Isoform-specific roles of AMPK catalytic α subunits in Alzheimer's disease

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AMPK is a heterotrimeric complex that serves as a major sensor of energy status in eukaryotic cells. Accumulating evidence depicts a complex role of dysregulated AMPK signaling in Alzheimer's disease (AD). In this issue of the *JCI*, Zimmermann et al. report on their investigation of AD-specific differential expression of AMPKα1 and AMPKα2 isoforms of the catalytic subunit and demonstrate that genetic reduction of AMPKα1, but not AMPKα2, rescued cognitive decline in AD mouse models. These findings reveal an isoform-specific role of AMPKα in the pathogenesis of AD, which likely provides a more precise target for future therapeutic development.

AMPK activation and outcome are likely context dependent

AMPK is an evolutionally conserved Ser/Thr kinase that serves a crucial physiological function as a cellular energy sensor (1). It is a heterotrimeric complex composed of a catalytic α subunit and two regulatory β and γ subunits at 1:1:1 ratio. The β subunit acts as a scaffold, interacting with the α subunit containing the kinase domain and the γ subunit containing four nucleotide-binding sites that allow AMPK to sense the status of cellular energy state. Two isoforms of α and β subunits and three isoforms of the γ subunit have been identified, giving rise to 12 distinct AMPK complexes (1). Despite functional redundancy, these complexes may differ in their tissue- and cell-specific distribution, regulation, subcellular localization, and biochemical properties and function (2). Cellular ATP concentration is kept at a constant level to ensure adequate ATP supply, which is essential to the survival of mammalian cells. Energy stress causes changes in AMP/ATP and ADP/ATP ratios. AMP binding to the γ subunit allosterically activates the complex and promotes the phosphorylation of Thr172 at the kinase domain of α subunit by upstream kinase of LKB1, which is a step required for catalytic activation of the complex (1). A rise in intracellular Ca^{2+} also activates AMPK through CAMKK2-mediated phosphorylation of Thr172. AMPK activation is instrumental in restoration of energy balance, acting by turning off energy-consuming processes, such as protein and lipid synthesis, and turning on energy-generating processes such as glucose metabolism, mitochondrial biogenesis, and autophagy, through a myriad of effectors (1).

As one of the high energy–consuming organs, the brain is vulnerable to disturbance in energy metabolism. A large body of evidence has demonstrated reduced energy metabolism as an early and consistent feature in Alzheimer's disease (AD) (3). Insulin resistance, abnormal glucose transport, mitochondrial dysfunction, dysregulated cholesterol metabolism, and calcium homeostasis are also prominent features in the AD brain (3, 4). These studies implicate a potential role of AMPK signaling in AD. Indeed, recent studies found putative pathogenic somatic mutations in the AD brain enriched in the AMPK pathway genes (5). Robust AMPK activation, as evidenced by increased Thr172 phosphorylation, was found in tangle- and pretangle-bearing neurons in AD brain (6). However, accumulating evidence appears to depict a complex role of dysregulated AMPK signaling in AD: AMPK directly phosphorylates tau protein, but it is also able to inhibit tau phosphorylation/aggregation through GSK3β inhibition or SIRT1 activation–mediated deacetylation of tau (7). AMPK activation could increase Aβ generation through ER stress (8) or transcriptional upregulation of BACE1 (9); however, it can also reduce Aβ levels by reducing lipid raft localization of APP protein through modulation of sphingomyelin levels or by enhancing Aβ clearance. AMPK activation by metformin alleviated mitochondrial, pathological, and cognitive deficits in AD models (10, 11), but AMPK inhibitor compound C treatment corrected toxic effects of Aβ on synaptic function (12, 13). While these studies underscore the critical roles of AMPK in the development of many aspects of AD, the controversy highlights the notion that AMPK activation and outcome are likely context dependent. It is possible that different isoforms and/or distinct AMPK complexes may contribute to the complexity (14).

AMPK isoform differences in Alzheimer's disease

Neurons show widespread expression of both AMPKα1 and AMPKα2 isoforms of the catalytic subunit. The AMPKα2 isoform is the predominant subunit in the brain, with constantly high neuronal expression, especially in the cortex and hippocampus, while AMPKα1 isoform expression peaks in embryos and declines during development (15, 16). Although AMPK is activated in AD, it is not known which AMPKα isoforms contribute to the

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disease. In this issue of the *JCI*, Zimmermann et al. (17) aimed to fill this important gap in our knowledge. The researchers found that expression of the AMPKα1 isoform was consistently elevated in the hippocampus from both sporadic and familial AD patients and in the Tg19959 APP transgenic mouse model. In contrast, AMPKα2 expression was decreased in the hippocampus from sporadic AD patients. These changes in AMPKα expression appeared to be AD specific, since AMPKα1/2 levels were unaffected in either Lewy body dementia (LBD) or frontotemporal dementia (FTD). Importantly, reduced expression of AMPKα1, but not AMPKα2, in hippocampal and cortical neurons effectively rescued synaptic deficits and memory decline in 2 different amyloid-β–bearing AD mouse models. However, it is somewhat surprising that no effects on amyloid pathology were found. Nevertheless, the reduction of AMPKα1, but not AMPKα2, corrected AD-associated hyperphosphorylation of eukaryotic elongation factor 2 (eEF2) and restored de novo protein synthesis (17). This change in protein synthesis is consistent with the rescuing effects on synaptic function and cognition, since nascent protein synthesis is essential for producing the synaptic modification needed for long-term memory storage (18). Given that prior studies from this same group demonstrated a critical role of aberrant eEF2 signaling in causing cognitive and pathological deficits in AD (19), these data suggest that upregulation of AMPKα1 in AD caused cognitive declines at least partly through eEF2-mediated chronic repression of protein synthesis. Therefore, this study provided a first set of evidence identifying an isoform-specific pathogenic role of AMPKα1 in AD, which paves the road for a more precise target development in the fight against AD (17).

Conclusions and future directions

While finding and characterizing an isoform-specific role of AMPKα in AD is a leap forward (17), future efforts to address some additional questions are certainly needed before we move ahead with translational studies: (a) reduced AMPKα1 rescued cognitive decline in APP/PS1 mice, yet metformin protected against cognitive and pathological deficits in this same mouse model, presumably through AMPK activation (10). How can we reconcile these contradictory findings? Does metformin selectively act on specific AMPK complexes other than those containing AMPKα1? Or, perhaps, did metformin mobilize additional pathways in concert with AMPK activation that are needed for protection? (b) It is unclear whether the expression pattern of AMPKα isoforms is the same in rodents and in humans, affecting whether this study (17) is applicable to human disease. This isoform expression ambiguity is not trivial, since prior studies demonstrated species-specific roles of different AMPK subunits. For example, AMPKγ2 is highly expressed in the human heart, but not in the mouse heart (2). AMPKγ2 mutations caused human Wolff-Parkinson-White syndrome, affecting the heart, but knockin mice with these mutations failed to recapitulate the heart phenotype (2). (c) The two APP transgenic mouse models (Tg19959 and APP/PS1) used in this study (17) are models with relatively rapidly progressive amyloid pathology and cognitive dysfunction. Slower progressive disease mouse models may need to be used to confirm the finding. It is worth noting that AMPK activation appears to be detrimental in a quickly developing mouse model of advanced Huntington disease (20), while it is protective in progressive animal models of early phase Huntington disease (21). Detailed characterization of AMPK signaling in different AD stages will also be of value.

Unlike reduced AMPKα1 expression, reduced AMPKα2 expression had no beneficial effect in AD mouse models. Does the result suggest AMPKα2 is not involved in AD? Probably not. It should be noted that the protein level of AMPKα2 was dramatically decreased in the hippocampus of sporadic AD patients. It is unlikely this decrease was a compensation for AMPKα1 elevation, since AMPKα2 levels were maintained when AMPKα1 levels were changed, as occurred in the brain from either familial AD patients or AMPKα1 haploinsufficient mice. More importantly, reduced AMPKα2 expression by itself impaired long-term synaptic plasticity and caused memory deficits, highlighting the importance of AMPKα2 as the predominant isoform in the brain. These data hint that AMPK2α reduction may contribute to AD. Indeed, reduced AMPKα2 expression exacerbated eEF2 hyperphosphorylation and protein synthesis inhibition in the AD mouse model. Therefore, future studies to restore AMPKα2 signaling in AD models are warranted to clarify the role of AMPKα2 in AD.

Given that changes in energy metabolism are implicated in many neurodegenerative diseases, it is puzzling that changes of AMPKα were only found in AD and not in other neurodegenerative diseases, such as FTD and DLB. This AD-specific effect may prompt one to speculate that different patterns of brain energy crisis accompany different types of diseases. However, prior studies demonstrated AMPK activation, measured by Thr172 phosphorylation, in cerebral neurons of multiple tauopathies, including Pick's disease and progressive supranuclear palsy (6). Since Zimmermann et al. (17) did not determine AMPK activation/phosphorylation in the human brain, there is a possibility that phosphorylation, rather than expressional change, may activate AMPK in FTD or DLB samples. Nevertheless, the isoform-specific dysregulation of AMPK in postmortem AD brain tissue raised the important question of how AMPKα1/2 is differentially regulated in AD. While Aβ could induce AMPKα phosphorylation via Ca^{2+}-mediated CAMKK2 activation (12), not much is known about how AMPKα expression may be modulated. In this regard, it is of interest to mention that AMPKα expression could be induced in activated astrocytes in the brain (15).

Zimmermann et al. (17) explored the mechanisms that underlie the protective effects of reduced AMPKα1 in APP Tg mice by focusing on protein synthesis. Given the distinct effects of AMPKα1 and AMPKα2 in the AD mouse, the authors performed mass spectrometry analysis and revealed that expression of ten proteins was uniquely restored by AMPKα1, but not by AMPKα2, which is worth further investigation. However, AMPK is a versatile signaling protein with multiple effectors that regulate various important cellular functions (7), many of which are altered in AD. Therefore, it remains to be determined what other downstream pathways, such as mitochondrial biogenesis (22), autophagy, cholesterol dyshomeostasis (23), etc., are selectively rescued by AMPKα1 reduction.

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