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An lncRNA switch for AMPK activation

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Cells and organisms have evolved elegant mechanisms to sense, respond, and adapt to fluctuations in energy and nutrient availability. One key mediator involved in such mechanisms is the adenosine monophosphate (AMP)-activated protein kinase (AMPK). AMPK functions as an energy sensor, and exists as a heterotrimeric complex which comprises one catalytic subunit (α subunit) and two regulatory subunits (β and γ subunits).[1] Under conditions of energy depletion with decreased cellular ATP level and increased AMP level, AMP directly binds to the γ regulatory subunit of the AMPK complex, resulting in allosteric activation of AMPK. In addition, full activation of AMPK requires phosphorylation of Thr172 in the α catalytic subunit. It has been proposed that AMP binding to the AMPK γ subunit leads to a conformational change of the AMPK complex, which facilitates Thr172 phosphorylation by the upstream kinase LKB1 and/or inhibits Thr172 de-phosphorylation by protein phosphatases, leading to full activation of AMPK in response to energy stress.[2] Once activated, AMPK phosphorylates many downstream substrates, which serves to switch off ATP-consuming anabolic processes and switch on ATP-generating catabolic processes, and eventually restores energy balance under conditions of energy stress.[3] In contrast to our deep understanding of AMPK regulation of downstream targets and biological functions, the underlying mechanisms of AMPK activation by energy stress remain much less understood.

Our recent study reported, for the first time, a long noncoding RNA (lncRNA)-involved mechanism in AMPK regulation.[4] Through RNA sequencing analysis, we identified *neighbor of BRCA1 gene 2* (*NBR2*), an uncharacterized noncoding gene,[5] as a glucose starvation–induced lncRNA. Further analyses revealed that energy stress induces *NBR2* expression through the LKB1-AMPK pathway. We then tested whether *NBR2* was also involved in feedback regulation of AMPK activation in response to energy stress. Indeed, *NBR2* knockdown attenuated AMPK activation upon energy stress, and overexpression of *NBR2* was sufficient to activate AMPK. AMPK activation was confirmed by phosphorylation of AMPK Thr172 and AMPK substrates involved in energy stress response, such as ACC, Raptor, and ULK1.[1] In response to energy stress, AMPK inactivates mammalian target of rapamycin complex 1 (mTORC1, also known as mechanistic TOR complex 1).[1] Accordingly, *NBR2* knockdown rendered cells partially resistant to energy stress–induced mTORC1 inactivation, while *NBR2* overexpression repressed mTORC1 activation.

Upon energy deprivation, AMPK also activates autophagy, a catabolic process which degrades cellular components (such as organelles and proteins) and recycles nutrients to maintain cell survival under metabolic stress conditions.[6,7] Autophagy is initiated by the ULK1 complex. Under energy rich conditions, mTORC1 is active and phosphorylates ULK1 to represses ULK1 function. Under energy deprivation conditions, AMPK, on one hand, inactivates mTORC1 and thus relieves mTORC1-mediated repression of ULK1, and on the other hand, directly phosphorylates ULK1 on a different set of sites and further promotes ULK1 function to induce autophagy.[6,7] We found that glucose starvation–induced ULK1 de-phosphorylation at Ser757 (an mTORC1 phosphorylation site) and phosphorylation at Ser555 (an AMPK phosphorylation site) were significantly compromised in *NBR2*-deficient cells, leading to defective GFP-LC3 punctate formation and p62 degradation under glucose deprivation, suggesting that energy stress–induced autophagy was defective in *NBR2* deficient cells. Consistent with the pro-survival role of AMPK and autophagy during energy stress conditions, we also observed that *NBR2* deficiency led to increased apoptosis under glucose starvation.

Mechanistically, we provide several lines of evidence to suggest a model that *NBR2* promotes AMPK signaling under energy stress through its direct interaction with AMPK and regulation of AMPK kinase activity. First, glucose starvation not only up-regulated *NBR2* expression, but also induced its interaction with AMPK. Further analyses revealed that *NBR2* directly interacted with the kinase domain of the α catalytic subunit of the AMPK complex; Second, in vitro kinase assay showed that *NBR2* promoted AMPK kinase activity; Third, functional characterization using a *NBR2* mutant which was incapable of interacting with AMPK showed that *NBR2* interaction with AMPK correlated with *NBR2* capability to promote AMPK kinase activity and to regulate AMPK downstream signaling events, suggesting that *NBR2* regulation of AMPK

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activation is likely mediated through *NBR2* interaction with AMPK.

Together, our data suggest a mechanism which involves an lncRNA switch to regulate AMPK function. Energy stress induces initial AMPK activation via AMP-mediated allosteric activation of AMPK and AMPK phosphorylation by LKB1 (as discussed earlier). Activated AMPK promotes *NBR2* expression in response to energy stress through an unknown mechanism. *NBR2* in turn interacts with AMPK and promotes AMPK activation under energy stress, forming a feed-forward loop to potentiate AMPK activation during long periods of energy stress. This model is consistent with our additional data showing that (1) glucose starvation–induced AMPK activation (typically within minutes) occurs earlier than glucose starvation–induced *NBR2* expression (usually after a couple of hours); and (2) *NBR2* deficiency only affects AMPK activation during long periods of energy stress (typically after several hours of glucose starvation). From a conceptual point of view, our study represents one of the first identifications of lncRNAs that regulate kinase function, and provides a broad framework to further understand the regulation of kinase signaling by lncRNAs.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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