New insights into MTORC1 amino acid sensing and activation

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Comment on: Jung J, Genau HM, Behrends C. Amino acid-dependent mTORC1 regulation by the lysosomal membrane protein SLC38A9. Mol Cell Biol 2015;35:2479-94.

The mechanistic target of rapamycin complex 1 (MTORC1) is an evolutionarily conserved eukaryotic Ser/Thr kinase that works as a master transducer of multiple cellular inputs involving growth factor sensing, and amino acid and ATP availability. Nutrient-rich conditions promote MTORC1 activation, which in turn leads to the stimulation of various anabolic processes such as protein and lipid synthesis, and cellular growth. Similarly, once activated MTORC1 also inhibits certain catabolic pathways such as macroautophagy/autophagy. In this regard, it is no surprise that a common feature in many cancer types is the deregulation of MTORC1 signaling (1). The close relationship between MTORC1 and cancer highlights the importance for a better understanding of the regulatory pathways by which MTORC1 is activated.

In recent years, enormous progress has been made in understanding the mechanisms by which MTORC1 can sense amino acid levels inside the cell. Under amino acid-rich conditions MTORC1 is translocated to the surface of the lysosome where it interacts directly with the active GTP-bound form of the small GTPase protein RHEB (2,3). The TSC complex, which acts as a RHEB GTPase-activating protein, regulates the transition of RHEB between its active GTP-bound and inactive GDP-bound forms. Thus, the TSC complex negatively regulates MTORC1 by inhibiting RHEB (4). Although RHEB binding is essential for MTORC1 activity, the RRAG family of small GTPases regulates MTORC1 translocation to the lysosomal surface. Mammals have 4 RRAG homologs (RRAGA, RRAGB, RRAGC and RRAGD) that work as heterodimeric complexes made of RRAGA or RRAGB with RRAGC or RRAGD. Amino acid stimulation promotes the formation of a GTP-bound RRAGA/B and GDP-bound RRAGC/D complex leading to MTORC1 recruitment to the lysosome where RHEB can subsequently activate it. Conversely, GDP-bound RRAGA/B and GTP-bound RRAGC/D inhibit MTORC1 recruitment to the lysosome (5,6). A major regulator of the RRAG GTPases includes the Ragulator complex formed by LAMTOR1 through LAMTOR5, which works as a guanine nucleotide exchange factor for RRAGA/B, thus promoting MTORC1 recruitment to the lysosome and its subsequent activation (3). In turn, Ragulator, and consequently MTORC1 lysosomal translocation, is controlled by the lysosomal vacuolar-type H^+-translocating ATPase (v-ATPase) protein complex, which pumps protons inside the lysosomal lumen; this complex directly interacts with Ragulator in an amino acid-dependent manner (7). Thus, the link between amino acid sensing and MTORC1 signaling requires the acidification of the lysosomal lumen dependent on the v-ATPase, which promotes the Ragulator guanine nucleotide exchange factor activity of RRAGA/B and translocates MTORC1 to the lysosome so it can be activated by RHEB binding.

The fact that the lysosomal lumenal pH plays an important role in MTORC1 activation opens the possibility for a model where, in order for MTORC1 to sense amino acids in the lysosome lumen, the amino acids first need to be transported out into the cytoplasm using the pH gradient generated by the v-ATPase pump. In a recent paper, Jung *et al.* identified SLC38A9, an 11-transmembrane domain protein that belongs to the family of sodium-coupled amino acid transporters as an interacting member of the RRAG-Ragulator pathway and regulator of MTORC1 signaling (8).

Through immunoprecipitation followed by both mass spectrometry and immunoblotting analysis, Jung *et al.* showed that tagged SLC38A9 interacts with RRAGA, RRAGB, RRAGC and Ragulator subunits LAMTOR1, LAMTOR2 and LAMTOR4, but not TSC2 (a member of the TSC complex that was used as a negative control). Immunoprecipitation assays using antibodies against SLC38A9 and RRAGC further confirm that these proteins associate when present at an endogenous level.

To determine the specific region of SLC38A9 that mediates the interaction with the RRAG-Ragulator complexes, the authors decided to immunoprecipitate a tagged version of full-length SLC38A9 and the N-terminal 119 amino-acid domain that faces the cytoplasm. Mass spectrometry results indicated that the SLC38A9 N-terminal region is adequate for maintaining the association with RRAGA, RRAGB, and LAMTOR1.

Next, the authors sought to determine SLC38A9 localization. To this end, immunofluorescence colocalization analysis between the lysosomal marker LAMP2 and GFP-tagged versions of full-length or N-terminal SLC38A9 were examined. Both full length and N-terminal SLC38A9 colocalize with LAMP2, with the soluble N-terminal version showing a more dispersed pattern across the cytoplasm.

Considering that amino acid levels in the cell play an important role in MTORC1 activation by the RRAG-Ragulator pathway, the authors sought to analyze if SLC38A9 protein interactions and cellular localization are dependent on the presence of amino acids. Through immunofluorescence colocalization analysis Jung *et al.* discovered that SLC38A9 interaction with RRAGC, LAMTOR2 and LAMTOR5, as well as its lysosomal localization is independent of the amino acid pool. However, immunoprecipitation analysis of full-length tagged SLC38A9 shows increased interaction with RRAGA and RRAGC under conditions of amino acid starvation. Furthermore, immunoprecipitation of GTP-locked RRAGB and GDP-locked RRAGC show a complete loss of SLC38A9 interaction, whereas mutants locked in the opposite nucleotide binding state show increased association.

To extend the analysis, the authors tried to determine if SLC38A9 is required for MTORC1 signaling. Whereas SLC38A9 depletion leads to decreased phosphorylation of standard MTORC1 substrates (EIF4EBP1, RPS6KB1/p70S6K and ULK1), overexpression of full-length SLC38A9 or its N-terminal domain increases ULK1 and RPS6KB1 phosphorylation, indicating that SLC38A9 might be involved in the activation of MTORC1. Notably, RHEB depletion inhibits the effects of SLC38A9 overexpression on RPS6KB1 phosphorylation.

To further investigate the effects of SLC38A9 on MTORC1 signaling, the authors tested ULK1 phosphorylation under conditions of amino acid starvation and replenishment. Cycloheximide treatment, which increases amino acid content by inhibiting translation, fails to increased ULK1 phosphorylation when SLC38A9 is depleted. Consistently, SLC38A9 overexpression partly prevents the amino acid starvation-dependent decrease in ULK1 phosphorylation. Moreover, after amino acid starvation, replenishment with amino acids that regulate MTORC1 activity—arginine, glutamine or leucine—fail to increase ULK1 phosphorylation to wild-type levels under SLC38A9-depleted conditions. These findings indicate that SLC38A9 is an important activator of MTORC1 under amino acid-rich conditions.

Finally, Jung *et al.* investigated if SLC38A9 can regulate MTORC1 translocation to the lysosome under amino acid-rich conditions. Unexpectedly, SLC38A9 depletion increases MTORC1 lysosomal localization both when amino acids are present and when they are lacking, indicating that SLC38A9 has a central function in amino acid-dependent MTORC1 localization, in particular allowing the complex to be released from the lysosome under amino acid-starvation conditions.

In summary, while searching for new regulators of MTORC1, Jung *et al.* came upon SLC38A9, a transmembrane domain protein involved in amino acid transport. Interaction with the RRAG-Ragulator complexes and lysosomal cellular localization, positions SLC38A9 as a prime candidate in the regulation of MTORC1 signaling. SLC38A9 depletion and overexpression experiments indicate SLC38A9 functions upstream of MTORC1, with the former decreasing and the latter increasing MTORC1 activity. Furthermore, SLC38A9 depletion during amino acid stimulation fails to allow complete activation of MTORC1, indicating that SLC38A9 is a key factor in amino acid sensing by MTORC1. Most intriguingly is the result showing SLC38A9 protein levels are critical for starvation-dependent movement of MTORC1 away from the lysosome. Further investigation will be needed to elucidate this point. Others have also implicated SLC38A9 as a new member of the MTORC1 amino acid-sensing regulatory machinery (9,10). In the future, additional studies on SLC38A9 and other possible members of the MTORC1

activation pathway could provide information allowing the development of new therapeutic drugs targeting cancers where MTORC1 is deregulated.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Editor-in-Chief Maorong Jiang (Laboratory Animal Center of Nantong University, Nanjing, China).

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/biotarget.2017.04.01). Dr. Klionsky reports grants from National Institutes of Health, during the conduct of the study. The other author has no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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