson, D. W., and Yen, S. H. (2013). Adenosine monophosphate-activated protein kinase overactivation leads to accumulation of alpha-synuclein oligomers and decrease of neurites. *Neurobiol Aging*, **34**(5), 1504-1515.
- Jiang, R., and Carlson, M. (1996). Glucose regulates protein interactions within the yeast SNF1 protein kinase complex. *Genes Dev*, **10**(24), 3105-3115.
- Jiang, R., and Carlson, M. (1997). The Snf1 protein kinase and its activating subunit, Snf4, interact with distinct domains of the Sip1/Sip2/Gal83 component in the kinase complex. *Mol Cell Biol*, **17**(4), 2099-2106.
- Ju, T.-C., Chen, H.-M., Lin, J.-T., Chang, C.-P., Chang, W.-C., Kang, J.-J., Sun, C.-P., Tao, M.-H., Tu, P.-H., and Chang, C. (2011). Nuclear translocation of AMPK- α 1 potentiates striatal neurodegeneration in Huntington's disease. *J Cell Biol*, **194**(2), 209-227.
- Kahn, B. B., Alquier, T., Carling, D., and Hardie, D. G. (2005). AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metab*, **1**(1), 15-25.
- Kazgan, N., Williams, T., Forsberg, L. J., and Brenman, J. E. (2010). Identification of a nuclear export signal in the catalytic subunit of AMP-activated protein kinase. *Mol Biol Cell*, **21**(19), 3433-3442.
- Kemp, B. E., Oakhill, J. S., and Scott, J. W. (2007). AMPK structure and regulation from three angles. *Structure*, **15**(10), 1161-1163.
- Kim, D., Sarbassov, D. D., Ali, S. M., King, J. E., Latek, R. R., Erdjument-Bromage, H., Tempst, P., and Sabatini, D. M. (2002). mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell*, **110**(2), 163-175.
- Kim, H., Kim, M.-J., Kim, E. J., Yang, Y., Lee, M.-S., and Lim, J.-S. (2012a). Berberine-induced AMPK activation inhibits the metastatic potential of melanoma cells via reduction of ERK activity and COX-2 protein expression. *Biochem Pharmacol*, **83**(3), 385-394.
- Kim, J., Kundu, M., Viollet, B., and Guan, K. L. (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol*, **13**(2), 132-141.
- Kim, M., Hunter, R. W., Garcia-Menendez, L., Gong, G., Yang, Y. Y., Kolwicz, S. C., Jr., Xu, J., Sakamoto, K., Wang, W., and Tian, R. (2014). Mutation in the gamma2-subunit of AMP-activated protein kinase stimulates cardiomyocyte proliferation and hypertrophy independent of glycogen storage. *Circ Res*, **114**(6), 966-975.
- Kim, S. Y., Jeong, S., Jung, E., Baik, K.-H., Chang, M., Kim, S., Shim, J., Chun, E., and Lee, K. (2012b). AMP-activated protein kinase- α 1 as an activating kinase of TGF- β -activated kinase 1 has a key role in inflammatory signals. *Cell Death Dis*, **3**(7), e357.
- Koay, A., Woodcroft, B., Petrie, E. J., Yue, H., Emanuelle, S., Bieri, M., Bailey, M. F., Hargreaves, M., Park, J. T., Park, K. H., Ralph, S., Neumann, D., Stapleton, D., and Gooley, P. R. (2010). AMPK beta subunits display isoform specific affinities for carbohydrates. *FEBS Lett*, **584**(15), 3499-3503.
- Kong, W., Wei, J., Abidi, P., Lin, M., Inaba, S., Li, C., Wang, Y., Wang, Z., Si, S., and Pan, H. (2004). Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. *Nat Med*, **10**(12), 1344-1351.
- Kramer, H. F., Witzczak, C. A., Fujii, N., Jessen, N., Taylor, E. B., Arnolds, D. E., Sakamoto, K., Hirshman, M. F., and Goodyear, L. J. (2006). Distinct signals regulate AS160 phosphorylation in response to insulin, AICAR, and contraction in mouse skeletal muscle. *Diabetes*, **55**(7), 2067-2076.
- Krishan, S., Richardson, D. R., and Sahni, S. (2014). AMP kinase (PRKAA1). *J Clin Pathol*, **67**(9), 758-763.
- Kurth-Kraczek, E. J., Hirshman, M. F., Goodyear, L. J., and Winder, W. (1999). 5'AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle. *Diabetes*, **48**(8), 1667-1671.
- Landstrom, M. (2010). The TAK1-TRAF6 signalling pathway. *Int J Biochem Cell Biol*, **42**(5), 585-589.
- LeBrasseur, N. K., Kelly, M., Tsao, T. S., Farmer, S. R., Saha, A. K., Ruderman, N. B., and Tomas, E. (2006). Thiazolidinediones can rapidly activate AMP-activated protein kinase in mammalian tissues. *Am J Physiol Endocrinol Metab*, **291**(1), E175-181.

- Lee, M., Lee, H.-J., Seo, W. D., Park, K. H., and Lee, Y.-S. (2010). Sialylation of integrin β 1 is involved in radiation-induced adhesion and migration in human colon cancer cells. *Int J Radiat Oncol Biol Phys* **76**(5), 1528-1536.
- Lee, Y. S., Kim, W. S., Kim, K. H., Yoon, M. J., Cho, H. J., Shen, Y., Ye, J.-M., Lee, C. H., Oh, W. K., and Kim, C. T. (2006). Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states. *Diabetes*, **55**(8), 2256-2264.
- Legro, R. S., Barnhart, H. X., Schlaff, W. D., Carr, B. R., Diamond, M. P., Carson, S. A., Steinkampf, M. P., Coutifaris, C., McGovern, P. G., Cataldo, N. A., Gosman, G. G., Nestler, J. E., Giudice, L. C., Ewens, K. G., Spielman, R. S., Leppert, P. C., and Myers, E. R. (2008). Ovulatory response to treatment of polycystic ovary syndrome is associated with a polymorphism in the STK11 gene. *J Clin Endocrinol Metab*, **93**(3), 792-800.
- Li, Y., Xu, S., Mihaylova, M. M., Zheng, B., Hou, X., Jiang, B., Park, O., Luo, Z., Lefai, E., and Shyy, J. Y.-J. (2011). AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. *Cell Metab*, **13**(4), 376-388.
- Light, P. E. (2006). Familial Wolff-Parkinson-White Syndrome: a disease of glycogen storage or ion channel dysfunction? *J Cardiovasc Electrophysiol*, **17 Suppl 1**, S158-S161.
- Lim, M. A., Selak, M. A., Xiang, Z., Krainc, D., Neve, R. L., Kraemer, B. C., Watts, J. L., and Kalb, R. G. (2012). Reduced activity of AMP-activated protein kinase protects against genetic models of motor neuron disease. *J Neurosci*, **32**(3), 1123-1141.
- Liu, B., Fan, Z., Edgerton, S. M., Yang, X., Lind, S. E., and Thor, A. D. (2011). Potent anti-proliferative effects of metformin on trastuzumab-resistant breast cancer cells via inhibition of erbB2/IGF-1 receptor interactions. *Cell Cycle*, **10**(17), 2959-2966.
- Longnus, S. L., Wambolt, R. B., Parsons, H. L., Brownsey, R. W., and Allard, M. F. (2003). 5-Aminoimidazole-4-carboxamide 1-beta -D-ribofuranoside (AICAR) stimulates myocardial glycogenolysis by allosteric mechanisms. *Am J Physiol Regul Integr Comp Physiol*, **284**(4), R936-944.
- Lopez-Lopez, C., Dietrich, M. O., Metzger, F., Loetscher, H., and Torres-Aleman, I. (2007). Disturbed cross talk between insulin-like growth factor I and AMP-activated protein kinase as a possible cause of vascular dysfunction in the amyloid precursor protein/presenilin 2 mouse model of Alzheimer's disease. *J Neurosci*, **27**(4), 824-831.
- Luptak, I., Shen, M., He, H., Hirshman, M. F., Musi, N., Goodyear, L. J., Yan, J., Wakimoto, H., Morita, H., Arad, M., Seidman, C. E., Seidman, J. G., Ingwall, J. S., Balschi, J. A., and Tian, R. (2007). Aberrant activation of AMP-activated protein kinase remodels metabolic network in favor of cardiac glycogen storage. *J Clin Invest*, **117**(5), 1432-1439.
- Marcus, A. I., and Zhou, W. (2010). LKB1 regulated pathways in lung cancer invasion and metastasis. *J Thorac Oncol*, **5**(12), 1883-1886.
- Martin-Castillo, B., Dorca, J., Vazquez-Martin, A., Oliveras-Ferraro, C., Lopez-Bonet, E., Garcia, M., Del Barco, S., and Menendez, J. A. (2010). Incorporating the antidiabetic drug metformin in HER2-positive breast cancer treated with neo-adjuvant chemotherapy and trastuzumab: an ongoing clinical-translational research experience at the Catalan Institute of Oncology. *Ann Oncol*, **21**(1), 187-189.
- McBride, A., Ghilagaber, S., Nikolaev, A., and Hardie, D. G. (2009). The glycogen-binding domain on the AMPK beta subunit allows the kinase to act as a glycogen sensor. *Cell Metab*, **9**(1), 23-34.
- Menon, S., Dibble, C. C., Talbott, G., Hoxhaj, G., Valvezan, A. J., Takahashi, H., Cantley, L. C., and Manning, B. D. (2014). Spatial control of the TSC complex integrates insulin and nutrient regulation of mTORC1 at the lysosome. *Cell*, **156**(4), 771-785.
- Milakovic, T., and Johnson, G. V. (2005). Mitochondrial respiration and ATP production are significantly impaired in striatal cells expressing mutant huntingtin. *J Biol Chem*, **280**(35), 30773-30782.
- Miller, R. A., Chu, Q., Xie, J., Foretz, M., Viollet, B., and Birnbaum, M. J. (2013). Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. *Nature*, **494**(7436), 256-260.
- Minokoshi, Y., Alquier, T., Furukawa, N., Kim, Y.-B., Lee, A., Xue, B., Mu, J., Fofelle, F., Ferre, P., and Birnbaum, M. J. (2004). AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature*, **428**(6982), 569-574.
- Mitchell, K. I., Michell, B. J., House, C. M., Stapleton, D., Dyck, J., Gamble, J., Ullrich, C., Witters, L. A., and Kemp, B. E. (1997). Posttranslational modifications of the 5'-AMP-activated protein kinase beta1 subunit. *J Biol Chem*, **272**(39), 24475-24479.
- Momcilovic, M., Hong, S. P., and Carlson, M. (2006). Mammalian TAK1 activates Snf1 protein kinase in yeast and phosphorylates AMP-activated protein kinase in vitro. *J Biol Chem*, **281**(35), 25336-25343.

- Momcilovic, M., Iram, S. H., Liu, Y., and Carlson, M. (2008). Roles of the glycogen-binding domain and Snf4 in glucose inhibition of SNF1 protein kinase. *J Biol Chem*, **283**(28), 19521-19529.
- Motoshima, H., Goldstein, B. J., Igata, M., and Araki, E. (2006). AMPK and cell proliferation—AMPK as a therapeutic target for atherosclerosis and cancer. *The Journal of physiology*, **574**(1), 63-71.
- Muller-Decker, K., and Furstenberger, G. (2007). The cyclooxygenase-2-mediated prostaglandin signaling is causally related to epithelial carcinogenesis. *Mol Carcinog*, **46**(8), 705-710.
- Nakano, A., Kato, H., Watanabe, T., Min, K.-D., Yamazaki, S., Asano, Y., Seguchi, O., Higo, S., Shintani, Y., and Asanuma, H. (2010). AMPK controls the speed of microtubule polymerization and directional cell migration through CLIP-170 phosphorylation. *Nat Cell Biol*, **12**(6), 583-590.
- Ninomiya-Tsuji, J., Kishimoto, K., Hiyama, A., Inoue, J., Cao, Z., and Matsumoto, K. (1999). The kinase TAK1 can activate the NIK-I kappaB as well as the MAP kinase cascade in the IL-1 signalling pathway. *Nature*, **398**(6724), 252-256.
- O'Neill, H. M. (2013). AMPK and exercise: glucose uptake and insulin sensitivity. *Diabetes Metab J*, **37**(1), 1-21.
- Oakhill, J. S., Chen, Z.-P., Scott, J. W., Steel, R., Castelli, L. A., Ling, N., Macaulay, S. L., and Kemp, B. E. (2010). β -Subunit myristoylation is the gatekeeper for initiating metabolic stress sensing by AMP-activated protein kinase (AMPK). *Proc Natl Acad Sci U S A*, **107**(45), 19237-19241.
- Oakhill, J. S., Steel, R., Chen, Z. P., Scott, J. W., Ling, N., Tam, S., and Kemp, B. E. (2011). AMPK is a direct adenylate charge-regulated protein kinase. *Science*, **332**(6036), 1433-1435.
- Oakhill, J. S., Scott, J. W., and Kemp, B. E. (2012). AMPK functions as an adenylate charge-regulated protein kinase. *Trends Endocrinol Metab*, **23**(3), 125-132.
- Ota, T., Suzuki, Y., Nishikawa, T., Otsuki, T., Sugiyama, T., Irie, R., Wakamatsu, A., Hayashi, K., Sato, H., Nagai, K., Kimura, K., Makita, H., Sekine, M., Obayashi, M., Nishi, T., Shibahara, T., Tanaka, T., Ishii, S., Yamamoto, J., Saito, K., Kawai, Y., Isono, Y., Nakamura, Y., Nagahari, K., Murakami, K., Yasuda, T., Iwayanagi, T., Wagatsuma, M., Shiratori, A., Sudo, H., Hosoiri, T., Kaku, Y., Kodaira, H., Kondo, H., Sugawara, M., Takahashi, M., Kanda, K., Yokoi, T., Furuya, T., Kikkawa, E., Omura, Y., Abe, K., Kamihara, K., Katsuta, N., Sato, K., Tanikawa, M., Yamazaki, M., Ninomiya, K., Ishibashi, T., Yamashita, H., Murakawa, K., Fujimori, K., Tanai, H., Kimata, M., Watanabe, M., Hiraoka, S., Chiba, Y., Ishida, S., Ono, Y., Takiguchi, S., Watanabe, S., Yosida, M., Hotuta, T., Kusano, J., Kanehori, K., Takahashi-Fujii, A., Hara, H., Tanase, T. O., Nomura, Y., Togiya, S., Komai, F., Hara, R., Takeuchi, K., Arita, M., Imose, N., Musashino, K., Yuuki, H., Oshima, A., Sasaki, N., Aotsuka, S., Yoshikawa, Y., Matsunawa, H., Ichihara, T., Shiohata, N., Sano, S., Moriya, S., Momiyama, H., Satoh, N., Takami, S., Terashima, Y., Suzuki, O., Nakagawa, S., Senoh, A., Mizoguchi, H., Goto, Y., Shimizu, F., Wakebe, H., Hishigaki, H., Watanabe, T., Sugiyama, A., Takemoto, M., Kawakami, B., Watanabe, K., Kumagai, A., Itakura, S., Fukuzumi, Y., Fujimori, Y., Komiyama, M., Tashiro, H., Tanigami, A., Fujiwara, T., Ono, T., Yamada, K., Fujii, Y., Ozaki, K., Hirao, M., Ohmori, Y., Kawabata, A., Hikiji, T., Kobatake, N., Inagaki, H., Ikema, Y., Okamoto, S., Okitani, R., Kawakami, T., Noguchi, S., Itoh, T., Shigeta, K., Senba, T., Matsumura, K., Nakajima, Y., Mizuno, T., Morinaga, M., Sasaki, M., Togashi, T., Oyama, M., Hata, H., Komatsu, T., Mizushima-Sugano, J., Satoh, T., Shirai, Y., Takahashi, Y., Nakagawa, K., Okumura, K., Nagase, T., Nomura, N., Kikuchi, H., Masuho, Y., Yamashita, R., Nakai, K., Yada, T., Ohara, O., Isogai, T., and Sugano, S. (2004). Complete sequencing and characterization of 21,243 full-length human cDNAs. *Nat Genet*, **36**(1), 40-45.
- Owen, M., DORAN, E., and Halestrap, A. (2000). Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *Biochem J*, **348**, 607-614.
- Panov, A. V., Gutekunst, C.-A., Leavitt, B. R., Hayden, M. R., Burke, J. R., Strittmatter, W. J., and Greenamyre, J. T. (2002). Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. *Nat Neurosci*, **5**(8), 731-736.
- Papanas, N., Maltezos, E., and Mikhailidis, D. P. (2010). Metformin and cancer: licence to heal? *Expert Opin Investig Drugs*, **19**(8), 913-917.
- Park, C. E., Kim, M. J., Lee, J. H., Min, B. I., Bae, H., Choe, W., Kim, S. S., and Ha, J. (2007). Resveratrol stimulates glucose transport in C2C12 myotubes by activating AMP-activated protein kinase. *Exp Mol Med*, **39**(2), 222-229.
- Park, J.-J., Seo, S.-M., Kim, E. J., Lee, Y.-J., Ko, Y.-G., Ha, J., and Lee, M. (2012). Berberine inhibits human colon cancer cell migration via AMP-activated protein kinase-mediated downregulation of integrin β 1 signaling. *Biochem Biophys Res Commun*, **426**(4), 461-467.
- Pehmoller, C., Treebak, J. T., Birk, J. B., Chen, S., Mackintosh, C., Hardie, D. G., Richter, E. A., and Wojtaszewski, J. F. (2009). Genetic disruption of AMPK signaling abolishes both contraction- and

- insulin-stimulated TBC1D1 phosphorylation and 14-3-3 binding in mouse skeletal muscle. *Am J Physiol Endocrinol Metab*, **297**(3), E665-675.
- Polekhina, G., Gupta, A., van Denderen, B. J., Feil, S. C., Kemp, B. E., Stapleton, D., and Parker, M. W. (2005). Structural basis for glycogen recognition by AMP-activated protein kinase. *Structure*, **13**(10), 1453-1462.
- Premanath, M., Basavanagowdappa, H., Mahesh, M., and Suresh, M. (2014). Correlation of abdominal adiposity with components of metabolic syndrome, anthropometric parameters and Insulin resistance, in obese and non obese, diabetics and non diabetics: A cross sectional observational study. (Mysore Visceral Adiposity in Diabetes Study). *Indian J Endocrinol Metab*, **18**(5), 676-682.
- Racanelli, A. C., Rothbart, S. B., Heyer, C. L., and Moran, R. G. (2009). Therapeutics by cytotoxic metabolite accumulation: pemetrexed causes ZMP accumulation, AMPK activation, and mammalian target of rapamycin inhibition. *Cancer Res*, **69**(13), 5467-5474.
- Ramratnam, M., Sharma, R. K., D'Auria, S., Lee, S. J., Wang, D., Huang, X. Y. N., and Ahmad, F. (2014). Transgenic Knockdown of Cardiac Sodium/Glucose Cotransporter 1 (SGLT1) Attenuates PRKAG2 Cardiomyopathy, Whereas Transgenic Overexpression of Cardiac SGLT1 Causes Pathologic Hypertrophy and Dysfunction in Mice. *Journal of the American Heart Association*, **3**(4), e000899.
- Rattan, R., Giri, S., Hartmann, L. C., and Shridhar, V. (2011). Metformin attenuates ovarian cancer cell growth in an AMP-kinase dispensable manner. *J Cell Mol Med*, **15**(1), 166-178.
- Roach, W. G., Chavez, J. A., Miinea, C. P., and Lienhard, G. E. (2007). Substrate specificity and effect on GLUT4 translocation of the Rab GTPase-activating protein Tbc1d1. *Biochem J*, **403**(2), 353-358.
- Rozengurt, E., Sinnott-Smith, J., and Kisfalvi, K. (2010). Crosstalk between insulin/insulin-like growth factor-1 receptors and G protein-coupled receptor signaling systems: a novel target for the antidiabetic drug metformin in pancreatic cancer. *Clin Cancer Res*, **16**(9), 2505-2511.
- Ruan, Q., Lesort, M., MacDonald, M. E., and Johnson, G. V. (2004). Striatal cells from mutant huntingtin knock-in mice are selectively vulnerable to mitochondrial complex II inhibitor-induced cell death through a non-apoptotic pathway. *Hum Mol Genet*, **13**(7), 669-681.
- Ruderman, N. B., Xu, X. J., Nelson, L., Cacicedo, J. M., Saha, A. K., Lan, F., and Ido, Y. (2010). AMPK and SIRT1: a long-standing partnership? *Am J Physiol Endocrinol Metab*, **298**(4), E751-760.
- Ruderman, N. B., Carling, D., Prentki, M., and Cacicedo, J. M. (2013). AMPK, insulin resistance, and the metabolic syndrome. *J Clin Invest*, **123**(7), 2764-2772.
- Russo, G. L., Russo, M., and Ungaro, P. (2013). AMP-activated protein kinase: a target for old drugs against diabetes and cancer. *Biochem Pharmacol*, **86**(3), 339-350.
- Sahni, S., Merlot, A. M., Krishan, S., Jansson, P. J., and Richardson, D. R. (2014). Gene of the month: BECN1. *J Clin Pathol*, **67**(8), 656-660.
- Salt, I., Celler, J., Hawley, S., Prescott, A., Woods, A., Carling, D., and Hardie, D. (1998). AMP-activated protein kinase: greater AMP dependence, and preferential nuclear localization, of complexes containing the $\alpha 2$ isoform. *Biochem J*, **334**, 177-187.
- Sanders, M. J., Grondin, P. O., Hegarty, B. D., Snowden, M. A., and Carling, D. (2007). Investigating the mechanism for AMP activation of the AMP-activated protein kinase cascade. *Biochem J*, **403**(1), 139-148.
- Sano, H., Kane, S., Sano, E., Miinea, C. P., Asara, J. M., Lane, W. S., Garner, C. W., and Lienhard, G. E. (2003). Insulin-stimulated phosphorylation of a Rab GTPase-activating protein regulates GLUT4 translocation. *J Biol Chem*, **278**(17), 14599-14602.
- Sapkota, G. P., Kieloch, A., Lizcano, J. M., Lain, S., Arthur, J. S., Williams, M. R., Morrice, N., Deak, M., and Alessi, D. R. (2001). Phosphorylation of the protein kinase mutated in Peutz-Jeghers cancer syndrome, LKB1/STK11, at Ser431 by p90(RSK) and cAMP-dependent protein kinase, but not its farnesylation at Cys(433), is essential for LKB1 to suppress cell growth. *J Biol Chem*, **276**(22), 19469-19482.
- Sapkota, G. P., Boudeau, J., Deak, M., Kieloch, A., Morrice, N., and Alessi, D. R. (2002a). Identification and characterization of four novel phosphorylation sites (Ser31, Ser325, Thr336 and Thr366) on LKB1/STK11, the protein kinase mutated in Peutz-Jeghers cancer syndrome. *Biochem J*, **362**(Pt 2), 481-490.
- Sapkota, G. P., Deak, M., Kieloch, A., Morrice, N., Goodarzi, A. A., Smythe, C., Shiloh, Y., Lees-Miller, S. P., and Alessi, D. R. (2002b). Ionizing radiation induces ataxia telangiectasia mutated kinase (ATM)-mediated phosphorylation of LKB1/STK11 at Thr-366. *Biochem J*, **368**(Pt 2), 507-516.
- Sawin, K. E., and Nurse, P. (1998). Regulation of cell polarity by microtubules in fission yeast. *J Cell Biol*, **142**(2), 457-471.

- Scholz, R., Sidler, C. L., Thali, R. F., Winssinger, N., Cheung, P. C., and Neumann, D. (2010). Autoactivation of transforming growth factor beta-activated kinase 1 is a sequential bimolecular process. *J Biol Chem*, **285**(33), 25753-25766.
- Shackelford, D. B., and Shaw, R. J. (2009). The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. *Nat Rev Cancer*, **9**(8), 563-575.
- Shaw, R. J. (2009). LKB1 and AMP-activated protein kinase control of mTOR signalling and growth. *Acta Physiol (Oxf)*, **196**(1), 65-80.
- Shim, J. H., Xiao, C., Paschal, A. E., Bailey, S. T., Rao, P., Hayden, M. S., Lee, K. Y., Bussey, C., Steckel, M., Tanaka, N., Yamada, G., Akira, S., Matsumoto, K., and Ghosh, S. (2005). TAK1, but not TAB1 or TAB2, plays an essential role in multiple signaling pathways in vivo. *Genes Dev*, **19**(22), 2668-2681.
- Sidhu, J. S., Rajawat, Y. S., Rami, T. G., Gollob, M. H., Wang, Z., Yuan, R., Marian, A. J., DeMayo, F. J., Weillbacher, D., Taffet, G. E., Davies, J. K., Carling, D., Khoury, D. S., and Roberts, R. (2005). Transgenic mouse model of ventricular preexcitation and atrioventricular reentrant tachycardia induced by an AMP-activated protein kinase loss-of-function mutation responsible for Wolff-Parkinson-White syndrome. *Circulation*, **111**(1), 21-29.
- Song, P., Xie, Z., Wu, Y., Xu, J., Dong, Y., and Zou, M. H. (2008). Protein kinase C ζ -dependent LKB1 serine 428 phosphorylation increases LKB1 nucleus export and apoptosis in endothelial cells. *J Biol Chem*, **283**(18), 12446-12455.
- Song, X. M., Fiedler, M., Galuska, D., Ryder, J. W., Fernstrom, M., Chibalin, A. V., Wallberg-Henriksson, H., and Zierath, J. R. (2002). 5-Aminoimidazole-4-carboxamide ribonucleoside treatment improves glucose homeostasis in insulin-resistant diabetic (ob/ob) mice. *Diabetologia*, **45**(1), 56-65.
- Srivastava, R. A. K., Pinkosky, S. L., Filippov, S., Hanselman, J. C., Cramer, C. T., and Newton, R. S. (2012). AMP-activated protein kinase: an emerging drug target to regulate imbalances in lipid and carbohydrate metabolism to treat cardio-metabolic diseases Thematic Review Series: New Lipid and Lipoprotein Targets for the Treatment of Cardiometabolic Diseases. *J Lipid Res*, **53**(12), 2490-2514.
- Steinberg, G. R., and Kemp, B. E. (2009). AMPK in Health and Disease. *Physiol Rev*, **89**(3), 1025-1078.
- Steinberg, G. R., O'Neill, H. M., Dzamko, N. L., Galic, S., Naim, T., Koopman, R., Jorgensen, S. B., Honeyman, J., Hewitt, K., Chen, Z. P., Schertzer, J. D., Scott, J. W., Koentgen, F., Lynch, G. S., Watt, M. J., van Denderen, B. J., Campbell, D. J., and Kemp, B. E. (2010). Whole body deletion of AMP-activated protein kinase β 2 reduces muscle AMPK activity and exercise capacity. *J Biol Chem*, **285**(48), 37198-37209.
- Strausberg, R. L., Feingold, E. A., Grouse, L. H., Derge, J. G., Klausner, R. D., Collins, F. S., Wagner, L., Shenmen, C. M., Schuler, G. D., Altschul, S. F., Zeeberg, B., Buetow, K. H., Schaefer, C. F., Bhat, N. K., Hopkins, R. F., Jordan, H., Moore, T., Max, S. I., Wang, J., Hsieh, F., Diatchenko, L., Marusina, K., Farmer, A. A., Rubin, G. M., Hong, L., Stapleton, M., Soares, M. B., Bonaldo, M. F., Casavant, T. L., Scheetz, T. E., Brownstein, M. J., Usdin, T. B., Toshiyuki, S., Carninci, P., Prange, C., Raha, S. S., Loquellano, N. A., Peters, G. J., Abramson, R. D., Mullahy, S. J., Bosak, S. A., McEwan, P. J., McKernan, K. J., Malek, J. A., Gunaratne, P. H., Richards, S., Worley, K. C., Hale, S., Garcia, A. M., Gay, L. J., Hulyk, S. W., Villalón, D. K., Muzny, D. M., Sodergren, E. J., Lu, X., Gibbs, R. A., Fahey, J., Helton, E., Kettman, M., Madan, A., Rodrigues, S., Sanchez, A., Whiting, M., Young, A. C., Shevchenko, Y., Bouffard, G. G., Blakesley, R. W., Touchman, J. W., Green, E. D., Dickson, M. C., Rodriguez, A. C., Grimwood, J., Schmutz, J., Myers, R. M., Butterfield, Y. S., Krzywinski, M. I., Skalska, U., Smailus, D. E., Schnerch, A., Schein, J. E., Jones, S. J., and Marra, M. A. (2002). Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. *Proc Natl Acad Sci U S A*, **99**(26), 16899-16903.
- Sullivan, J. E., Brocklehurst, K. J., Marley, A. E., Carey, F., Carling, D., and Beri, R. K. (1994). Inhibition of lipolysis and lipogenesis in isolated rat adipocytes with AICAR, a cell-permeable activator of AMP-activated protein kinase. *FEBS Lett*, **353**(1), 33-36.
- Suter, M., Riek, U., Tuerk, R., Schlattner, U., Wallimann, T., and Neumann, D. (2006). Dissecting the role of 5'-AMP for allosteric stimulation, activation, and deactivation of AMP-activated protein kinase. *J Biol Chem*, **281**(43), 32207-32216.
- Taliaferro-Smith, L., Nagalingam, A., Zhong, D., Zhou, W., Saxena, N., and Sharma, D. (2009). LKB1 is required for adiponectin-mediated modulation of AMPK-S6K axis and inhibition of migration and invasion of breast cancer cells. *Oncogene*, **28**(29), 2621-2633.
- Thampy, K. (1989). Formation of malonyl coenzyme A in rat heart. Identification and purification of an isozyme of A carboxylase from rat heart. *J Biol Chem*, **264**(30), 17631-17634.

- Thiery, J. P. (2003). Epithelial-mesenchymal transitions in development and pathologies. *Curr Opin Cell Biol*, **15**(6), 740-746.
- Thornton, C., Snowden, M. A., and Carling, D. (1998). Identification of a novel AMP-activated protein kinase beta subunit isoform that is highly expressed in skeletal muscle. *J Biol Chem*, **273**(20), 12443-12450.
- Thornton, C., Bright, N., Sastre, M., Muckett, P., and Carling, D. (2011). AMP-activated protein kinase (AMPK) is a tau kinase, activated in response to amyloid beta-peptide exposure. *Biochem J*, **434**, 503-512.
- Tillhon, M., Guamn Ortiz, L. M., Lombardi, P., and Scovassi, A. I. (2012). Berberine: new perspectives for old remedies. *Biochem Pharmacol*, **84**(10), 1260-1267.
- Tosca, L., Rame, C., Chabrolle, C., Tesseraud, S., and Dupont, J. (2010). Metformin decreases IGF1-induced cell proliferation and protein synthesis through AMP-activated protein kinase in cultured bovine granulosa cells. *Reproduction*, **139**(2), 409-418.
- Turner, N., Li, J.-Y., Gosby, A., To, S. W., Cheng, Z., Miyoshi, H., Taketo, M. M., Cooney, G. J., Kraegen, E. W., and James, D. E. (2008). Berberine and Its More biologically available derivative, dihydroberberine, inhibit mitochondrial respiratory complex IA mechanism for the action of berberine to activate AMP-activated protein kinase and improve insulin action. *Diabetes*, **57**(5), 1414-1418.
- Um, J. H., Park, S. J., Kang, H., Yang, S., Foretz, M., McBurney, M. W., Kim, M. K., Viollet, B., and Chung, J. H. (2010). AMP-activated protein kinase-deficient mice are resistant to the metabolic effects of resveratrol. *Diabetes*, **59**(3), 554-563.
- Vingtdeux, V. r., Davies, P., Dickson, D. W., and Marambaud, P. (2011). AMPK is abnormally activated in tangle-and pre-tangle-bearing neurons in Alzheimer's disease and other tauopathies. *Acta Neuropathol (Berl)*, **121**(3), 337-349.
- Viollet, B., Guigas, B., Sanz Garcia, N., Leclerc, J., Foretz, M., and Andreelli, F. (2012). Cellular and molecular mechanisms of metformin: an overview. *Clin Sci (Lond)*, **122**(6), 253-270.
- Wakil, S. J., Titchener, E. B., and Gibson, D. M. (1958). Evidence for the participation of biotin in the enzymic synthesis of fatty acids. *Biochim Biophys Acta*, **29**(1), 225-226.
- Wakil, S. J., Stoops, J. K., and Joshi, V. C. (1983). Fatty acid synthesis and its regulation. *Annu Rev Biochem*, **52**(1), 537-579.
- Wang, L., Di, L., and Noguchi, C. T. (2014). AMPK is involved in mediation of erythropoietin influence on metabolic activity and reactive oxygen species production in white adipocytes. *Int J Biochem Cell Biol*, **54**, 1-9.
- Warden, S., Richardson, C., O'DONNELL, J. J., Stapleton, D., Kemp, B., and Witters, L. (2001). Post-translational modifications of the $\beta 1$ subunit of AMP-activated protein kinase affect enzyme activity and cellular localization. *Biochem J*, **354**, 275-283.
- Winder, W. (2001). Energy-sensing and signaling by AMP-activated protein kinase in skeletal muscle. *J Appl Physiol*, **91**(3), 1017-1028.
- Woods, A., Johnstone, S. R., Dickerson, K., Leiper, F. C., Fryer, L. G., Neumann, D., Schlattner, U., Wallimann, T., Carlson, M., and Carling, D. (2003). LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr Biol*, **13**(22), 2004-2008.
- Woods, A., Dickerson, K., Heath, R., Hong, S.-P., Momcilovic, M., Johnstone, S. R., Carlson, M., and Carling, D. (2005). Ca^{2+} calmodulin-dependent protein kinase kinase- β acts upstream of AMP-activated protein kinase in mammalian cells. *Cell Metab*, **2**(1), 21-33.
- Wullschleger, S., Loewith, R., and Hall, M. N. (2006). TOR signaling in growth and metabolism. *Cell*, **124**(3), 471-484.
- Xiao, B., Heath, R., Saiu, P., Leiper, F. C., Leone, P., Jing, C., Walker, P. A., Haire, L., Eccleston, J. F., Davis, C. T., Martin, S. R., Carling, D., and Gamblin, S. J. (2007). Structural basis for AMP binding to mammalian AMP-activated protein kinase. *Nature*, **449**(7161), 496-500.
- Xiao, B., Sanders, M. J., Underwood, E., Heath, R., Mayer, F. V., Carmena, D., Jing, C., Walker, P. A., Eccleston, J. F., and Haire, L. F. (2011). Structure of mammalian AMPK and its regulation by ADP. *Nature*, **472**(7342), 230-233.
- Xiao, B., Sanders, M. J., Carmena, D., Bright, N. J., Haire, L. F., Underwood, E., Patel, B. R., Heath, R. B., Walker, P. A., Hallen, S., Giordanetto, F., Martin, S. R., Carling, D., and Gamblin, S. J. (2013). Structural basis of AMPK regulation by small molecule activators. *Nat Commun*, **4**, 3017.
- Xie, M., Zhang, D., Dyck, J. R., Li, Y., Zhang, H., Morishima, M., Mann, D. L., Taffet, G. E., Baldini, A., Khoury, D. S., and Schneider, M. D. (2006). A pivotal role for endogenous TGF-beta-activated kinase-1 in the LKB1/AMP-activated protein kinase energy-sensor pathway. *Proc Natl Acad Sci U S A*, **103**(46), 17378-17383.

- Xie, Z., Dong, Y., Zhang, J., Scholz, R., Neumann, D., and Zou, M. H. (2009). Identification of the serine 307 of LKB1 as a novel phosphorylation site essential for its nucleocytoplasmic transport and endothelial cell angiogenesis. *Mol Cell Biol*, **29**(13), 3582-3596.
- Xin, F. J., Wang, J., Zhao, R. Q., Wang, Z. X., and Wu, J. W. (2013). Coordinated regulation of AMPK activity by multiple elements in the alpha-subunit. *Cell Res*, **23**(10), 1237-1240.
- Yamaguchi, K., Shirakabe, K., Shibuya, H., Irie, K., Oishi, I., Ueno, N., Taniguchi, T., Nishida, E., and Matsumoto, K. (1995). Identification of a member of the MAPKKK family as a potential mediator of TGF-beta signal transduction. *Science*, **270**(5244), 2008-2011.
- Yin, J., Xing, H., and Ye, J. (2008). Efficacy of berberine in patients with type 2 diabetes mellitus. *Metabolism*, **57**(5), 712-717.
- Yoon, M. J., Lee, G. Y., Chung, J. J., Ahn, Y. H., Hong, S. H., and Kim, J. B. (2006). Adiponectin increases fatty acid oxidation in skeletal muscle cells by sequential activation of AMP-activated protein kinase, p38 mitogen-activated protein kinase, and peroxisome proliferator-activated receptor alpha. *Diabetes*, **55**(9), 2562-2570.
- Yu, X., McCorkle, S., Wang, M., Lee, Y., Li, J., Saha, A., Unger, R., and Ruderman, N. (2004). Leptinomimetic effects of the AMP kinase activator AICAR in leptin-resistant rats: prevention of diabetes and ectopic lipid deposition. *Diabetologia*, **47**(11), 2012-2021.
- Zakikhani, M., Dowling, R. J., Sonenberg, N., and Pollak, M. N. (2008). The effects of adiponectin and metformin on prostate and colon neoplasia involve activation of AMP-activated protein kinase. *Cancer Prev Res (Phila)*, **1**(5), 369-375.
- Zang, M., Xu, S., Maitland-Toolan, K. A., Zuccollo, A., Hou, X., Jiang, B., Wierzbicki, M., Verbeuren, T. J., and Cohen, R. A. (2006). Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice. *Diabetes*, **55**(8), 2180-2191.
- Zhang, B. L., Xu, R. L., Zhang, J., Zhao, X. X., Wu, H., Ma, L. P., Hu, J. Q., Zhang, J. L., Ye, Z., Zheng, X., and Qin, Y. W. (2013a). Identification and functional analysis of a novel PRKAG2 mutation responsible for Chinese PRKAG2 cardiac syndrome reveal an important role of non-CBS domains in regulating the AMPK pathway. *J Cardiol*, **62**(4), 241-248.
- Zhang, B. L., Ye, Z., Xu, R. L., You, X. H., Qin, Y. W., Wu, H., Cao, J., Zhang, J. L., Zheng, X., and Zhao, X. X. (2013b). Overexpression of G100S mutation in PRKAG2 causes Wolff-Parkinson-White syndrome in zebrafish. *Clin Genet*, **86**(3), 287-291.
- Zhang, C. S., Jiang, B., Li, M., Zhu, M., Peng, Y., Zhang, Y. L., Wu, Y. Q., Li, T. Y., Liang, Y., Lu, Z., Lian, G., Liu, Q., Guo, H., Yin, Z., Ye, Z., Han, J., Wu, J. W., Yin, H., Lin, S. Y., and Lin, S. C. (2014). The lysosomal v-ATPase-Ragulator complex is a common activator for AMPK and mTORC1, acting as a switch between catabolism and anabolism. *Cell Metab*, **20**(3), 526-540.
- Zheng, B., and Cantley, L. C. (2007). Regulation of epithelial tight junction assembly and disassembly by AMP-activated protein kinase. *Proc Natl Acad Sci U S A*, **104**(3), 819-822.
- Zhou, G., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyk-Melody, J., Wu, M., Ventre, J., Doebber, T., and Fujii, N. (2001a). Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest*, **108**(108 (8)), 1167-1174.
- Zhou, G., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyk-Melody, J., Wu, M., Ventre, J., Doebber, T., Fujii, N., Musi, N., Hirshman, M. F., Goodyear, L. J., and Moller, D. E. (2001b). Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest*, **108**(8), 1167-1174.
- Zhu, L., Chen, L., Zhou, X. M., Zhang, Y. Y., Zhang, Y. J., Zhao, J., Ji, S. R., Wu, J. W., and Wu, Y. (2011). Structural insights into the architecture and allostery of full-length AMP-activated protein kinase. *Structure*, **19**(4), 515-522.

Footnotes

D.R.R. is the recipient of a National Health and Medical Research Council Senior Principal Research Fellowship and Project Grants.

*D.R.R. and S.S. contributed equally as co-corresponding senior authors.

Reprint requests to be sent to Dr. Sumit Sahni (Molecular Pharmacology and Pathology Program, Department of Pathology, Blackburn Building D06, University of Sydney, NSW 2006, Australia).
Email: sumit.sahni@sydney.edu.au.

The authors declare no conflict of interest.

FIGURE LEGENDS

Figure 1. Structure of AMPK. (A) Crystal structure of AMPK. The α -subunit is indicated in yellow, orange and cyan, the β -subunit is in green with the CBM in bright green, and the γ -subunit is in pink. For this structure, an AMPK complex phosphorylated on Thr172, was crystallized in the presence of AMP and the kinase inhibitor staurosporine. Additionally AMPK activator 991 shown in magenta is bound in a pocket generated at the interface between the CBM and the kinase domain. Only AMP-binding at CBS site 3 and 4 is shown (marked as AMP-3 and AMP-4, respectively). Reprinted with permission from Macmillan Publishers Ltd: [Nature] (Xiao et al., 2013), (<http://creativecommons.org/licenses/by/3.0/legalcode>). (B) The α -subunit is a 550 amino acid long catalytic subunit, and is composed of the kinase domain, α -linker region, the C-terminal β/γ - subunit interacting domain (α -CTD) and a nuclear export signal (NES). The α -linker region contains the auto-inhibitory sequence (AIS) and “ α -hook” domain. The kinase domain is phosphorylated at Thr172 in the activation loop. The β -subunit is 270 amino acids long and has a carbohydrate-binding motif (CBM) and the C-terminal domain of the β - subunit (β -CTD). The N-terminal contains a more variable sequence including the N-myristoylation site. The γ -subunit contains 4 tandem repeats of the CBS, AMP-binding pockets (*i.e.*, CBS 1-4).

Figure 2. Regulation of AMPK activity. AMPK is phosphorylated by three kinases, LKB1, CaMKK β and TAK1. LKB1 forms a complex with STRAD and MO25, which then allows it to translocate from the nucleus to the cytoplasm. Once in the cytoplasm, LKB1 can phosphorylate AMPK at Thr172. CaMKK β phosphorylates AMPK as a response to increased cytosolic Ca²⁺ levels. TAK1 is activated by IL-1, TGF- β , TLR, CD40 and B cell receptors. Furthermore, AMP-binding to the γ -subunit of AMPK prevents the dephosphorylation of Thr172 in the α -subunit.

Figure 3. Regulation of energy homeostasis by AMPK. AMPK phosphorylates acetyl CoA carboxylase 1 (ACC1) and inactivates it. ACC1 is required to convert acetyl CoA to malonyl CoA for

fatty acid (FA) synthesis. Malonyl CoA inhibits carnitine palmitoyltransferase 1 (CPT1), a protein that enables the entry of FAs into the mitochondria for β -oxidation. Phosphorylation of ACC1 by activated AMPK prevents acetyl CoA from being converted to malonyl CoA, and thus, prevents the inhibition of β -oxidation. Furthermore, inhibition of malonyl CoA synthesis also prevents FA synthesis. AMPK also suppresses ACC1 levels by inhibiting sterol regulatory element-binding protein-1 (SREBP1), a transcription-factor involved in ACC1 transcription. Similarly, inhibition of hydroxymethylglutaryl CoA reductase (HMGCR) *via* phosphorylation by AMPK prevents cholesterol synthesis by interrupting the conversion of hydroxymethylglutaryl CoA (HMGCoA) to mevalonate. In addition to this, AMPK also regulates glucose uptake by increasing GLUT4 translocation to the membrane.

Figure 4. Regulation of mTOR complex 1 (mTORC1) by AMPK. AMPK inhibits mTOR signaling *via* a dual mechanism. First, AMPK inhibits raptor, and prevents the formation of mTORC1. Second, AMPK also interferes with PI3K/AKT signaling by phosphorylating and activating the tuberous sclerosis 1/2 (TSC1/TSC2) dimer, which inhibits Ras homolog enriched in brain (Rheb). Rheb is an activator of mTORC1. Hence, activation of TSC1/TSC2 dimer by AMPK, results in suppression of Rheb, which is then unable to activate mTORC1. Suppression of mTORC1 leads to inhibition of ribosomal S6 kinase (S6K) and eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) phosphorylation. Non-phosphorylated 4EBP1 can bind to translation factor eIF4E, and prevent protein synthesis. This leads to decreased protein translation, which then results in decreased cell proliferation.

Figure 5. Regulation of autophagy by AMPK. AMPK regulates autophagy through a dual mechanism. First, AMPK phosphorylates Unc 51-like kinase (ULK1) at Ser317/777, which results in initiation of autophagy. Second, AMPK also indirectly enhances ULK1 phosphorylation at these sites by suppression of mTORC1. mTORC1 phosphorylates ULK1 at Ser757, but this phosphorylation has

an inhibitory effect on the phosphorylation of Ser317/777. Hence, suppression of mTORC1 removes the inhibitory phosphorylation on ULK1 and allows it to be phosphorylated at Ser317/777.

Figure 6. AMPK regulates cell polarity and directional migration. AMPK phosphorylates the microtubule plus end protein, CLIP-170, which is involved in binding of newly polymerized distal ends of growing microtubules and rapidly dissociating from the older microtubule lattice. This results in directional migration of cells.

Figure 7. Activators of AMPK. Line drawings of the structures of some of the pharmacological and natural compounds that activate AMPK.

TABLES

Table 1: AMPK-subunits. The gene encoding the subunits, their chromosomal location, molecular weight (MW) and transcript size.

Subunit	Gene	Location	MW (Da)	Transcript	
AMPKα1	<i>PRKAA1</i>	5p12	64009	1929 bp	(Krishan et al., 2014; Strausberg et al., 2002)
AMPKα2	<i>PRKAA2</i>	1p31	62320	1703 bp	(Strausberg et al., 2002)
AMPKβ1	<i>PRKAB1</i>	12q24.1-q24.3	30382	813 bp	(Gao et al., 1996; Mitchelhill et al., 1997)
AMPKβ2	<i>PRKAB2</i>	1q21.1	30302	819 bp	(Gao et al., 1996)
AMPKγ1	<i>PRKAG1</i>	12q12-q14	37579	996 bp	(Gao et al., 1996)
AMPKγ2	<i>PRKAG2</i>	7q36.1	63066	1962 bp	(Ota et al., 2004)
AMPKγ3	<i>PRKAG3</i>	2q35	54258	1599 bp	(Gao et al., 1996)

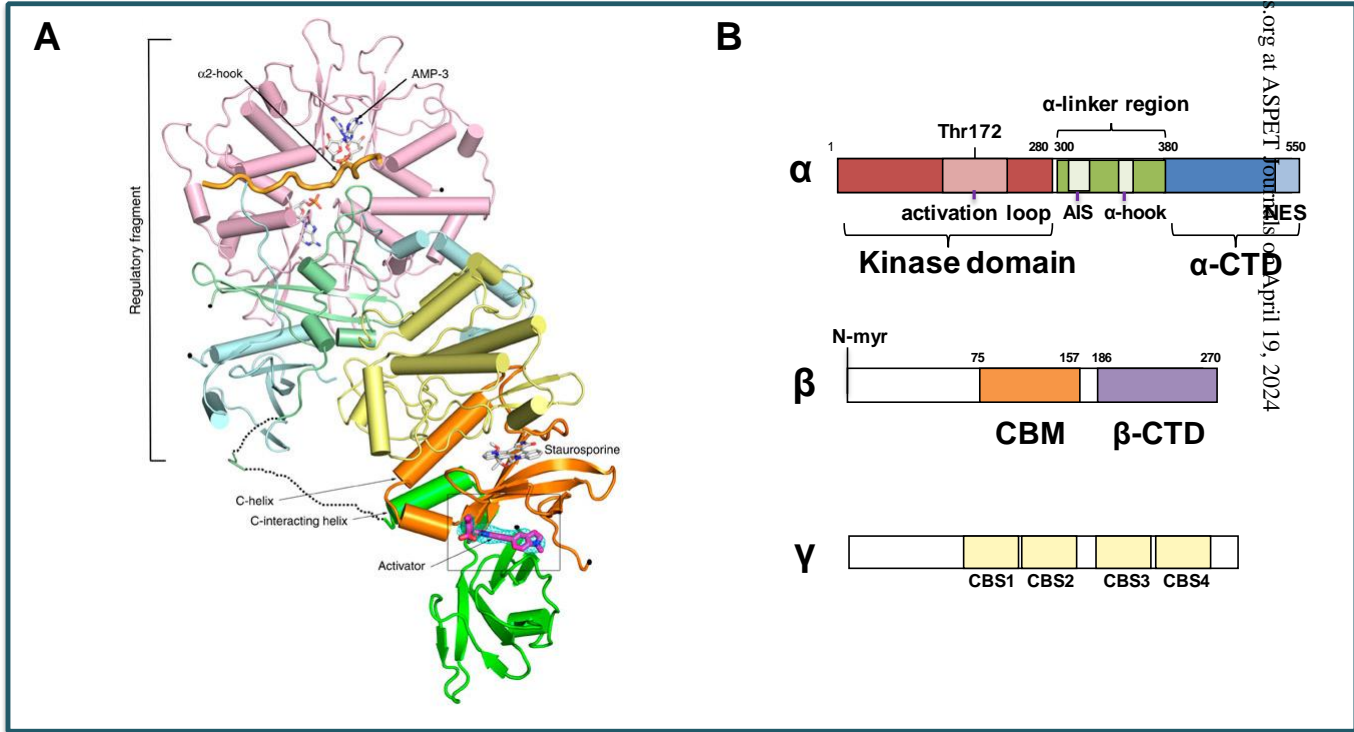


Figure 1

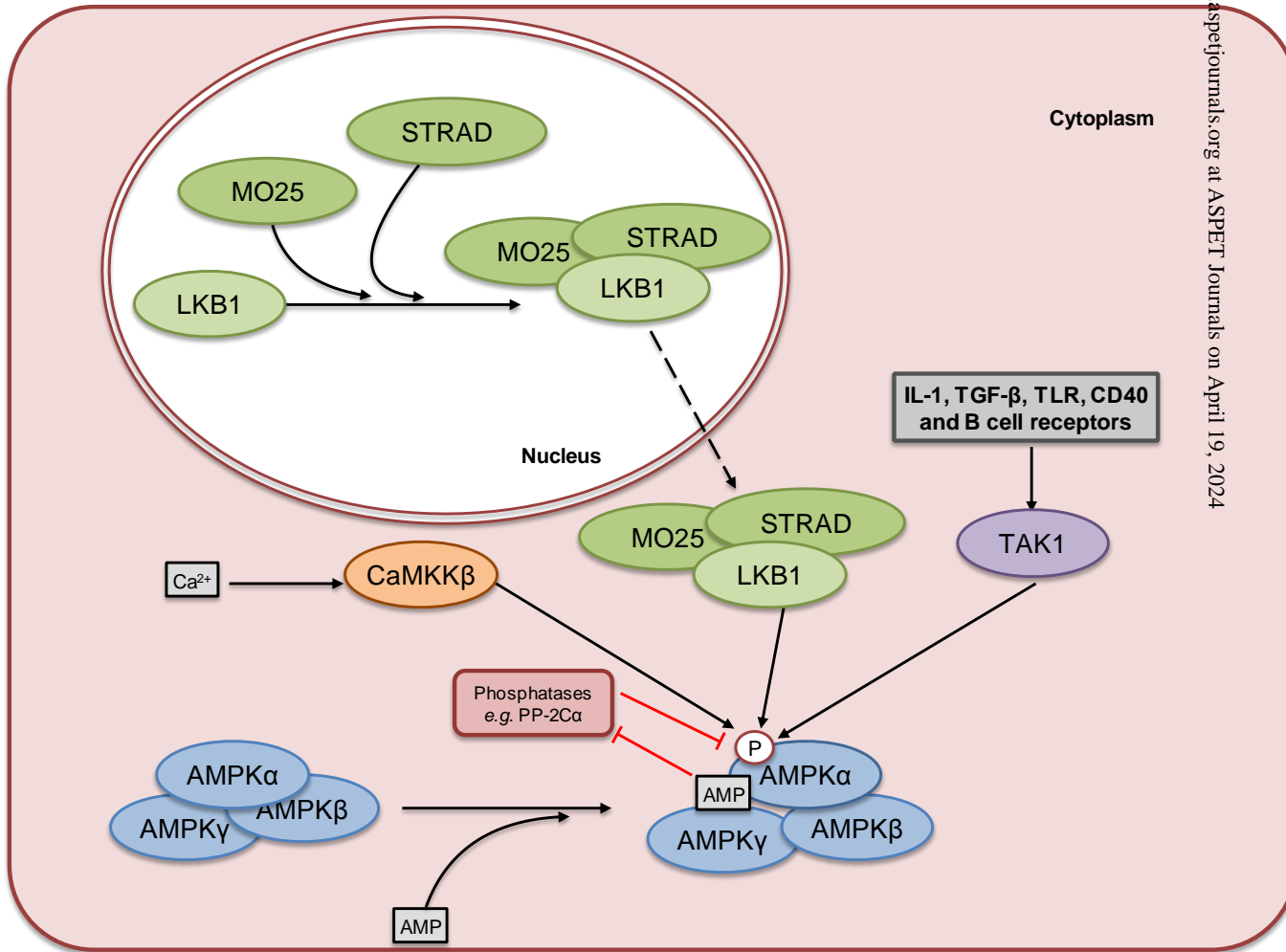


Figure 2

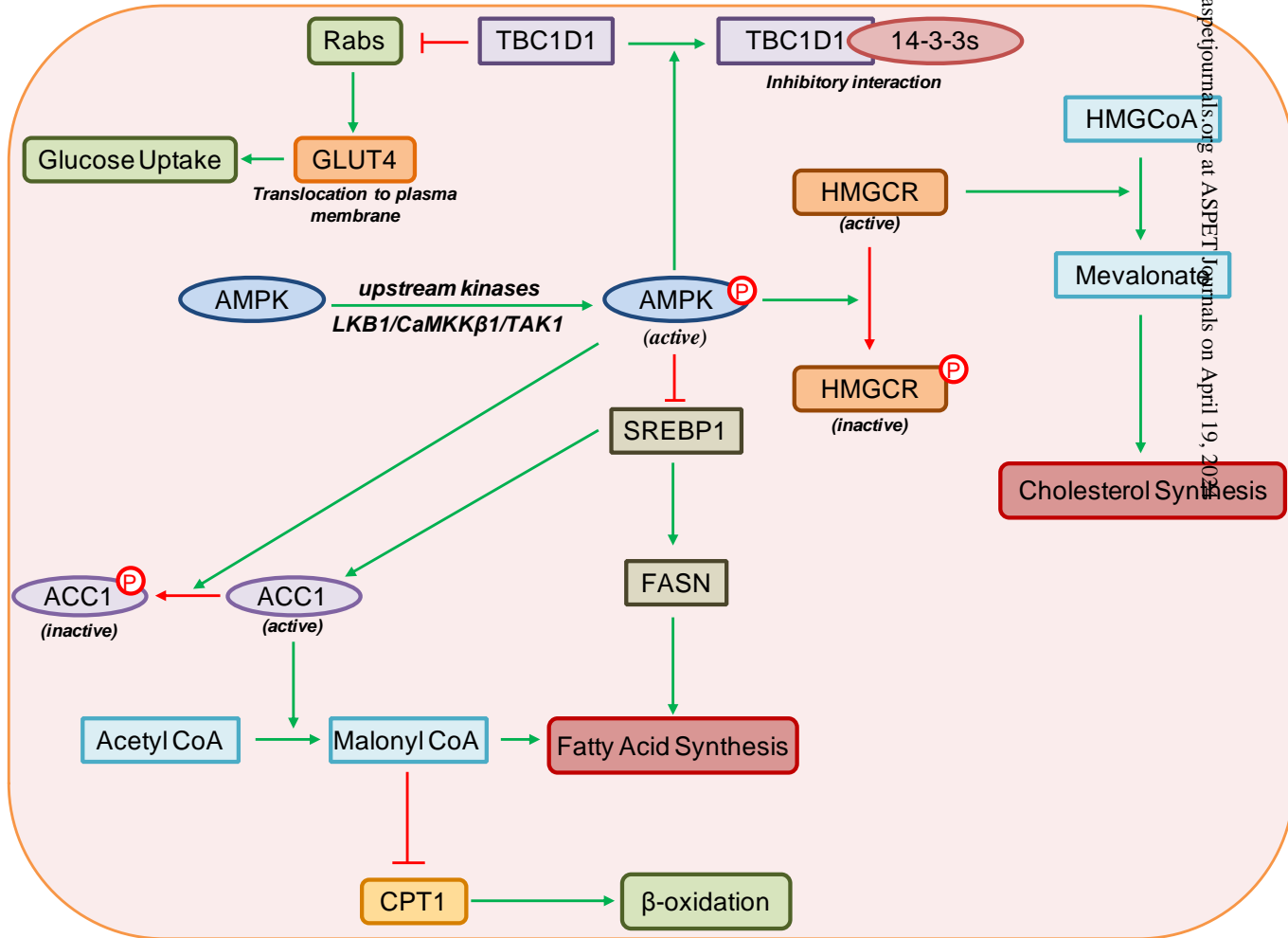
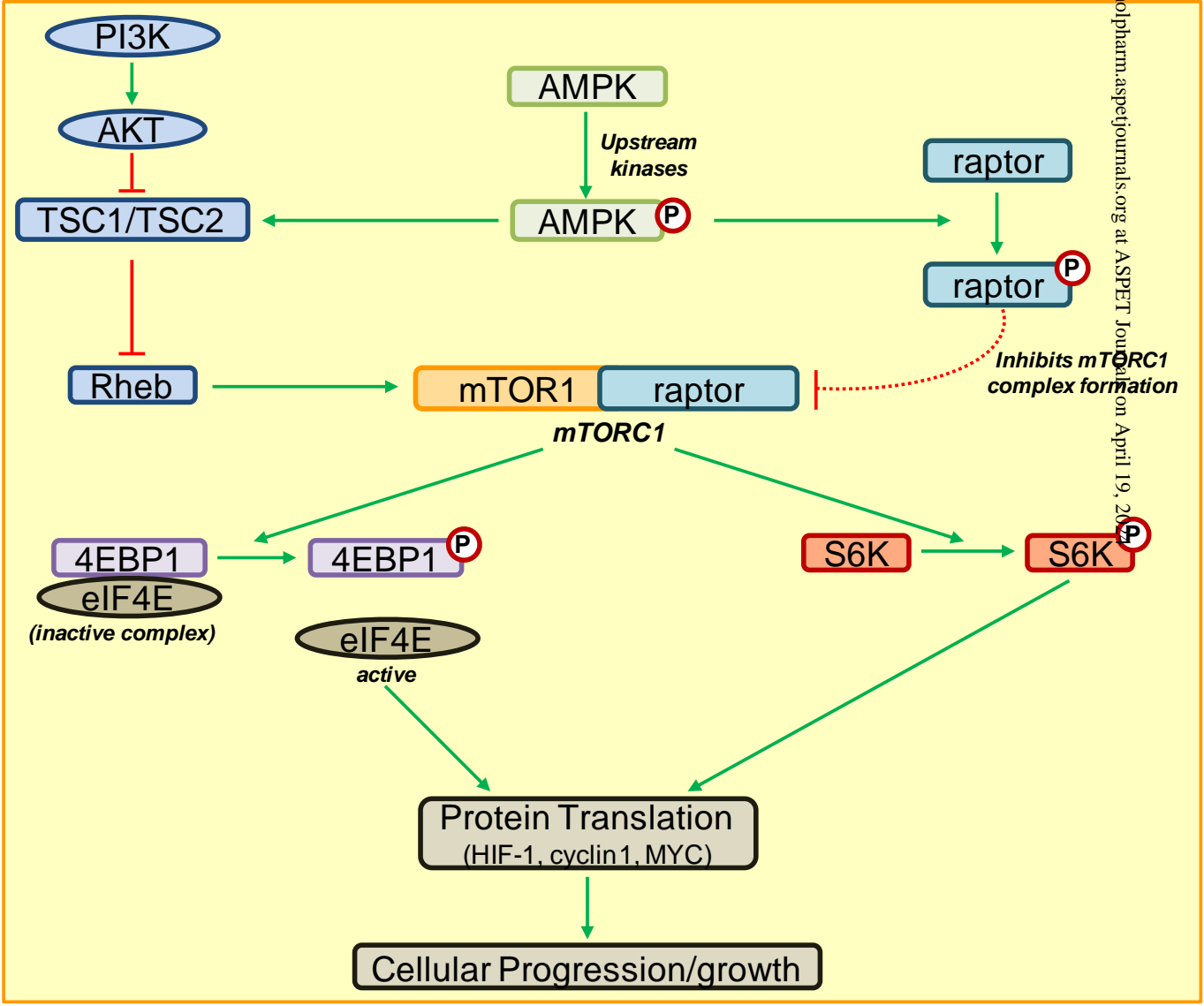


Figure 3



Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 19, 2015

Figure 4

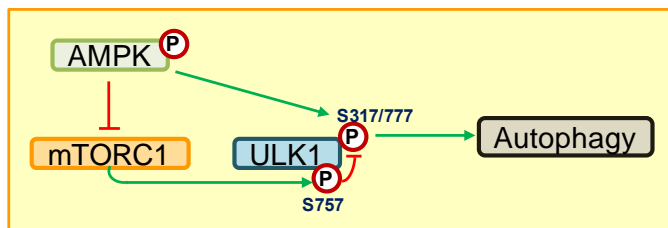


Figure 5

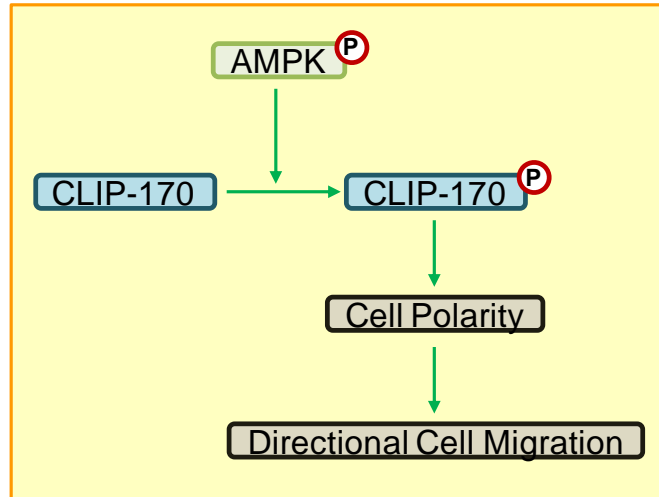


Figure 6

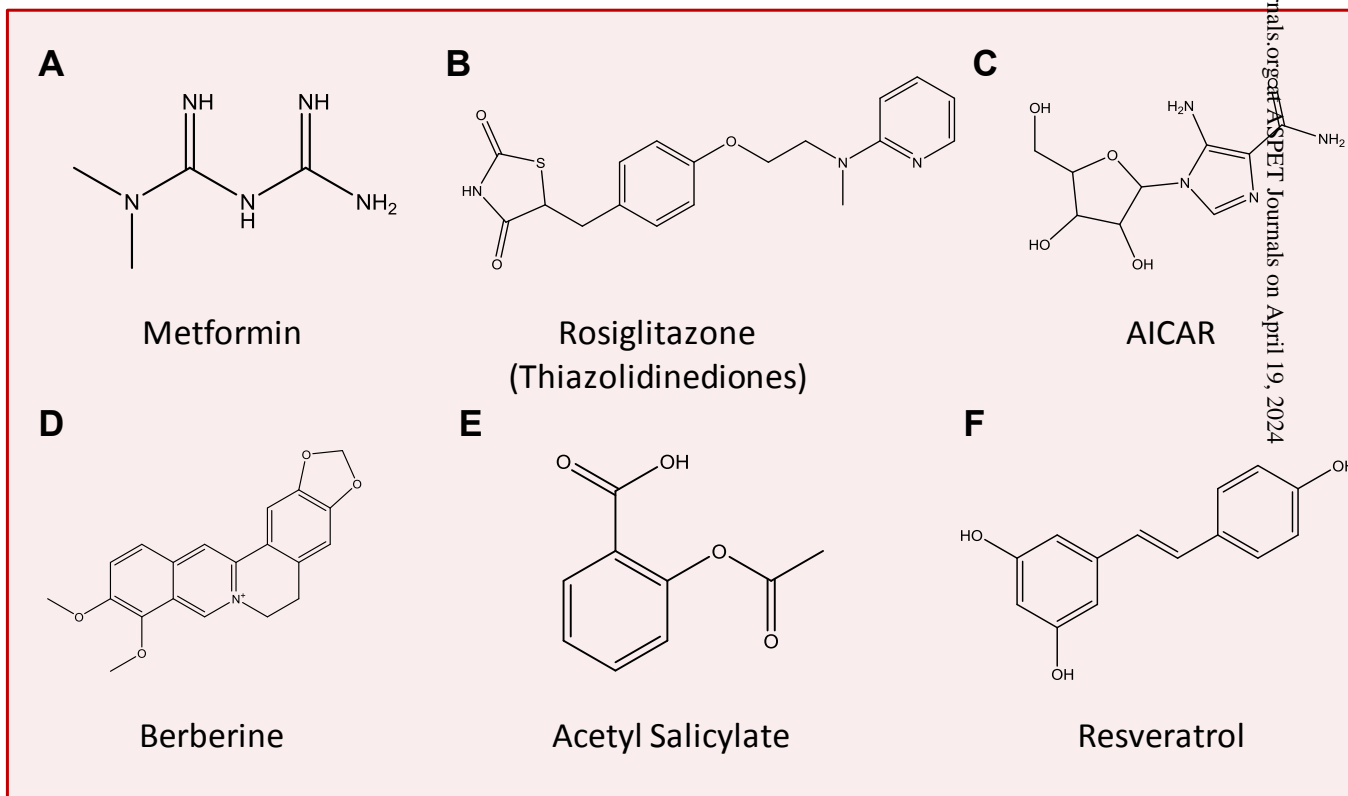


Figure 7