

## Mini-review

# AMPK: An emerging target for modification of injury-induced pain plasticity

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## H I G H L I G H T S

- We review the form, function and pharmacology of AMPK.
- We review emerging evidence for a role of AMPK in neuronal plasticity and disease.
- We describe the potential role of AMPK in manipulation of pain plasticity.
- We describe novel opportunities to target AMPK for pain therapy.

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## A B S T R A C T

Chronic pain is a critical medical problem afflicting hundreds of millions of people worldwide with costly effects on society and health care systems. Novel therapeutic avenues for the treatment of pain are needed that are directly targeted to the molecular mechanisms that promote and maintain chronic pain states. Recent evidence suggests that peripheral pain plasticity is promoted and potentially maintained via changes in translation control that are mediated by mTORC1 and MAPK pathways. While these pathways can be targeted individually, stimulating the AMPK pathway with direct or indirect activators achieves inhibition of these pathways via engagement of a single kinase. Here we review the form, function and pharmacology of AMPK with special attention to its emerging role as a potential target for pain therapeutics. We present the existing evidence supporting a role of AMPK activation in alleviating symptoms of peripheral nerve injury- and incision-induced pain plasticity and the blockade of the development of chronic pain following surgery. We argue that these preclinical findings support a strong rationale for clinical trials of currently available AMPK activators and further development of novel pharmacological strategies for more potent and efficacious manipulation of AMPK in the clinical setting. Finally, we posit that AMPK represents a unique opportunity for drug development in the kinase area for pain because it is pharmacologically manipulated via activation rather than inhibition potentially offering a wider therapeutic window with interesting additional pharmacological opportunities. Altogether, the physiology, pharmacology and therapeutic opportunities surrounding AMPK make it an attractive target for novel intervention for chronic pain and its prevention.

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## 1. Introduction

The Institute of Medicine report on “Pain in America” published in 2011 highlights the urgent need for a better understanding of mechanisms that drive chronic pain and the development of therapeutics that target these mechanisms for the improved management of clinical pain disorders [1]. Pain is the most prominent reason that Americans seek medical attention and the lifetime population incidence of chronic pain in this country is 33%. This creates an enormous burden on medical care systems and society and leads to human suffering. An important goal of research in the pain area is to further understand mechanisms driving chronic pain and develop therapeutic strategies to treat pain based on these molecular insights. Herein we will focus on a novel target, AMP-activated protein kinase (AMPK), which directly targets signaling pathways that are involved in promoting chronic pain through the sensitization of peripheral nociceptors. This target is unique among kinases under investigation in the pain area because it can be pharmacologically targeted through an “agonist” based approach. The mechanism also provides a unique opportunity for immediate translation into the clinic because it can be engaged via activation by clinically available drugs, metformin and phenformin.

AMPK is a ubiquitous kinase endogenously activated by AMP and ADP and exogenously regulated by a variety of pharmacological entities including the widely prescribed anti-diabetes drug metformin [20]. AMPK is best known as a target for intervention in diabetes, largely due to the discovery that the kinase is a target, albeit indirectly, for metformin [92]. Work in the AMPK area has accelerated dramatically in the past decade and it is now clear that the kinase may play a central role in inflammation, cancer, neurological degenerative disorders and in the area of this review, pain. Accompanying advances in the basic understanding of AMPK function and its role in physiology have provided major inroads into pharmacological mechanisms for engaging AMPK activity [40]. While drug discovery around most kinases has focused on inhibition of kinase activity, largely through active site inhibitors, the role of AMPK as a negative regulator of other kinase pathways has long made it clear that pharmacological targeting of the kinase should involve activation, rather than inhibition [20,40]. This gives AMPK a somewhat unique position among kinases in the drug discovery area. The emergence of AMPK as a potential target for pain therapeutics is a relatively new development for the field, hence, our purposes for this review are to (1) introduce the form and function of the kinase, (2) to describe how the kinase can be targeted pharmacologically and then (3) to describe the work suggesting that targeting AMPK may lead to beneficial effects in the treatment of chronic pain.

## 2. Role of AMPK in cellular metabolism

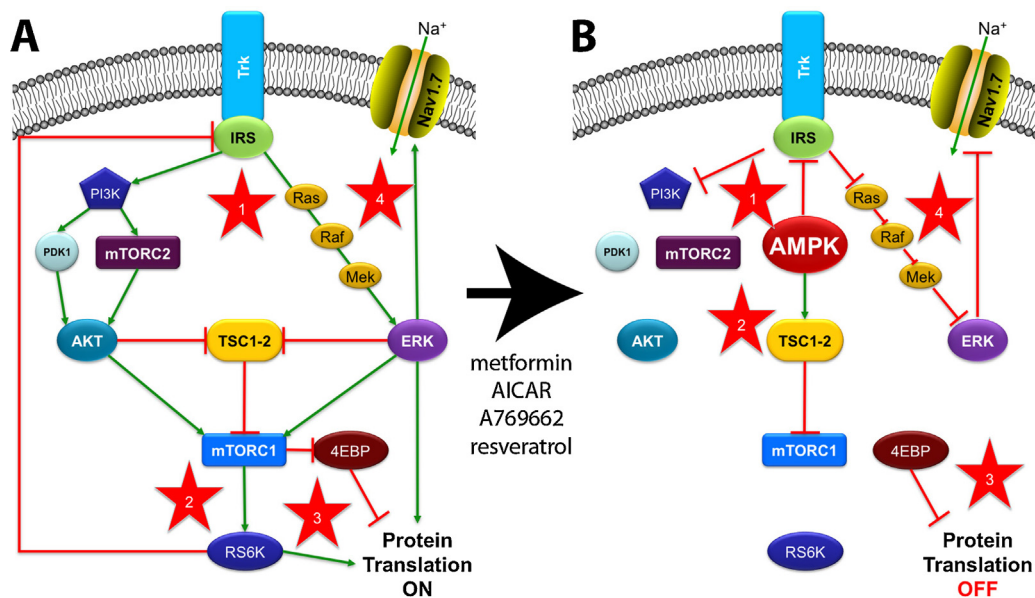
Anabolic processes, such as protein synthesis, are orchestrated by upstream kinases that signal to the translation machinery [95] such as mammalian target of rapamycin complex 1 (mTORC1) and extracellular signal-regulated kinase (ERK) which signals via mitogen activated interacting kinases (MNK1 and MNK2) to the

eukaryotic initiation factor (eIF) 4E. These kinases can be pharmacologically targeted individually by selective inhibitors or they can be negatively modulated by endogenous signaling factors that act on these pathways [113]. A crucial kinase for negative regulation of translation is the ubiquitous, energy-sensing kinase AMPK (Fig. 1, [20]). Activation of AMPK by depletion of cellular nutrients or through pharmacological intervention results in a dampening of signaling to the translation machinery [113]. This is the natural cellular response to energy deprivation wherein high AMP levels signal to AMPK thereby shutting down anabolic processes when nutrient levels are low [20]. AMPK is not solely regulated by cellular homeostatic mechanisms as it can also be targeted pharmacologically via a number of investigational compounds (e.g. AICAR and A769662 [24]), natural products (resveratrol [27,110]) and by the widely clinically available and safe drug metformin [79,92]. AMPK negatively regulates mTOR via activation of mTOR's negative regulator tuberous sclerosis complex 2 (TSC2) [25]. This results in a profound inhibition of mTOR and its downstream targets involved in translation control (e.g. eIF4E binding protein (4EBP) and ribosomal S6 kinase [25], Fig. 1). Activation of AMPK also negatively regulates ERK activity induced by growth factors and cytokines [54]. This likely occurs via phosphorylation of the insulin receptor substrate 1 (IRS1) protein at Ser 794 [101]. Because IRS-1 is a critical component of the signaling module of all tyrosine kinase receptors (Trks) [6], AMPK activation is likely to hinder Trk signaling (Fig. 1).

AMPK activation is not exclusively linked to negative regulation of protein synthesis. The kinase is also intimately associated with changes in glucose handling, fatty acid metabolism and plays an important role in the regulation of autophagy in cells. Hence, it is clear that AMPK plays a central role in regulation of diverse aspects of cellular metabolism. This central role likely emerges from AMPK's essential position as a cellular sensor of AMP and ADP. Because ATP is used as the proximal energy source in all cells, this mechanism is necessary to monitor cellular needs. Therefore, it appears that AMPK is essential for the monitoring of ATP levels and the diversion of cellular resources to the replenishment of ATP when cellular stores are depleted [20].

## 3. Physiological and pharmacological activation of AMPK in cells

To understand how AMPK is regulated it is necessary to understand the structure of the kinase. AMPK is made up of three subunits comprised of an  $\alpha$ ,  $\beta$  and  $\gamma$  subunit [20]. There are two isoforms of the  $\alpha$  subunit, two isoforms of the  $\beta$  subunit and three  $\gamma$  subunit isoforms. The  $\alpha$  subunit contains the serine/threonine kinase domain and the  $\beta$  and  $\gamma$  subunits are involved in regulation of the kinase containing subunit. Both the  $\alpha$  and  $\beta$  subunits contain phosphorylation sites but the key site for upstream regulation appears to localize to the  $\alpha$  subunit at threonine 172 which is phosphorylated by liver kinase B1 (LKB1). Phosphorylation of AMPK is a key regulator of kinase activity and the central endogenous regulator of AMPK, AMP, increases phosphorylation of the kinase. While the precise mechanisms through which this occurs are still not completely understood, it is clear that AMP binding to the  $\gamma$



**Fig. 1.** Regulation of ERK/mTOR signaling by AMPK. (A) mTOR and ERK signaling downstream of tyrosine kinase receptors (Trks). Star 1: IRS-1 stimulation via Trks leads to stimulation of PI3K and small GTPase signaling cascades resulting in activation of mTORC1 and ERK. Star 2: mTORC1 activation leads to phosphorylation of RS6K. RS6K activation leads to negative feedback on IRS-1 resulting in a termination of signaling. Star 3: mTORC1 activation relieves 4EBP-mediated inhibition of translation resulting in an increase in gene expression. Star 4: ERK activation downstream of Trk signaling mediated by IRS-1 may result in increased activity in Nav1.7 mediated by a direct action of ERK1. (B) Activation of AMPK by metformin, AICAR, A769662 or resveratrol results in AMPK-mediated inhibition of IRS-1 (star 1) resulting in blockade of Trk (or GP130) signaling to ERK. AMPK activation likewise modulates TSC2 activity resulting in an inhibition of mTORC1 (star 2). This negative regulation of mTORC1 ultimately results in a decrease in translation (star 3) due to maintenance of tonic inhibition of ribosome recruitment by 4EBP. Star 4: AMPK-mediated regulation of IRS-1 also results in a decrease in activity-dependent regulation of ERK and possibly a concomitant blockade of activity-dependent modulation of Nav1.7 by ERK1.

subunit of the kinase protects the kinase from dephosphorylation by a diverse array of phosphatases including protein phosphatase (PP) 1, 2A and 2C [20]. The AMPK activating tool compound AICAR is a prodrug that is converted to the AMP analog 5-aminoimidazole-4-carboxamide ribotide (AICA-ribotide or more commonly ZMP) in cells. Because the AMP site in AMPK also binds ZMP, AICAR mimics the effects of high AMP levels to activate AMPK [48].

Recent advances in pharmacological targeting of AMPK have taken advantage of this feature of the  $\gamma$  subunit in protecting the kinase from dephosphorylation. Two recently described, potent and efficacious AMPK activators, A769662 and OSU-53, appear to bind allosterically to AMPK presumably causing the  $\gamma$  subunit to assume a conformation that protects the  $\alpha$  subunit from protein phosphatase activity. In the case of A769662, specific subunit knockdown studies have demonstrated that the mechanism of action of the compound at AMPK requires the presence of a specific  $\gamma$  subunit [24]. Likewise, OSU-53 has direct activity on AMPK that may also be linked to inhibition of dephosphorylation, although this has not been clearly elucidated to this point [60]. Hence a strategy of activating AMPK directly via positive allosteric modulation, perhaps through engagement of the  $\gamma$  subunit, appears to offer a potent and potentially selective mechanism for pharmacological manipulation of AMPK without the requirement for stimulation of upstream kinases [20]. Several other examples of positive allosteric modulators have been described in the patent literature [40].

This pharmacological opportunity stands in contrast to two other major classes of AMPK activators, the biguanides (e.g. metformin) and the polyphenols (e.g. resveratrol). Metformin and its structural analog, phenformin, have been used as first line treatment for type II diabetes for decades. While metformin is still in wide clinical use (in fact it is the most widely prescribed drug in the world), phenformin has been pulled from the market in much of the world due to toxicities. Although these drugs have been in use for decades, their relation to AMPK has only recently been elucidated. Metformin is not a direct AMPK activator because it has

no effect on the purified kinase [111]. Rather, metformin interferes with mitochondrial function leading to activation of LKB1 and subsequent AMPK phosphorylation [92]. Polyphenols (including resveratrol) may act via a similar mechanism [10,27], however, there is also evidence that, in neurons, AMPK activation by polyphenols involves calcium/calmodulin-dependent protein kinase kinase  $\beta$  (CaMKK $\beta$ , [27]). Neither biguanides nor polyphenols have particularly potent actions at AMPK ( $EC_{50}$ s of at least 100  $\mu$ M levels for short time courses) so these compounds also have other targets that may be important for their physiological effects. A prime example of this is SIRT1 and resveratrol. While there is still ample controversy over the relative contribution of SIRT1 or AMPK for the actions of resveratrol, it is now clear that AMPK plays an important role in resveratrol's effects [22,27,36,102].

#### 4. Targets of AMPK phosphorylation

Activation of AMPK, either through raised cellular AMP levels or pharmacological activation sets off a diverse number of cellular processes controlled largely through the regulation of AMPK-mediated phosphorylation of downstream targets. One of the best-known targets of AMPK, acetyl-CoA carboxylase (ACC), plays an important function in AMPK's regulation of fatty acid synthesis [20]. Other direct targets are largely involved in the regulation of protein synthesis or pathways that ultimately regulate protein synthesis. AMPK directly phosphorylates the negative regulators of mTOR, the tuberous sclerosis complex (TSC) proteins, thereby enhancing their negative regulation of mTOR [25]. AMPK also phosphorylates mTOR itself adding an additional level of negative mTOR regulation [20]. AMPK also phosphorylates IRS1 [48] and AKT at negative regulatory sites [20]. This phosphorylation is likely to play a key role in shunting of activation of feedback signaling that occurs when mTORC1 is inactivated. These negative phosphorylation events may also play a key role in negatively regulating feedback signaling that can be engaged by pharmacological

inhibition of mTORC1 with rapamycin or structural analogues suggesting that AMPK activators might be important co-therapeutics for disease states, like cancer, where strong mTORC1 inhibition is therapeutically desired. AMPK also phosphorylates a key target for autophagy, Unc-51-like kinase (ULK1). This event is likely to play a key role in AMPK's ability to induce autophagy in a diverse array of cell types [31]. Hence, AMPK phosphorylation targets provide a mechanistic rationale for AMPK regulation of fatty acid synthesis, protein synthesis and autophagy.

There is also strong evidence that activation of AMPK leads to inhibition of mitogen activated protein kinase (MAPK) signaling pathways, including ERK [20,71,99]. This is important because the ERK pathway is linked to changes in neuronal excitability and is known to play an important role along the pain neuronal axis to as an inducer of pain plasticity [65]. While the mechanisms through which AMPK activation achieves inhibition of ERK activity are still not completely understood, this is likely linked to AMPK-mediated phosphorylation of IRS1 and concomitant inhibition of small GTPase signaling molecules that link Trk signaling to MAPK activity.

## 5. AMPK regulation of ion channels

While much work has focused on AMPK regulation of intracellular signaling pathways, relatively few studies have examined AMPK regulation of ion channel function. Given the general energy conservation role of the kinase during low glucose availability, modulation of channel activity that ultimately leads to decreased cellular activity might be predicted. One of the earliest identified channel targets of AMPK was the cystic fibrosis transmembrane conductance regulator (CFTR). Phosphorylation of CFTR by AMPK results in a decrease in open probability [42] and also prevents PKA stimulation of the channel [55]. Several years following the discovery of CFTR modulation, it was found that AMPK negatively regulates epithelial Na<sup>+</sup> channel (ENaC) membrane expression, although not through direct phosphorylation but through phosphorylation of the ubiquitin ligase Nedd4-2 which then decreases ENaC expression [13,19]. AMPK can also increase K<sub>ATP</sub> (Kir6.2) channel activity and membrane expression [98,108]. And AMPK appears to participate in increased BK<sub>Ca</sub> channel expression in outer hair cells of the ear [35]. However, AMPK is known to contribute to hyperpolarizing shifts in the voltage dependence of Nav1.5, decreased amplitudes of BK<sub>Ca</sub>, TASK, and TREK channel currents, and decreased membrane expression of Kv7.1 and Kir2.1 channels [5], all effects that would presumably enhance cellular activity. So the ultimate role of AMPK channel modulation in cellular activity is not entirely clear.

All of the effects of AMPK on ion channels described above have been documented in non-neuronal cells or cell lines. While many of these channels are present in neurons and could be subject to similar regulation by AMPK, whether direct modulation of channels by this kinase occurs in neurons is largely unknown. At the time of this writing, only a single study has examined direct AMPK effects on channels in neurons. In rat hippocampal neurons, activation of AMPK caused hyperpolarizing shifts in K<sup>+</sup> channel currents, an effect that was abolished by including antibodies against Kv2.1 in the recording pipette [47]. A direct effect of AMPK on Kv2.1 was also confirmed in this study in HEK cells where direct phosphorylation of Kv2.1 was observed as were similar shifts in channel properties to those in neurons. Thus, there is precedence for the concept that AMPK activation in neurons can dampen excitability, in this case via increased K<sup>+</sup> channel activity. Clearly, more study is required to determine whether and which channels are directly modulated by AMPK within the nervous system.

The potential also exists that AMPK activation can indirectly modulate channel activity or expression in neurons. As mentioned above, AMPK phosphorylates Nedd4-2, which is present in neurons and may lead to modulation of expression of multiple channel types on neuronal membranes. Nedd4-2 is known to interact with numerous channel types including voltage gated Na<sup>+</sup> channels, KCNQ channels, and ClC-2 chloride channels [16] suggesting that an AMPK/Nedd4-2 modulatory mechanism may occur in neurons although this has not been tested experimentally.

Another potential indirect modulatory mechanism mediated by AMPK is via the decrease in ERK activity described above. ERK modulation of several channel types has been documented including Kv4.2 [4,90], a K<sup>+</sup> channel subtype contributing to transient outward or A-type K<sup>+</sup> currents, and Cav2.2 channels [67] mediating N-type Ca<sup>2+</sup> current and the target of the analgesic ziconotide. Another is Nav1.7, a channel thought to contribute to amplification of generator potentials to near threshold for action potential firing. ERK is known to phosphorylate Nav1.7 on specific residues on an intracellular loop of the channel [97]. We have recently published that ERK1 specifically interacts with Nav1.7 after IL-6 exposure [107]. ERK phosphorylation of Nav1.7 mediates an increase in action potential firing and a decrease in latency to first action potential in sensory neurons stimulated with depolarizing ramp current injections [97]. Importantly for the purposes of this review, Nav1.7 is linked to human pain conditions wherein loss-of-function results in insensitivity to pain while gain-of-function causes erythromelalgia and paroxysmal extreme pain disorder [28]. Hence, genetic evidence clearly demonstrates an important role for Nav1.7 in human pain disorders. Other lines of evidence implicate Nav1.7 in acquired pain states. Inhibition of Nav1.7 with small molecules decreases sensory neuron excitability [33,89]. Genetic deletion of Nav1.7 in mice leads to marked decreases in acute and inflammatory pain [75] although neuropathic pain is unaffected [74]. Despite a lack of evidence for a neuropathic pain phenotype in Nav1.7 conditional knockout mice, pharmacological inhibition of Nav1.7 with several distinct classes of inhibitors leads to a reduction of allodynia in preclinical neuropathic pain models [45,46,63,100]. Hence, human clinical findings and pharmacological inhibition of Nav1.7 creates a compelling rationale for targeting Nav1.7 in human pain disorders. We have recently shown that AMPK activators both decrease ERK phosphorylation and decrease excitability of sensory neurons in response to ramp currents, an effect consistent with decreased phosphorylation of the voltage-gated sodium channel Nav1.7 [71]. Although not a direct effect of AMPK on Nav1.7, the negative regulation of ERK activity nonetheless contributes to the overall decrease in neuronal excitability due to AMPK (Fig. 1).

## 6. AMPK as a regulator of neuronal function, plasticity and neurodegeneration

As noted above, AMPK is known to affect several ion channel targets. Although relatively few studies have examined the effect of these targets in neurons, there is a wealth of emerging evidence that AMPK activation plays a significant role in important neuronal processes. The mTORC1 pathway is now an extensively studied target for the regulation of activity-dependent protein synthesis and neuronal plasticity [53]. Emerging evidence suggests that AMPK activation induces an effect on neuronal plasticity via mTORC1. For instance, activation of AMPK suppresses mTORC1 activity induced by long-term potentiation (LTP) and therefore negatively regulates LTP [85]. It still not known if this manifests as a physiological consequence of pharmacological activation of AMPK at clinically relevant doses of AMPK activators, such as metformin. This is somewhat unlikely given that metformin is so widely used and this potential side effect is not reported in the clinical literature.



AMPK activity in specific brain regions, in particular the hypothalamus, has been shown to play an important role in feeding behavior [14]. In this regard, it is clear that hypothalamic AMPK responds, both negatively and positively to a variety of hormones that play a key role in brain-mediated metabolic functions. For instance, thyroid hormone is a negative regulator of hypothalamic AMPK and this negative regulation plays a key role in metabolic changes induced by hyperthyroidism [64]. Similarly, the orexigenic hormone leptin strongly suppresses hypothalamic AMPK activity. On the other hand, ghrelin stimulates hypothalamic AMPK activity [29]. Hence, hypothalamic AMPK plays a central role in the regulation of hypothalamic function by peripheral hormones.

There is also strong evidence that AMPK plays an important role in protection of brain neurons from excitotoxicity and may be an important target for the alleviation of neurodegenerative disorders [96]. Several studies have now shown that AMPK activators suppress neurotoxicity associated with factors that are increased in the brains of humans with Alzheimer's disease. Activation of AMPK with resveratrol stimulates increased clearance of amyloid  $\beta$  protein in preclinical models, an effect linked to autophagy induction via AMPK activation [102]. There is also evidence that AMPK activation may be linked to neuroprotection in Huntington's disease [81] and to protection against axonal degeneration in diabetes [88]. Hence, in addition to pain, the topic of this review, AMPK activators may have use in a variety of neurological disorders.

## 7. Rationale for targeting AMPK in pain

The unique positioning of AMPK as a negative regulator of mTORC1-mediated, activity-dependent protein synthesis and other signaling pathways that are linked to chronic pain make this kinase an ideal target to harness an endogenous regulatory mechanism that may dampen nociceptor excitability and sensitization [77]. Two major targets for AMPK are the mTORC1 pathway and MAPKs. Both of these targets play an important role in pain plasticity. The role of MAPKs has been extensively reviewed [49,65,77], hence, we will not touch on this topic here except where there is direct evidence for AMPK-mediated negative regulation of MAPKs in pain. The mTORC1 pathway and its role in activity-dependent protein synthesis, on the other hand, has only recently received attention in the field so we will briefly review the evidence for a role of mTORC1 in pain plasticity here.

Over the past decade, work from our and other laboratories has clearly demonstrated that translation control of gene expression is a key factor in initiation and maintenance of pain hypersensitivity in a wide range of preclinical pain models [15,34,38,50,70,71,86,87,99]. This work has shown that translation control, via mTORC1 contributes to the basal sensitivity of a subset of nociceptors [50] and, perhaps more importantly, contributes to local changes in gene expression in response to injury [15,34,38,50,87,99] or specific algogens [70,99] to promote nociceptive hypersensitivity. This latter point demonstrates an important role for local, activity-dependent translation in peripheral nociceptive plasticity [86]. This is a crucial distinction because activity-dependent translation is controlled by a specific set of kinases, mainly mTORC1 and MAPKs, that provide therapeutic opportunities for pharmacological intervention [95]. In fact, there is already compelling evidence that mTOR is a key kinase controlling plasticity in the nociceptive system [38,50,62,71,87,93]. Because these experiments have largely relied on rapamycin, a specific inhibitor of mTORC1, these effects appear to be linked to translation control, the major function of mTORC1. Unfortunately, while local, short-term inhibition of mTORC1 leads to blockade of pain hypersensitivity, we have recently shown that long-term inhibition of mTORC1 (either pharmacologically or genetically) leads to

feedback activation of ERK causing nociceptive hypersensitivity [72]. These feedback mechanisms in mTORC1 signaling have long been recognized [39] and have almost certainly contributed to the failure of mTORC1 inhibitors in several cancer clinical trials [21]. Hence, although mTORC1 is an obvious target for pain therapeutics due to its mechanistic importance, it is unlikely that clinicians will advantageously target this specific mechanism because of its side-effect profile (which includes immune suppression). Importantly, this rapamycin-induced nociceptive hypersensitivity is alleviated by AMPK activators due to their ability to suppress feedback-signaling emerging from prolonged direct inhibition of mTORC1.

The picture emerging from this body of work is that activity-dependent translation control, either controlled by mTORC1 or ERK/MNK/eIF4E, plays a key role in the hypersensitivity developing in the nociceptive system following injury. However, pharmacological targeting of these pathways must be carefully controlled due to the extensive feedback kinase signaling networks activated by targeting individual kinases. In this regard, it is potentially advantageous to utilize "agonists" of a kinase that can negatively regulate activity-dependent translation and block feedback signaling mechanisms. Based on the extensive work described above in other systems, one kinase, AMPK, fits this potential therapeutic opportunity. To this end, we have recently shown that AMPK activators reduce peripheral nerve injury-induced allodynia and decrease excitability of sensory neurons *in vitro* [69]. We have also shown that resveratrol, a natural product AMPK activator, reduces incision-induced allodynia in mice via an AMPK-mediated mechanism [99]. These findings create a compelling case for the further exploration of AMPK activators for the treatment of a variety of chronic pain states.

## 8. Neuropathic pain and AMPK

The first evidence for a role of AMPK in pain modulation came from studies in animals with traumatic injury to peripheral nerves [71]. As noted above, it was already known that mTORC1 activity [7,38,76], and translation control in general [86,87], plays an important nerve injury-induced excitability and allodynia. To this end, we demonstrated that peripheral nerve injury (PNI) induces a fundamental reorganization of the translation machinery in the peripheral nervous system such that signaling pathway mediators (e.g. mTOR and ERK) and their downstream partners (4EBP and eIF4E) are increased. Moreover, RNA binding proteins and upstream regulators of translational machinery signaling are increased by PNI and these molecular events are linked to an increase in nascent protein synthesis in the sciatic nerve. We then asked if treatment with AMPK activators led to a decrease a PNI-induced allodynia in rats and mice. Both metformin and A769662 significantly reduced nerve injury-induced allodynia after 2–3 days of systemic treatment. Remarkably, a 7 day treatment with either of these compounds leads to a resolution of neuropathic allodynia that is not reversed following cessation of treatment, even in animals with long-standing PNI (up to 60 days, [71,72]). Hence, these findings suggest that prolonged treatment with AMPK activators had disease-modifying properties on neuropathic allodynia. Because metformin treatment also reduces the PNI-induced increase in nascent protein synthesis in the peripheral nerve we hypothesize that these effects are functionally linked to a restoration of protein synthesis homeostasis following prolonged treatment.

This does not mean that AMPK stimulation with metformin or other AMPK activators leads to a complete block of protein synthesis in peripheral nerves or other tissues. In terms of overall protein synthesis rate, metformin simply normalizes this to rates seen in uninjured nerves [71]. Moreover, proteomic profiling of injured sciatic nerves after PNI indicates that metformin

changes the proteomic profile of the injured nerve such that certain gene clusters associated with increased excitability are decreased whereas clusters associated with regeneration are increased [73]. This may be associated with an emerging role, as discussed above, for AMPK activation in neuroprotection. In this regard, we found that apolipoprotein E (ApoE), a gene associated with Alzheimer's disease is increased by nerve injury and further increased by metformin treatment. In fact, in naïve mice, metformin treatment increases ApoE expression in the peripheral nervous system [73]. Because ApoE is associated with functional recovery of peripheral nervous system function after injury [61], this finding may suggest that in addition to metformin's anti-allodynic effects the drug may also promote functional recovery after PNI.

Importantly, AMPK activators also have profound effects on sensory neurons in culture, both in terms of signaling pathways and excitability. Treatment of dorsal root ganglion (DRG) or trigeminal ganglion (TG) neurons in culture leads to a time- and concentration-dependent inhibition of mTORC1 signaling and, in some cases, a reduction of ERK activity [71]. And as described above, AMPK activators reduce the excitability of these neurons in response to ramp-evoked spiking protocols [71], an effect consistent with voltage-gated sodium channel modulation. The time- and concentration-dependence of these events are correlated with biochemical signaling events suggesting an AMPK-mediated effect. Therefore, AMPK activation reverses neuropathic allodynia and pathologies in protein synthesis rates in response to PNI and modifies signaling pathways and excitability in relevant neuronal systems in vitro.

An important question arises with respect to these findings from the long-standing use of metformin in the clinic for type II diabetes. Type II diabetes is a well-known cause of chronic neuropathic pain due to the toxic effects of prolonged hyperglycemia on peripheral nerves. Does metformin have an influence on diabetes-induced painful neuropathy? In many ways this is a confounded experiment because metformin is glucose lowering and would therefore be expected to reduce the incidence of diabetic neuropathy due to its anti-hyperglycemic effects. There are, however, ways to address this question when comparing metformin to other anti-hyperglycemics. Interestingly, one study has shown that the incidence of diabetic neuropathic pain is reduced in patients treated with metformin versus those treated with insulin [84]. While more work is clearly needed to understand how this effect might be mediated or if it can be reproduced in different or larger populations, the finding suggests that an anti-neuropathic pain signature for metformin might already be present in a large clinical population. Another key piece of evidence comes from two case reports showing that treatment with metformin for newly diagnosed type II diabetes reverses previously established chronic pain conditions unrelated to type II diabetes [58,59]. While only prospective trials with metformin in non-diabetic populations will be able to determine the potential clinical utility of this drug, several recent trials with metformin for cancer suggest that this common and safe drug can achieve success in the clinic for non-traditional uses.

Recently salicylate has been shown to activate AMPK [44]. Interestingly, while salicylate and other NSAIDs show little efficacy for neuropathic pain when given systemically, small trials have suggested that topical salicylate, where concentrations can reach much higher levels than can be tolerated systemically, is effective for certain types of localized neuropathic pain [56]. The mechanism of action of salicylate has not been explained for this apparent clinical action. Several other NSAIDs also demonstrate efficacy for neuropathic pain when given at high concentrations topically [68]. It is not known if this salicylate effect is mediated by AMPK or if other NSAIDs have a generalized action on AMPK.

## 9. Post-surgical pain and AMPK

Despite the proliferation of analgesics and the development of innovative pharmacological strategies to treat post-surgical pain, chronic pain still occurs in up to 50% of surgical patients [51,52] and up to a quarter of all chronic pain patients suffer from persistent pain because of a prior surgery [26]. This chronic pain can be debilitating in 2–10% of this population [51,52] highlighting the importance of gaining a better understanding of chronic pain following surgery and developing novel therapeutics. Population-based studies suggest that both pre-surgery pain state and acute post-surgical pain are predictors of chronic pain following surgery [43,52,82]. Surveys of hospital care of post-surgical pain demonstrate clear deficiencies in adherence to protocols and utilization of multimodal analgesia, however, the occurrence rate of these shortcomings (~15% [12]) are incongruent with the notion that this is the primary reason that surgery patients develop chronic pain (as high as 40–50% in some populations [51,52]). Furthermore, this area is still highly reliant on opioid analgesics and this class of drugs is fraught with serious side-effects, potential for exacerbation of chronic pain (as has been shown preclinically with fentanyl [57]) and abuse liabilities. As a result, it is not clear whether chronic post-surgical pain is due to inadequate treatment of acute post-surgical pain or whether the currently available therapeutics used to treat acute post-surgical pain do not prevent the transition to chronic pain. Novel therapeutics that directly target molecular events responsible for driving incision-induced pain and its chronification may relieve the burden that this problem presents for patients, clinicians and healthcare systems [91].

The development of a preclinical model for post-surgical pain, the hindpaw incision model [17,18], has greatly enhanced our understanding of how endogenous mediators released as a result of incision lead to activation and sensitization of nociceptors. Two of the major endogenous mediators released following incision are NGF and IL-6 [23]. Of these, the effects of NGF are best understood as NGF is clearly increased at the site of incision and its release is directly linked to post-surgical pain and nociceptor sensitization [9,104,105,109]. These findings led to the view that anti-NGF therapies might be useful in the treatment of post-surgical pain and, by extension, in avoiding the transition to chronic pain after surgery [109]. While the fate of anti-NGF trials remains to be seen, mechanisms that target downstream mediators of NGF might also find clinic utility for the treatment of post-surgical pain.

NGF and IL6 lead to engagement of the mTORC1 and ERK pathways, respectively, in nociceptors and their axons [70]. Engagement of these pathways is functionally linked to the development of mechanical allodynia [70] and to the presence of persistent nociceptive sensitization to subsequent noxious stimuli following recovery from the initial NGF and/or IL-6 injection [8]. A role for ERK in the sensitization of nociceptors is well established [32,49,66,78,112] but a critical role for mTORC1 has only recently been recognized as described above [8,38,50,70,86,87].

We hypothesized that AMPK activators might represent a unique class of drugs for the attenuation of post-surgical pain and the prevention of chronic pain brought on by surgery. Regional application of local anesthetics has long been a mainstay of post-surgical pain control and recent treatments (e.g. capsaicin instillation) have successfully demonstrated that targeting the activity of peripheral nociceptors at the site of incision is a viable approach for the alleviation of post-surgical pain [2,3]. Along these lines, we evaluated whether local treatment with resveratrol would alleviate incision-induced allodynia and hyperalgesic priming. Resveratrol, given locally at the time of incision and/or 24 h following incision, leads to a significant reversal of incision-induced allodynia in either treatment regime. Moreover, it completely blocks the development of hyperalgesic priming suggesting an

important protective effect from the development of susceptibility to a chronic pain state following surgery [99]. Resveratrol also completely blocked the allodynic effects of IL-6 and/or NGF in mice suggesting a role of AMPK in alleviating pro-nociceptive signaling induced by these important pain-promoting factors. In vitro studies revealed that resveratrol potentially activates AMPK in sensory neurons and inhibits mTORC1 and ERK activity leading to a reduction of cap-dependent translation. These effects of resveratrol were not mediated by SIRT1 as they were unaffected by SIRT1 modulators [99]. Hence, resveratrol shares biochemical properties with other AMPK activators in vitro and inhibits allodynia development in vivo consistent with properties of metformin and A769662 described above. Interestingly, several previous investigations have demonstrated an anti-hyperalgesic property of peripherally administered resveratrol but none had investigated its precise mechanism of action [11,37,83,106]. The available biochemical evidence points in the direction of AMPK. While more work is needed in this area, the available evidence points to an important opportunity for AMPK as a preventive measure for the development of chronic post-surgical pain.

In the case of post-surgical pain, it can again be asked why a role for metformin in inhibition of the development of chronic pain induced by surgery has not already been indicated due to its widespread use. Here it is important to note that metformin has been contra-indicated for decades in the 48 h around surgery due to risk of possible lactic acidosis. This clinical guideline is based largely on case reports and is not supported by population-based studies [41,94,103]. In fact, there is strong evidence that recent metformin ingestion is not a risk factor after cardiac surgery and may actually be beneficial due to its cardioprotective qualities [30]. Hence, several investigators have suggested that risk of lactic acidosis be evaluated before discontinuation of metformin for surgery [30,94]. While this controversy is clearly still unresolved, we wish to make several points: (1) the existing evidence does not absolutely rule out the use of metformin as a treatment for post-surgical pain [30,94], (2) it is unlikely that this rare effect of metformin is an AMPK class effect as metformin directly inhibits mitochondrial respiratory complex 1 [80] and (3) surgery offers unique opportunities for local application of drug, as we have already demonstrated preclinically with resveratrol.

## 10. Pharmacological opportunities for AMPK in pain

Based on the preclinical findings discussed above, we propose that there are several important opportunities for targeting AMPK in the clinic for the treatment of chronic pain. The most obvious is to design and conduct prospective trials for metformin or phenformin (in countries where it is available) for neuropathic pain or post-surgical pain. Although these drugs have been widely used as anti-hyperglycemics for type II diabetes, there are no indications that they cause hypoglycemia in individuals with normal glucose handling capacity, therefore it is unlikely that they will cause adverse effects in a normoglycemic populations. Moreover, there is already some evidence that these compounds might be effective for neuropathic pain based on the lower incidence of neuropathic diabetic pain in patients taking metformin [84] and limited case reports for other types of chronic pain [58,59]. This rationale and evidence aside, metformin and phenformin are only weak activators of AMPK and it is as yet unclear that they reach therapeutic levels in relevant tissues (e.g. peripheral nervous tissue) in humans. There are several strategies that might be employed to achieve better AMPK activation in the clinic for the alleviation of chronic pain.

One route would include the further development of positive allosteric modulators for AMPK. These compounds exist (e.g.

A769662 and OSU-53) and we have shown efficacy for A769662 in neuropathic pain models [71]. This compound also profoundly reduces mTORC1 and ERK activity and excitability in peripheral sensory neurons. A wide variety of potential positive allosteric modulators have been published in the patent literature [40]. The developmental stage of these compounds is unclear, as is their clinical safety profile. However, in our view, this class of compounds holds great potential in the pain arena for several important reasons. (1) These compounds are generally at least 100 times more potent in activating AMPK than metformin and they have clear advantages in terms of efficacy [40]. (2) They can target specific subunits (e.g.  $\gamma 2$  for A769662) therefore achieving strong AMPK activation in certain tissues while avoiding potentially negative effects in other tissues that have unique AMPK subunit expression like skeletal muscle [20,40]. (3) Indirect activators of AMPK have diverse targets arising largely from their lack of potency [20,40] whereas direct AMPK activators offer the opportunity for very specific AMPK-mediated effect because they target the kinase through allosteric binding. (4) Finally, these compounds have “agonist”-like properties at AMPK [40] suggesting that clinically meaningful target engagement may not require full target coverage as is so often the case for the antagonist or active site inhibitor approach. Hence, the therapeutic window for this class of compounds may be broader than would otherwise be expected for kinase inhibitors.

A final pharmacological opportunity arises from the diverse mechanisms and pharmacology through which AMPK can be activated. It is formally possible that allosteric activators of AMPK will have dose-limiting toxicities due to the ubiquitous nature of AMPK (ignoring the possibility for subunit-specific activation in only desired tissues). Having noted this, it is possible that combining indirect and direct AMPK activators will be able to achieve pharmacological synergism. Indirect activators enhance AMPK phosphorylation whereas direct allosteric activators protect the kinase from dephosphorylation via  $\gamma$  or  $\beta$  subunits. Based on this mechanistic understanding, it is possible that combining upstream LKB1 or CaMKK $\beta$  activation with indirect activators leading to enhanced AMPK phosphorylation will synergize with protection from dephosphorylation with direct activators in terms of enhancement of AMPK activity. While this idea has not been tested it does reveal several interesting therapeutic scenarios. The most obvious is oral treatment with metformin (an indirect activator) combined with local treatment with a direct activator at the site of pain or surgery. If synergism can be achieved at AMPK, this treatment regime would be expected to promote strong local AMPK activation while sparing AMPK activation systemically that might lead to undesired side effects.

## 11. Concluding remarks

The past decade has seen enormous growth in our understanding of the physiology and pharmacology of AMPK. A growing understanding has paralleled these advances that cellular processes that are under the control of AMPK play a key role in sensitizing pain pathways following peripheral injury. These same pathways may also play a key role in the development of chronic pain that often persists after the resolution of injury induced by the precipitating insult. Therefore, the evidence presented above suggests that AMPK stands in a prime position for the development of novel therapeutic strategies for the treatment and/or prevention of chronic pain. Clearly time will tell, but we look forward to exciting discoveries in this area of work in the coming years.

## Conflicts of interest

The authors declare no conflicts of interest.



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## References

- [1] Institute of Medicine, Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research, Washington (DC), 2011.
- [2] E.K. Aasvang, J.B. Hansen, H. Kehlet, Late sensory function after intraoperative capsaicin wound instillation, *Acta Anaesthesiol. Scand.* 54 (2010) 224–231.
- [3] E.K. Aasvang, J.B. Hansen, J. Malmstrom, T. Asmussen, D. Gennevois, M.M. Struys, H. Kehlet, The effect of wound instillation of a novel purified capsaicin formulation on postherniotomy pain: a double-blind, randomized, placebo-controlled study, *Anesth. Analg.* 107 (2008) 282–291.
- [4] J.P. Adams, A.E. Anderson, A.W. Varga, K.T. Dineley, R.G. Cook, P.J. Pfaffinger, J.D. Sweatt, The A-type potassium channel Kv4.2 is a substrate for the mitogen-activated protein kinase ERK, *J. Neurochem.* 75 (2000) 2277–2287.
- [5] M.N. Andersen, H.B. Rasmussen, AMPK, A regulator of ion channels, *Commun. Integr. Biol.* 5 (2012) 480–484.
- [6] L.S. Argentsinger, G.W. Hsu, M.G. Myers, N. Billestrup Jr., M.F. White, C. Carter-Su, Growth hormone, interferon-gamma, and leukemia inhibitory factor promoted tyrosyl phosphorylation of insulin receptor substrate-1, *J. Biol. Chem.* 270 (1995) 14685–14692.
- [7] C.O. Asante, V.C. Wallace, A.H. Dickenson, Mammalian target of rapamycin signaling in the spinal cord is required for neuronal plasticity and behavioral hypersensitivity associated with neuropathy in the rat, *J. Pain* (2010).
- [8] M.N. Asiedu, D.V. Tillu, O.K. Melemedjian, A. Shy, R. Sanoja, B. Bodell, S. Ghosh, F. Porreca, T.J. Price, Spinal protein kinase M {zeta} underlies the maintenance mechanism of persistent nociceptive sensitization, *J. Neurosci.* 31 (2011) 6646–6653.
- [9] R.K. Banik, A.R. Subieta, C. Wu, T.J. Brennan, Increased nerve growth factor after rat plantar incision contributes to guarding behavior and heat hyperalgesia, *Pain* 117 (2005) 68–76.
- [10] J.A. Baur, K.J. Pearson, N.L. Price, H.A. Jamieson, C. Lerin, A. Kalra, V.V. Prabhu, J.S. Allard, G. Lopez-Lluch, K. Lewis, P.J. Pistell, S. Poosala, K.G. Becker, O. Boss, D. Gwinn, M. Wang, S. Ramaswamy, K.W. Fishbein, R.G. Spencer, E.G. Lakatta, D. Le Couteur, R.J. Shaw, P. Navas, P. Puigserver, D.K. Ingram, R. de Cabo, D.A. Sinclair, Resveratrol improves health and survival of mice on a high-calorie diet, *Nature* 444 (2006) 337–342.
- [11] K.O. Bazzo, A.A. Souto, T.G. Lopes, R.F. Zanin, M.V. Gomez, A.H. Souza, M.M. Campos, Evidence for the analgesic activity of resveratrol in acute models of nociception in mice, *J. Nat. Prod.* 76 (2013) 13–21.
- [12] D. Benhamou, M. Berti, G. Brodner, J. De Andres, G. Draisci, M. Moreno-Azcoita, E.A. Neugebauer, W. Schwenk, L.M. Torres, E. Viel, Postoperative Analgesic Therapy Observational Survey (PATHOS): a practice pattern study in 7 central/southern European countries, *Pain* 136 (2008) 134–141.
- [13] V. Bhalla, N.M. Oyster, A.C. Fitch, M.A. Wijngaarden, D. Neumann, U. Schlatter, D. Pearce, K.R. Hallows, AMP-activated kinase inhibits the epithelial Na<sup>+</sup> channel through functional regulation of the ubiquitin ligase Nedd4-2, *J. Biol. Chem.* 281 (2006) 26159–26169.
- [14] P. Blanco Martinez de Morentin, C.R. Gonzalez, A.K. Saha, L. Martins, C. Dieguez, A. Vidal-Puig, M. Tena-Sempere, M. Lopez, Hypothalamic AMP-activated protein kinase as a mediator of whole body energy balance, *Rev. Endocr. Metab. Disord.* 12 (2011) 127–140.
- [15] O. Bogen, N. Alessandri-Haber, C. Chu, R.W. Gear, J.D. Levine, Generation of a pain memory in the primary afferent nociceptor triggered by PKCepsilon activation of CPBE, *J. Neurosci.* Off. J. Soc. Neurosci. 32 (2012) 2018–2026.
- [16] D. Bongiorno, F. Schuetz, P. Poronnik, D.J. Adams, Regulation of voltage-gated ion channels in excitable cells by the ubiquitin ligases Nedd4 and Nedd4-2, *Channels (Austin)* 5 (2011) 79–88.
- [17] T.J. Brennan, Postoperative models of nociception, *Ilar. J.* 40 (1999) 129–136.
- [18] T.J. Brennan, E.P. Vandermeulen, G.F. Gebhart, Characterization of a rat model of incisional pain, *Pain* 64 (1996) 493–501.
- [19] M.D. Carattino, R.S. Edinger, H.J. Grieser, R. Wise, D. Neumann, U. Schlatter, J.P. Johnson, T.R. Kleyman, K.R. Hallows, Epithelial sodium channel inhibition by AMP-activated protein kinase in oocytes and polarized renal epithelial cells, *J. Biol. Chem.* 280 (2005) 17608–17616.
- [20] D. Carling, C. Thornton, A. Woods, M.J. Sanders, AMP-activated protein kinase: new regulation, new roles? *Biochem. J.* 445 (2012) 11–27.
- [21] A. Carracedo, L. Ma, J. Teruya-Feldstein, F. Rojo, L. Salmena, A. Alimonti, A. Egia, A.T. Sasaki, G. Thomas, S.C. Kozma, A. Papa, C. Nardella, L.C. Cantley, J. Baselga, P.P. Pandolfi, Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer, *J. Clin. Invest.* 118 (2008) 3065–3074.
- [22] A.Y. Chan, V.W. Dolinsky, C.L. Soltys, B. Viollet, S. Baksh, P.E. Light, J.R. Dyck, Resveratrol inhibits cardiac hypertrophy via AMP-activated protein kinase and Akt, *J. Biol. Chem.* 283 (2008) 24194–24201.
- [23] J.D. Clark, X. Shi, X. Li, Y. Qiao, D. Liang, M.S. Angst, D.C. Yeomans, Morphine reduces local cytokine expression and neutrophil infiltration after incision, *Mol. Pain* 3 (2007) 28.
- [24] B. Cool, B. Zinker, W. Chiou, L. Kifle, N. Cao, M. Perham, R. Dickinson, A. Adler, G. Gagne, R. Iyengar, G. Zhao, K. Marsh, P. Kym, P. Jung, H.S. Camp, E. Frevert, Identification and characterization of a small molecule AMPK activator that treats key components of type 2 diabetes and the metabolic syndrome, *Cell Metab.* 3 (2006) 403–416.
- [25] M.N. Corradetti, K. Inoki, N. Bardeesy, R.A. DePinho, K.L. Guan, Regulation of the TSC pathway by LKB1: evidence of a molecular link between tuberous sclerosis complex and Peutz-Jeghers syndrome, *Genes Dev.* 18 (2004) 1533–1538.
- [26] I.K. Crombie, H.T. Davies, W.A. Macrae, Cut and thrust: antecedent surgery and trauma among patients attending a chronic pain clinic, *Pain* 76 (1998) 167–171.
- [27] B. Dasgupta, J. Milbrandt, Resveratrol stimulates AMP kinase activity in neurons, *Proc. Natl. Acad. Sci. U.S.A.* 104 (2007) 7217–7222.
- [28] S.D. Dib-Hajj, T.R. Cummins, J.A. Black, S.G. Waxman, Sodium channels in normal and pathological pain, *Annu. Rev. Neurosci.* 33 (2010) 325–347.
- [29] C. Dieguez, M.J. Vazquez, A. Romero, M. Lopez, R. Nogueiras, Hypothalamic control of lipid metabolism: focus on leptin, ghrelin and melanocortins, *Neuroendocrinology* 94 (2011) 1–11.
- [30] A.I. Duncan, C.G. Koch, M. Xu, M. Manlapaz, B. Batdorf, G. Pitas, N. Starr, Recent metformin ingestion does not increase in-hospital morbidity or mortality after cardiac surgery, *Anesth. Analg.* 104 (2007) 42–50.
- [31] D.F. Egan, D.B. Shackelford, M.M. Mihaylova, S. Gelino, R.A. Kohnz, W. Mair, D.S. Vasquez, A. Joshi, D.M. Gwinn, R. Taylor, J.M. Asara, J. Fitzpatrick, A. Dillin, B. Viollet, M. Kundu, M. Hansen, R.J. Shaw, Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy, *Science* 331 (2011) 456–461.
- [32] N. Eijkelkamp, H. Wang, A. Garza-Carbajal, H.L. Willems, F.J. Zwartkruis, J.N. Wood, R. Dantzer, K.W. Kelley, C.J. Heijnen, A. Kavelaars, Low nociceptor GRK2 prolongs prostaglandin E2 hyperalgesia via biased cAMP signaling to Epac/Rap1, protein kinase Cepsilon, and MEK/ERK, *J. Neurosci.* Off. J. Soc. Neurosci. 30 (2010) 12806–12815.
- [33] M. Estacion, S.G. Waxman, S.D. Dib-Hajj, Effects of ranolazine on wild-type and mutant hNav1.7 channels and on DRG neuron excitability, *Mol. Pain* 6 (2010) 35.
- [34] L.F. Ferrari, O. Bogen, C. Chu, J.D. Levine, Peripheral administration of translation inhibitors reverses increased hyperalgesia in a model of chronic pain in the rat, *J. Pain* (2013).
- [35] M. Foller, M. Jaumann, J. Dettling, A. Saxena, T. Pakladok, C. Munoz, P. Ruth, M. Sopjani, G. Seeböhm, L. Rüttger, M. Knipper, F. Lang, AMP-activated protein kinase in BK-channel regulation and protection against hearing loss following acoustic overstimulation, *FASEB J.* Off. Publ. Feder. Am. Soc. Exp. Biol. 26 (2012) 4243–4253.
- [36] M.D. Fullerton, G.R. Steinberg, SIRT1 takes a backseat to AMPK in the regulation of insulin sensitivity by resveratrol, *Diabetes* 59 (2010) 551–553.
- [37] M. Gentilli, J.X. Mazoit, H. Bouaziz, D. Fletcher, R.F. Casper, D. Benhamou, J.F. Savouret, Resveratrol decreases hyperalgesia induced by carrageenan in the rat hind paw, *Life Sci.* 68 (2001) 1317–1321.
- [38] S.M. Geranton, L. Jimenez-Diaz, C. Torsney, K.K. Tochiki, S.A. Stuart, J.L. Leith, B.M. Lumb, S.P. Hunt, A rapamycin-sensitive signaling pathway is essential for the full expression of persistent pain states, *J. Neurosci.* 29 (2009) 15017–15027.
- [39] S. Ghosh, V. Tergaonkar, C.V. Rothlin, R.G. Correa, V. Bottero, P. Bist, I.M. Verma, T. Hunter, Essential role of tuberous sclerosis genes TSC1 and TSC2 in NF-kappaB activation and cell survival, *Cancer Cell* 10 (2006) 215–226.
- [40] F. Giordanetto, D. Karis, Direct AMP-activated protein kinase activators: a review of evidence from the patent literature, *Expert Opin. Ther. Pat.* 22 (2012) 1467–1477.
- [41] J.R. Gowardman, J. Havill, Fatal metformin induced lactic acidosis: case report, *N. Z. Med. J.* 108 (1995) 230–231.
- [42] K.R. Hallows, V. Raghuram, B.E. Kemp, L.A. Witters, J.K. Foskett, Inhibition of cystic fibrosis transmembrane conductance regulator by novel interaction with the metabolic sensor AMP-activated protein kinase, *J. Clin. Invest.* 105 (2000) 1711–1721.
- [43] M.A. Hanley, M.P. Jensen, D.G. Smith, D.M. Ehde, W.T. Edwards, L.R. Robinson, Pre-amputation pain and acute pain predict chronic pain after lower extremity amputation, *J. Pain* 8 (2007) 102–109.
- [44] S.A. Hawley, M.D. Fullerton, F.A. Ross, J.D. Schertzer, C. Chevtzoff, K.J. Walker, M.W. Pegg, D. Zibrova, K.A. Green, K.J. Mustard, B.E. Kemp, K. Sakamoto, G.R. Steinberg, D.G. Hardie, The ancient drug salicylate directly activates AMP-activated protein kinase, *Science* 336 (2012) 918–922.
- [45] S.B. Hoyt, C. London, D. Gorin, M.J. Wyvratt, M.H. Fisher, C. Abbadi, J.P. Felix, M.L. Garcia, X. Li, K.A. Lyons, E. McGowan, D.E. MacIntyre, W.J. Martin, B.T. Priest, A. Ritter, M.M. Smith, V.A. Warren, B.S. Williams, G.J. Kaczorowski, W.H. Parsons, Discovery of a novel class of benzazepinone Nav1.7 blockers: potential treatments for neuropathic pain, *Bioorg. Med. Chem. Lett.* 17 (2007) 4630–4634.
- [46] S.B. Hoyt, C. London, H. Ok, E. Gonzalez, J.L. Duffy, C. Abbadi, B. Dean, J.P. Felix, M.L. Garcia, N. Jochowitz, B.V. Karanam, X. Li, K.A. Lyons, E. McGowan, D.E. MacIntyre, W.J. Martin, B.T. Priest, M.M. Smith, R. Tschirret-Guth, V.A. Warren, B.S. Williams, G.J. Kaczorowski, W.H. Parsons, Benzazepinone Nav1.7 blockers: potential treatments for neuropathic pain, *Bioorg. Med. Chem. Lett.* 17 (2007) 6172–6177.
- [47] N. Ikematsu, M.L. Dallas, F.A. Ross, R.W. Lewis, J.N. Rafferty, J.A. David, R. Suman, C. Peers, D.G. Hardie, A.M. Evans, Phosphorylation of the



- voltage-gated potassium channel Kv2.1 by AMP-activated protein kinase regulates membrane excitability, *Proc. Natl. Acad. Sci. U.S.A.* 108 (2011) 18132–18137.
- [48] S.N. Jakobsen, D.G. Hardie, N. Morrice, H.E. Tornqvist, 5'-AMP-activated protein kinase phosphorylates IRS-1 on Ser-789 in mouse C2C12 myotubes in response to 5-aminoimidazole-4-carboxamide riboside, *J. Biol. Chem.* 276 (2001) 46912–46916.
- [49] R.R. Ji, R.W.T. Gereau, M. Malcangio, G.R. Strichartz, MAP kinase and pain, *Brain Res. Rev.* 60 (2009) 135–148.
- [50] L. Jimenez-Diaz, S.M. Geranton, G.M. Passmore, J.L. Leith, A.S. Fisher, L. Berliocchi, A.K. Sivasubramanian, A. Sheasby, B.M. Lumb, S.P. Hunt, Local translation in primary afferent fibers regulates nociception, *PLoS One* 3 (2008) e1961.
- [51] A. Johansen, L. Romundstad, C.S. Nielsen, H. Schirmer, A. Stubhaug, Persistent postsurgical pain in a general population: prevalence and predictors in the Tromsø study, *Pain* 153 (2012) 1390–1396.
- [52] H. Kehlet, T.S. Jensen, C.J. Woolf, Persistent postsurgical pain: risk factors and prevention, *Lancet* 367 (2006) 1618–1625.
- [53] R.J. Kelleher 3rd, A. Govindarajan, S. Tonegawa, Translational regulatory mechanisms in persistent forms of synaptic plasticity, *Neuron* 44 (2004) 59–73.
- [54] J. Kim, M.Y. Yoon, S.L. Choi, I. Kang, S.S. Kim, Y.S. Kim, Y.K. Choi, J. Ha, Effects of stimulation of AMP-activated protein kinase on insulin-like growth factor 1- and epidermal growth factor-dependent extracellular signal-regulated kinase pathway, *J. Biol. Chem.* 276 (2001) 19102–19110.
- [55] J.D. King, A.C. Fitch Jr., J.K. Lee, J.E. McCane, D.O. Mak, J.K. Foskett, K.R. Hallows, AMP-activated protein kinase phosphorylation of the R domain inhibits PKA stimulation of CFTR, *Am. J. Physiol. Cell Physiol.* 297 (2009) C94–C101.
- [56] R.B. King, Topical aspirin in chloroform and the relief of pain due to herpes zoster and postherpetic neuralgia, *Arch. Neurol.* 50 (1993) 1046–1053.
- [57] E. Laboureyras, J. Chateauraynaud, P. Richebe, G. Simonnet, Long-term pain vulnerability after surgery in rats: prevention by nefopam, an analgesic with antihyperalgesic properties, *Anesth. Analg.* 109 (2009) 623–631.
- [58] K. Labuzek, S. Liber, W. Marcol, B. Okopien, Controlling newly diagnosed type 2 diabetes mellitus with metformin managed pain symptoms in a patient affected with Dercum's disease, *Pain Med.* 13 (2012) 1526–1527.
- [59] K. Labuzek, S. Liber, D. Suchy, B. Okopien, A successful case of pain management using metformin in a patient with adipositis dolorosa, *Int. J. Clin. Pharmacol. Ther.* (2013).
- [60] K.H. Lee, E.C. Hsu, J.H. Guh, H.C. Yang, D. Wang, S.K. Kulp, C.L. Shapiro, C.S. Chen, Targeting energy metabolic and oncogenic signaling pathways in triple-negative breast cancer by a novel adenosine monophosphate-activated protein kinase (AMPK) activator, *J. Biol. Chem.* 286 (2011) 39247–39258.
- [61] F.Q. Li, K.A. Fowler, J.E. Neil, C.A. Colton, M.P. Vitek, An apolipoprotein E-mimetic stimulates axonal regeneration and remyelination after peripheral nerve injury, *J. Pharmacol. Exp. Ther.* 334 (2010) 106–115.
- [62] L. Liang, B. Tao, L. Fan, M. Yaster, Y. Zhang, Y.X. Tao, mTOR and its downstream pathway are activated in the dorsal root ganglion and spinal cord after peripheral inflammation, but not after nerve injury, *Brain Res.* 1513 (2013) 17–25.
- [63] C. London, S.B. Hoyt, W.H. Parsons, B.S. Williams, V.A. Warren, R. Tschirret-Guth, M.M. Smith, B.T. Priest, E. McGowan, W.J. Martin, K.A. Lyons, X. Li, B.V. Karanam, N. Jochowitz, M.L. Garcia, J.P. Felix, B. Dean, C. Abbadie, G.J. Kaczorowski, J.L. Duffy, Imidazopyridines: a novel class of hNav1.7 channel blockers, *Bioorg. Med. Chem. Lett.* 18 (2008) 1696–1701.
- [64] M. Lopez, L. Varela, M.J. Vazquez, S. Rodriguez-Cuenca, C.R. Gonzalez, V.R. Velagapudi, D.A. Morgan, E. Schoenmakers, K. Agassandian, R. Lage, P.B. Martinez de Morentin, S. Tovar, R. Nogueiras, D. Carling, C. Lelliott, R. Gallego, M. Oresic, K. Chatterjee, A.K. Saha, K. Rahmouni, C. Dieguez, A. Vidal-Puig, Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance, *Nat. Med.* 16 (2010) 1001–1008.
- [65] W. Ma, R. Quirion, The ERK/MAPK pathway, as a target for the treatment of neuropathic pain, *Expert Opin. Ther. Targets* 9 (2005) 699–713.
- [66] M. Malik-Hall, O.A. Dina, J.D. Levine, Primary afferent nociceptor mechanisms mediating NGF-induced mechanical hyperalgesia, *Eur. J. Neurosci.* 21 (2005) 3387–3394.
- [67] S.W. Martin, A.J. Butcher, N.S. Berrow, M.W. Richards, R.E. Paddon, D.J. Turner, A.C. Dolphin, T.S. Sihra, E.M. Fitzgerald, Phosphorylation sites on calcium channel  $\alpha_1$  and  $\beta$  subunits regulate ERK-dependent modulation of neuronal N-type calcium channels, *Cell Calcium* 39 (2006) 275–292.
- [68] P. Matthews, S. Derry, R.A. Moore, H.J. McQuay, Topical rubefacients for acute and chronic pain in adults, *Cochr. Database Syst. Rev.* (2009) CD007403.
- [69] O. Melemedjian, M.N. Asiedu, D.V. Tillu, K.A. Sanoja, J. Yan, A. Lark, A. Khoutorsky, J. Johnson, K.A. Peebles, T. Lepow, N. Sonenberg, G.O. Dussor, T.J. Price, Targeting adenosine monophosphate-activated protein kinase (AMPK) in preclinical models reveals a potential mechanism for the treatment of neuropathic pain, *Mol. Pain* 21 (7) (2011) 70, <http://dx.doi.org/10.1186/1744-8069-7-70>.
- [70] O.K. Melemedjian, M.N. Asiedu, D.V. Tillu, K.A. Peebles, J. Yan, N. Ertz, G.O. Dussor, T.J. Price, IL-6- and NGF-induced rapid control of protein synthesis and nociceptive plasticity via convergent signaling to the eIF4F complex, *J. Neurosci.* 30 (2010) 15113–15123.
- [71] O.K. Melemedjian, M.N. Asiedu, D.V. Tillu, R. Sanoja, J. Yan, A. Lark, A. Khoutorsky, J. Johnson, K.A. Peebles, T. Lepow, N. Sonenberg, G. Dussor, T.J. Price, Targeting adenosine monophosphate-activated protein kinase (AMPK) in preclinical models reveals a potential mechanism for the treatment of neuropathic pain, *Mol. Pain* 7 (2011) 70.
- [72] O.K. Melemedjian, A. Khoutorsky, R.E. Sorge, J. Yan, M.N. Asiedu, A. Valdez, S. Ghosh, G. Dussor, J.S. Mogil, N. Sonenberg, T.J. Price, mTORC1 inhibition induces pain via IRS-1-dependent feedback activation of ERK, *Pain* 154 (7) (2013) 1080–1091, <http://dx.doi.org/10.1016/j.pain.2013.03.021>.
- [73] O.K. Melemedjian, H.N. Yassine, A. Shy, T.J. Price, Proteomic and functional annotation analysis of injured peripheral nerves reveals ApoE as a protein upregulated by injury that is modulated by metformin treatment, *Mol. Pain* 9 (2013) 14.
- [74] M.A. Nassar, A. Levato, L.C. Stirling, J.N. Wood, Neuropathic pain develops normally in mice lacking both Na(v)1.7 and Na(v)1.8, *Mol. Pain* 1 (2005) 24.
- [75] M.A. Nassar, L.C. Stirling, G. Forlani, M.D. Baker, E.A. Matthews, A.H. Dickenson, J.N. Wood, Nociceptor-specific gene deletion reveals a major role for Nav1.7 (PN1) in acute and inflammatory pain, *Proc. Natl. Acad. Sci. U.S.A.* 101 (2004) 12706–12711.
- [76] E. Norsted Gregory, S. Codeluppi, J.A. Gregory, J. Steinauer, C.I. Svensson, Mammalian target of rapamycin in spinal cord neurons mediates hypersensitivity induced by peripheral inflammation, *Neuroscience* 169 (2010) 1392–1402.
- [77] I. Obara, S.M. Geranton, S.P. Hunt, Axonal protein synthesis: a potential target for pain relief? *Curr. Opin. Pharmacol.* 12 (2012) 42–48.
- [78] K. Obata, H. Yamanaka, Y. Dai, T. Mizushima, T. Fukuoka, A. Tokunaga, K. Noguchi, Differential activation of MAPK in injured and uninjured DRG neurons following chronic constriction injury of the sciatic nerve in rats, *Eur. J. Neurosci.* 20 (2004) 2881–2895.
- [79] J. Ouyang, R.A. Parakhia, R.S. Ochs, Metformin activates AMP kinase through inhibition of AMP deaminase, *J. Biol. Chem.* 286 (2011) 1–11.
- [80] M.R. Owen, E. Doran, A.P. Halestrap, Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain, *Biochem. J.* 348 (Pt 3) (2000) 607–614.
- [81] G.M. Pasinetti, J. Wang, P. Marambaud, M. Ferruzzi, P. Gregor, L.A. Knable, L. Ho, Neuroprotective and metabolic effects of resveratrol: Therapeutic implications for Huntington's disease and other neurodegenerative disorders, *Exp. Neurol.* 232 (1) (2011) 1–6, <http://dx.doi.org/10.1016/j.expneurol.2011.08.014>.
- [82] F.M. Perkins, H. Kehlet, Chronic pain as an outcome of surgery. A review of predictive factors, *Anesthesiology* 93 (2000) 1123–1133.
- [83] T.A. Pham-Marcou, H. Beloel, X. Sun, M. Gentili, G. Benoit, D. Benhamou, J.X. Mazoit, Antinociceptive effect of resveratrol in carrageenan-evoked hyperalgesia in rats: prolonged effect related to COX-2 expression impairment, *Pain* 140 (2008) 274–283.
- [84] R. L.J. Pop-Busui, N. Lopes, T.L. Jones, BARI 2D Investigators, Prevalence of diabetic peripheral neuropathy and relation to glycemic control therapies at baseline in the BARI 2D cohort, *Peripher. Nerv. Syst.* 14 (2009) 1–13.
- [85] W.B. Potter, K.J. O'Riordan, D. Barnett, S.M. Osting, M. Wagoner, C. Burger, A. Roopra, Metabolic regulation of neuronal plasticity by the energy sensor AMPK, *PLoS One* 5 (2010) e8996.
- [86] T.J. Price, S.M. Geranton, Translating nociceptor sensitivity: the role of axonal protein synthesis in nociceptor physiology, *Eur. J. Neurosci.* 29 (2009) 2253–2263.
- [87] T.J. Price, M.H. Rashid, M. Millemcamp, R. Sanoja, J.M. Entrena, F. Cervero, Decreased nociceptive sensitization in mice lacking the fragile X mental retardation protein: role of mGluR1/5 and mTOR, *J. Neurosci.* 27 (2007) 13958–13967.
- [88] S.K. Roy Chowdhury, D.R. Smith, A. Saleh, J. Schapansky, A. Marquez, S. Gomes, E. Akude, D. Morrow, N.A. Calcutt, P. Fernyhough, Impaired adenosine monophosphate-activated protein kinase signalling in dorsal root ganglia neurons is linked to mitochondrial dysfunction and peripheral neuropathy in diabetes, *Brain* 135 (2012) 1751–1766.
- [89] W.A. Schmalhofer, J. Calhoun, R. Burrows, T. Bailey, M.G. Kohler, A.B. Wein-glass, G.J. Kaczorowski, M.L. Garcia, M. Koltzenburg, B.T. Priest, ProTx-II, a selective inhibitor of Nav1.7 sodium channels, blocks action potential propagation in nociceptors, *Mol. Pharmacol.* 74 (2008) 1476–1484.
- [90] L.A. Schrader, S.G. Birnbaum, B.M. Nadin, Y. Ren, D. Bui, A.E. Anderson, J.D. Sweatt, ERK/MAPK regulates the Kv4.2 potassium channel by direct phosphorylation of the pore-forming subunit, *Am. J. Physiol. Cell Physiol.* 290 (2006) C852–C861.
- [91] S.A. Schug, Persistent post-surgical pain: a view from the other side of the fence, *Pain* 153 (2012) 1344–1345.
- [92] R.J. Shaw, K.A. Lamia, D. Vasquez, S.H. Koo, N. Bardeesy, R.A. Depinho, M. Montminy, L.C. Cantley, The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin, *Science* 310 (2005) 1642–1646.
- [93] M.H. Shih, S.C. Kao, W. Wang, M. Yaster, Y.X. Tao, Spinal cord NMDA receptor-mediated activation of mammalian target of rapamycin is required for the development and maintenance of bone cancer-induced pain hypersensitivities in rats, *J. Pain* 13 (2012) 338–349.
- [94] E. Sirvinskis, S. Kinduris, J. Kapturauskas, R. Samalavicius, Perioperative use of metformin in cardiac surgery, *Medicina (Kaunas)* 46 (2010) 723–729.
- [95] N. Sonenberg, A.G. Hinnebusch, Regulation of translation initiation in eukaryotes: mechanisms and biological targets, *Cell* 136 (2009) 731–745.
- [96] M.R. Spasic, P. Callaerts, K.K. Norga, AMP-activated protein kinase (AMPK) molecular crossroad for metabolic control and survival of neurons, *The Neuroscientist: A Review Journal Bringing Neurobiology Neurology and Psychiatry* 15 (2009) 309–316.
- [97] S. Stamboulis, J.S. Choi, H.S. Ahn, Y.W. Chang, L. Tyrrell, J.A. Black, S.G. Waxman, S.D. Dib-Hajj, ERK1/2 mitogen-activated protein kinase phosphorylates sodium channel Na(v)1.7 and alters its gating properties, *J. Neurosci.* 30 (2010) 1637–1647.

- [98] A. Sukhodub, S. Jovanovic, Q. Du, G. Budas, A.K. Clelland, M. Shen, K. Sakamoto, R. Tian, A. Jovanovic, AMP-activated protein kinase mediates preconditioning in cardiomyocytes by regulating activity and trafficking of sarcolemmal ATP-sensitive K(+) channels, *J. Cell. Physiol.* 210 (2007) 224–236.
- [99] D.V. Tiillu, O.K. Melemedjian, M.N. Asiedu, N. Qu, M. De Felice, G. Dussor, T.J. Price, Resveratrol engages AMPK to attenuate ERK and mTOR signaling in sensory neurons and inhibits incision-induced acute and chronic pain, *Mol. Pain* 8 (2012) 5.
- [100] S. Tyagarajan, P.K. Chakravarty, B. Zhou, B. Taylor, M.H. Fisher, M.J. Wyvratt, K. Lyons, T. Klatt, X. Li, S. Kumar, B. Williams, J. Felix, B.T. Priest, R.M. Brochu, V. Warren, M. Smith, M. Garcia, G.J. Kaczorowski, W.J. Martin, C. Abbadie, E. McGowan, N. Jochnowitz, W.H. Parsons, Substituted biaryl pyrazoles as sodium channel blockers, *Bioorg. Med. Chem. Lett.* 20 (2010) 5480–5483.
- [101] A. Tzatsos, P.N. Tschlis, Energy depletion inhibits phosphatidylinositol 3-kinase/Akt signaling and induces apoptosis via AMP-activated protein kinase-dependent phosphorylation of IRS-1 at Ser-794, *J. Biol. Chem.* 282 (2007) 18069–18082.
- [102] V. Vingtdoux, L. Giliberto, H. Zhao, P. Chandakkar, Q. Wu, J.E. Simon, E.M. Janle, J. Lobo, M.G. Ferruzzi, P. Davies, P. Marambaud, AMP-activated protein kinase signaling activation by resveratrol modulates amyloid-beta peptide metabolism, *J. Biol. Chem.* 285 (2010) 9100–9113.
- [103] R. Vreven, M. De Kock, Metformin lactic acidosis and anaesthesia: myth or reality? *Acta Anaesthesiol. Belg.* 56 (2005) 297–302.
- [104] C. Wu, L. Boustany, H. Liang, T.J. Brennan, Nerve growth factor expression after plantar incision in the rat, *Anesthesiology* 107 (2007) 128–135.
- [105] C. Wu, M.A. Erickson, J. Xu, K.D. Wild, T.J. Brennan, Expression profile of nerve growth factor after muscle incision in the rat, *Anesthesiology* 110 (2009) 140–149.
- [106] K. Wuerzt, L. Quero, M. Sekiguchi, M. Klawitter, A. Nerlich, S. Konno, S. Kikuchi, N. Boos, The red wine polyphenol resveratrol shows promising potential for the treatment of nucleus pulposus-mediated pain in vitro and in vivo, *Spine (Phila Pa 1976)* 36 (2011) E1373–E1384.
- [107] J. Yan, O.K. Melemedjian, T.J. Price, G. Dussor, Sensitization of dural afferents underlies migraine-related behavior following meningeal application of interleukin-6 (IL-6), *Mol. Pain* 8 (2012) 6.
- [108] H. Yoshida, L. Bao, E. Kefaloyianni, E. Taskin, U. Okorie, M. Hong, P. Dhar-Chowdhury, M. Kaneko, W.A. Coetzee, AMP-activated protein kinase connects cellular energy metabolism to KATP channel function, *J. Mol. Cell Cardiol.* 52 (2012) 410–418.
- [109] P.K. Zahn, A. Subieta, S.S. Park, T.J. Brennan, Effect of blockade of nerve growth factor and tumor necrosis factor on pain behaviors after plantar incision, *J. Pain: Off. J. Am. Pain Soc.* 5 (2004) 157–163.
- [110] M. Zang, S. Xu, K.A. Maitland-Toolan, A. Zuccollo, X. Hou, B. Jiang, M. Wierzbicki, T.J. Verbeuren, R.A. Cohen, Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice, *Diabetes* 55 (2006) 2180–2191.
- [111] G. Zhou, R. Myers, Y. Li, Y. Chen, X. Shen, J. Fenyk-Melody, M. Wu, J. Ventre, T. Doebber, N. Fujii, N. Musi, M.F. Hirshman, L.J. Goodyear, D.E. Moller, Role of AMP-activated protein kinase in mechanism of metformin action, *J. Clin. Invest.* 108 (2001) 1167–1174.
- [112] Z.Y. Zhuang, H. Xu, D.E. Clapham, R.R. Ji, Phosphatidylinositol 3-kinase activates ERK in primary sensory neurons and mediates inflammatory heat hyperalgesia through TRPV1 sensitization, *J. Neurosci.: Off. J. Soc. Neurosci.* 24 (2004) 8300–8309.
- [113] R. Zoncu, A. Efeyan, D.M. Sabatini, mTOR: from growth signal integration to cancer, diabetes and ageing, *Nat. Rev. Mol. Cell Biol.* 12 (2011) 21–35.