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Review

Age-related changes in AMPK activation: Role for AMPK phosphatases and inhibitory phosphorylation by upstream signaling pathways

Antero Salminen[a,*], Kai Kaarniranta[b,c], Anu Kauppinen[d]

[a] Department of Neurology, Institute of Clinical Medicine, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland
[b] Department of Ophthalmology, Institute of Clinical Medicine, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland
[c] Department of Ophthalmology, Kuopio University Hospital, P.O. Box 100, FI-70029 KYS, Finland
[d] School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland

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ABSTRACT

AMP-activated protein kinase (AMPK) is a fundamental regulator of energy metabolism, stress resistance, and cellular proteostasis. AMPK signaling controls an integrated signaling network which is involved in the regulation of healthspan and lifespan e.g. via FoxO, mTOR/ULK1, CRCT-1/CREB, and SIRT1 signaling pathways. Several studies have demonstrated that the activation capacity of AMPK signaling declines with aging, which impairs the maintenance of efficient cellular homeostasis and enhances the aging process. However, it seems that the aging process affects AMPK activation in a context-dependent manner since occasionally, it can also augment AMPK activation, possibly attributable to the type of insult and tissue homeostasis. Three protein phosphatases, PP1, PP2A, and PP2C, inhibit AMPK activation by dephosphorylating the Thr172 residue of AMPKα, required for AMPK activation. In addition, several upstream signaling pathways can phosphorylate Ser/Thr residues in the β/γ interaction domain of the AMPKα subunit that subsequently blocks the activation of AMPK. These inhibitory pathways include the insulin/AKT, cyclic AMP/PKA, and RAS/MEK/ERK pathways. We will examine the evidence whether the efficiency of AMPK responsiveness declines during the aging process. Next, we will review the mechanisms involved in curtailing the activation of AMPK. Finally, we will elucidate the potential age-related changes in the inhibitory regulation of AMPK signaling that might be a part of the aging process.

Contents

* Corresponding author.
E-mail address: antero.salminen@uef.fi (A. Salminen)

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1. Introduction

The AMPK-activated protein kinase (AMPK) has a fundamental role in the cellular and organismal energy metabolism (Hardie, 2007; Lage et al., 2008; Hardie and Ashford, 2014). ATP depletion induced by energy deficiency activates AMPK signaling which in turn stimulates catabolic processes to maintain energy homeostasis. Accordingly, AMPK switches off many energy consuming reactions, such as protein and lipid syntheses. AMPK is an evolutionarily conserved energy sensor, e.g. it has been identified in yeast (SNF1), *Caenorhabditis elegans* (AAK-2), and plants (SnRK1) (Curtis et al., 2006; Hardie, 2007; Halford and Hey, 2009; Ghillebert et al., 2011). The mammalian heterotrimeric AMPK complex consists of a catalytic α unit and regulatory β and γ subunits. The subunits are made up two α (α1 and α2) and β (β1 and β2) isoforms as well as three γ (γ1-3) isoforms which are differentially expressed in tissues. The AMPK complex is activated allosterically by increasing AMP and ADP concentrations and also via the phosphorylation of the Thr172 residue on the α subunits (Fig. 1). There are three upstream kinases which can activate AMPK, (i) liver kinase B (LKB), also known as serine/threonine kinase 11 (STK11), (ii) Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ), and transforming growth factor-β-activated kinase 1 (TAK1) (Hardie, 2014). Correspondingly, the phosphorylated Thr172 residue can be dephosphorylated by three AMPK phosphatases, which include protein phosphatase 1 (PP1), protein phosphatase 2A (PP2A), and protein phosphatase 2C (PP2C) (Section 3.1). Moreover, some protein kinases of upstream signaling pathways can target the Ser/Thr residues at the β/γ interacting domain and prevent the AMPK activation by inhibiting the assembly of the AMPK complex (Section 3.2) (Figs. 1 and 2). The activation of AMPK is dependent on the energy status as well as on the activity of upstream stimulatory and inhibitory signaling pathways.

There is convincing data indicating that AMPK/SNF1/AAK-2 are key players in the aging process, extending the lifespan of yeast (Lorenz et al., 2009; Friis et al., 2014), *C. elegans* (Apfeld et al., 2004; Curtis et al., 2006; Mair et al., 2011), and *Drosophila* (Ulgherait et al., 2014; Yang et al., 2015). Moreover, the AMPK-mediated signaling can also increase the so-called healthspan in mice (Edwards et al., 2010; Martin-Montalvo et al., 2013; Kobilo et al., 2014). The AMPK signaling acts downstream to control a complex signaling network which includes several longevity pathways, such as FoxO, mTOR/ULK1, and SIRT1 signaling (Canto and Auwerx, 2010; Salminen and Kaarniranta, 2012; Burkewitz et al., 2014). Interestingly, several different approaches have indicated that there is a decline in the extent of AMPK activation with aging (Section 2). Consequently, impaired activation of AMPK signaling disturbs the function of the downstream signaling network thus causing problems in the maintenance of cellular homeostasis. We will examine the studies indicating that the aging process affects the activation capacity of AMPK signaling, and then provide a detailed review of the mechanisms capable of inhibiting the activation of AMPK signaling. In particular, we will focus on the age-related changes in the upstream inhibitory signaling which might disturb the activation of AMPK.

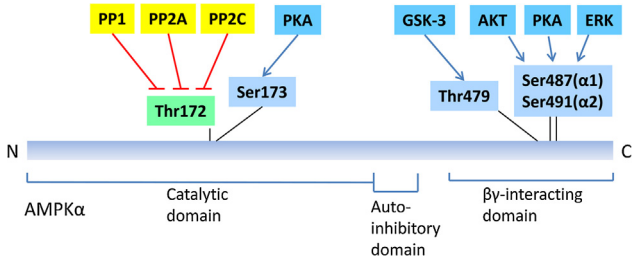

Fig. 1. Inhibition of AMPK activation both through protein phosphatases and via inhibitory phosphorylation induced by upstream protein kinases. The figure depicts the catalytic, autoinhibitory, and β,γ-interacting domains of AMPKα subunit. PP1, PP2A, and PP2C inhibit AMPK activity by dephosphorylating the Thr172 residue. AKT, PKA, and ERK phosphorylate Ser487 in the AMPKα1 subunit and Ser491 in the AMPKα2 subunit. GSK-3 phosphorylates Thr479 in the β,γ-interacting domain of AMPKα1. All of these phosphorylation reactions inhibit AMPK activation. PKA also phosphorylates Ser173 in the catalytic domain and this disturbs the phosphorylation of Thr172, thus also inhibiting AMPK activation. *Abbreviations:* AKT, protein kinase B; AMPKα, AMP-activated protein kinase α-subunit; ERK, extracellular signal-regulated kinase; GSK-3, glycogen synthase kinase-3; PKA, protein kinase A; PP, protein phosphatase; Ser, serine; Thr, threonine.

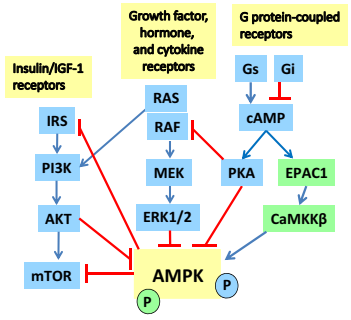

Fig. 2. Schematic illustration of the upstream signaling pathways which control the activity of AMPK signaling. Activating interactions are marked with blue arrows and inhibitory connections with red stoppers. Insulin/IGF-1 pathway inhibits AMPK via AKT-mediated inhibition, whereas AMPK inhibits insulin/IGF-1 pathway through IRS and mTOR inhibition. Ligands activating G protein-coupled receptors either inhibit (PKA) or activate (EPAC1) the AMPK signaling. The RAS/RAF pathway can inhibit AMPK activation via ERK1/2-mediated phosphorylation. *Abbreviations*: CaMKKβ, Ca2+/calmodulin-dependent protein kinase kinase β; cAMP, cyclic AMP; EPAC1, exchange protein directly activated by cAMP; Gi, inhibitory G protein; Gs, stimulatory G protein; IGF-1; insulin-like growth factor-1; IRS, insulin receptor substrate; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; PI3 K, phosphoinositide 3-kinase; RAF, rapid accelerated fibrosarcoma kinase; RAS, rat sarcoma kinase; other abbreviations as in Fig. 1.

2. Age-related changes in AMPK activation

In their seminal study, Reznick et al. (2007) demonstrated that the aging process was associated with a decline in the capacity of AMPK signaling to respond to different activation insults. This is an important observation since effective activation of AMPK is required to adjust the metabolism of organism to cope with both inherent and environmental stresses. The loss of that capacity can disturb the maintenance of homeostasis and enhance the aging process (Section 4). Reznick et al. (2007) compared the activation capabilities of the AMPK α2-subunit in the extensor digitorum longus (EDL) muscle of young and old rats by determining how robustly it reacted to physical exercise, AICAR infusion, and feeding with β-guanidinopropionic acid (β-GPA), all of which are well-known inducers of AMPK activation. They observed that although aging itself did not affect the activity of AMPK in EDL muscle, in contrast, its capacity to respond to these three stimuli was lost in EDL of old rats. Moreover, they reported that the stimuli-evoked responses in AMPK downstream targets, i.e. the phosphorylation of acetyl-CoA carboxylase (ACC) and mitochondrial biogenesis, were impaired with aging. However, the activity of LKB1, an upstream kinase, was not affected by either aging or AICAR treatment. Ljubicic and Hood (2009) studied the age-related responses of rat tibialis anterior (TA) muscle by provoking *in situ* contractions using electric stimulation. After monitoring the contraction-evoked effects separately in both the red (mitochondria-rich, oxidative) and white (glycolytic) muscle regions, they reported that the electric stimulation-induced increase in AMPKα phosphorylation at Thr172 was equal in the red part of TA both in young and old