Commentary on "LncRNA NBR2 engages a metabolic checkpoint by regulating AMPK under energy stress"

Sara Malih, Massoud Saidijam

Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

Correspondence to: Massoud Saidijam, Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran. Email: sjam110@yahoo.com.

Provenance: This is a Guest Commentary commissioned by Section Editor Zi-Guo Yang (Key Laboratory of Carcinogenesis and Translational Research, Breast Center, Peking University Cancer Hospital & Institute, Beijing, China).

Comment on: Liu X, Xiao ZD, Han L, *et al.* LncRNA NBR2 engages a metabolic checkpoint by regulating AMPK under energy stress. Nat Cell Biol 2016;18:431-42.

Submitted Nov 12, 2016. Accepted for publication Nov 24, 2016.

doi: 10.21037/cco.2017.01.02

View this article at: http://dx.doi.org/10.21037/cco.2017.01.02

Introduction

Recent whole genome and RNA deep sequencing technologies have represented long noncoding RNAs (lncRNAs) as a novel major field in various diseases investigations especially in cancers. LncRNAs, usually known as non-protein-coding RNA molecules longer than 200 nucleotides, are often involved in a spectrum of biological processes, including alternative splicing, regulation of protein activity, nuclear import, epigenetic modulation, precursors of small RNAs and silencing the microRNAs. Furthermore, some lncRNAs can regulate the expression of genes in trans or cis via inducing chromatin remodeling or affecting RNA polymerase II recruitment. Mechanistic studies have demonstrated that lncRNAs might perform these molecular functions through direct interactions with proteins, DNA sites and other RNA molecules. Hence, lncRNAs dysregulation can result in diverse diseases and disorders (1,2). Despite abundant newly discovered lncRNAs, additional functions and detailed signaling pathways of lncRNAs remain to be elucidated.

It is essential to maintain energy homeostasis for the proper functioning of all living cells. AMP-activated protein kinase (AMPK) is a central sensor of cellular energy status and regulator of organismal metabolism in eukaryotes and recently has been linked to some cellular processes such as cell polarity and autophagy (3). AMPK is a heterotrimer complex, composed of a catalytic subunit (α), and two regulatory subunits (β and γ). Under lowered intracellular ATP levels, AMP or ADP direct binding to the γ regulatory subunits, results in a conformational change that allosterically activates the complex. In addition to nucleotide binding, Thr172 phosphorylation in the α catalytic subunit is required for AMPK full activation, and some studies have demonstrated that this activation is directly through the serine/threonine kinase LKB1 (4). It could also be activated by drugs such as metformin and a wide variety of xenobiotics including Resveratrol and curcumine (3). AMPK activation enhances catabolic pathways and inhibits anabolic pathways to maintain energy balance.

Discussion of the results

Recently, Liu *et al.* reported that *NBR2*, a lncRNA, is involved in AMPK regulation (5). *BRCA1* and *NBR2* lie head-to-head and it is observed that these genes share a bi-directional promoter. It is also demonstrated that non-tissue specific repressor proteins interacting with DNA elements provide a uni-directional control on the respective gene (6).

There are two types of theories providing evidences on lncRNAs biological functions: (I) conservation of transcription and primary sequence across species and (II) Loss-of-function approaches. The combination of these approaches supports the essential roles of IncRNAs in mammalian biology.

Various techniques are used to conduct loss-of-function approaches, although they may represent some defects.

For example, RNA interference can result in off-target knockdown. Genetic knockouts could be used to overcome this challenge. To this end, it is required to manipulate DNA sequences that could serve as *cis* regulatory elements for neighboring genes; so, it is not easy to distinguish and relate the effects to loss of the RNA or to loss of the *cis* regulatory DNA elements. Therefore, gain-of-function experiments using heterologous vectors expressing lncRNAs can be utilized to overcome these challenges (7).

In the present study done by Liu *et al.* regarding these challenges, it is identified that glucose starvation induces the expression of NBR2 at least partly through the LKB1-AMPK pathway. It is also observed that NBR2 knockdown attenuates energy stress-induced phosphorylation of AMPK and mammalian target of rapamycin complex 1 (mTORC1) inactivation, thus proposes a feed-forward mechanism on NBR2_AMPK regulation.

Studies have displayed that ULK1 is a substrate for AMPK phosphorylation (8). In line with previous findings, present study results demonstrated that NBR2 deficiency promotes cell cycling, reduces autophagy and increases apoptosis which are initiated by ULK1 under glucose starvation.

There is a growing body of evidence demonstrating that AMPK is a metabolic tumor suppressor (9). In consistent with this, present study displayed that NBR2 knockdown enhances anchorage- independent growth, especially under energy stress. NBR2 deficiency also promotes tumor development in xenograft model, *in vivo*.

Mechanistic studies have demonstrated that lncRNAs might perform their molecular functions through direct interactions with proteins, DNA sites and other RNA molecules (1). In the current study, a new conceptual framework is provided to elucidate the regulation of kinase signaling by lncRNAs that is quite interesting.

Many of the interactions between lncRNAs and proteins investigated in various studies are obtained by RIP experiments. These approaches have represented some defects resulted from *in vitro* reassociation between RNAs and proteins (non-crosslinked RIP) or from indirect interactions mediated by protein (formaldehyde crosslinked RIP). Therefore, further identifications including UV CLIP and perturbation approaches are recommended to examine direct RNA-protein interactions and to disrupt interaction domains on both the RNA and interacting protein, respectively (7).

Additionally, current biochemical techniques for investigating RNA-chromatin or RNA-protein interactions are restricted to *in vitro* studies, which are unable to demonstrate *in vivo* interactions, precisely. Current computational analysis have demonstrated that, most of the lncRNAs characterized from human cells/tissues do not appear to have corresponding ortholog genes in invertebrate organisms, and in some cases, even in mouse. Such characteristics significantly restricts the application of relevant model systems, such as genetically engineered mouse models, to investigate the lncRNAs identified in humans. Eventually, translating the findings of lncRNAs made from other model organisms into human setting could become controversial if not conserved between different organisms (10).

These challenges indicate a need for novel approaches to determine the molecular components of lncRNA complexes *in vivo*. Further mechanistic studies are still required to clarify the exact regulatory mechanisms of AMPK by NBR2. To this end, determining crystal structure of the NBR2-AMPK complex is of significant importance.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Malih S, Saidijam M. Commentary on "LncRNA NBR2 engages a metabolic checkpoint by regulating AMPK under energy stress". Chin Clin Oncol 2017;6(1):10. doi: 10.21037/cco.2017.01.02

