

MTORC1 determines autophagy through ULK1 regulation in skeletal muscle

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Autophagy impairment has been implicated in several muscle disorders and in age-related dysfunction. Although previous reports pointed to FOXO as a positive regulator of autophagy in skeletal muscle, it remained unclear what is triggering autophagy. We found that TSC muscle knockout (TSCmKO) mice, characterized by specific depletion of TSC1 in skeletal muscle, and thus constant activation of MTORC1, develop a late-onset myopathy marked by the accumulation of autophagic substrates. In those mice, autophagy induction is blocked despite FOXO activation because of constant MTORC1-dependent inhibition of ULK1. Treatment of TSCmKO mice with rapamycin is sufficient to restore autophagy and to alleviate, at least in part, the myopathy. Inversely, inactivation of the MTORC1 pathway in RPTOR-depleted muscles triggers LC3B lipidation in spite of FOXO inhibition. In conclusion, MTORC1 constitutes the master regulator of autophagy induction in skeletal muscle and its deregulation leads to pathologic alterations of muscle homeostasis.

Autophagy is an essential, catabolic process that ensures the clearance of damaged organelles and misfolded proteins. In most cell types, inhibition of MTORC1 via fasting or administration of rapamycin induces autophagy. Several lines of evidence indicate that this induction is based on the relief of MTORC1-mediated phosphorylation of ULK1 at Ser757 and the subsequent activation of the

ULK1-ATG13-RB1CC1/FIP200 complex by AMPK. Previous reports showed that constitutively active FOXO3, but not rapamycin, induces autophagy in skeletal muscle, suggesting that in these cells autophagy is independent of MTORC1 but instead is regulated by FOXO signaling through the transcriptional regulation of autophagy genes. However, the control of autophagy by FOXO would not allow instantaneous activation of the process, and the failure of rapamycin to induce autophagy in skeletal muscle might be based on insufficient inhibition of MTORC1.

To analyze the role of MTORC1 in autophagy regulation directly, we generated TSCmKO mice, which lack the main endogenous inhibitor of MTORC1 in muscle. As expected, the immediate MTORC1 target RP6KB/ribosomal protein p70S6 kinase is constantly activated and, as a consequence of the negative feedback onto IRS1, the FOXO pathway is simultaneously activated in mutant mice. This unique configuration allowed testing the role of MTORC1 and FOXO pathways on autophagy as they are expected to have reciprocal effects on the flux. Examination of muscles from aging TSCmKO mice revealed accumulation of autophagic substrates and abnormal organelles. Concomitantly, muscles display a loss of mass and a reduction of force, indicating that autophagy impairment causes progressive myopathy in TSCmKO mice (Fig. 1).

To better understand these defects, 2-mo-old TSCmKO mice were submitted to starvation, which is a well-described trigger of autophagy. The first interesting

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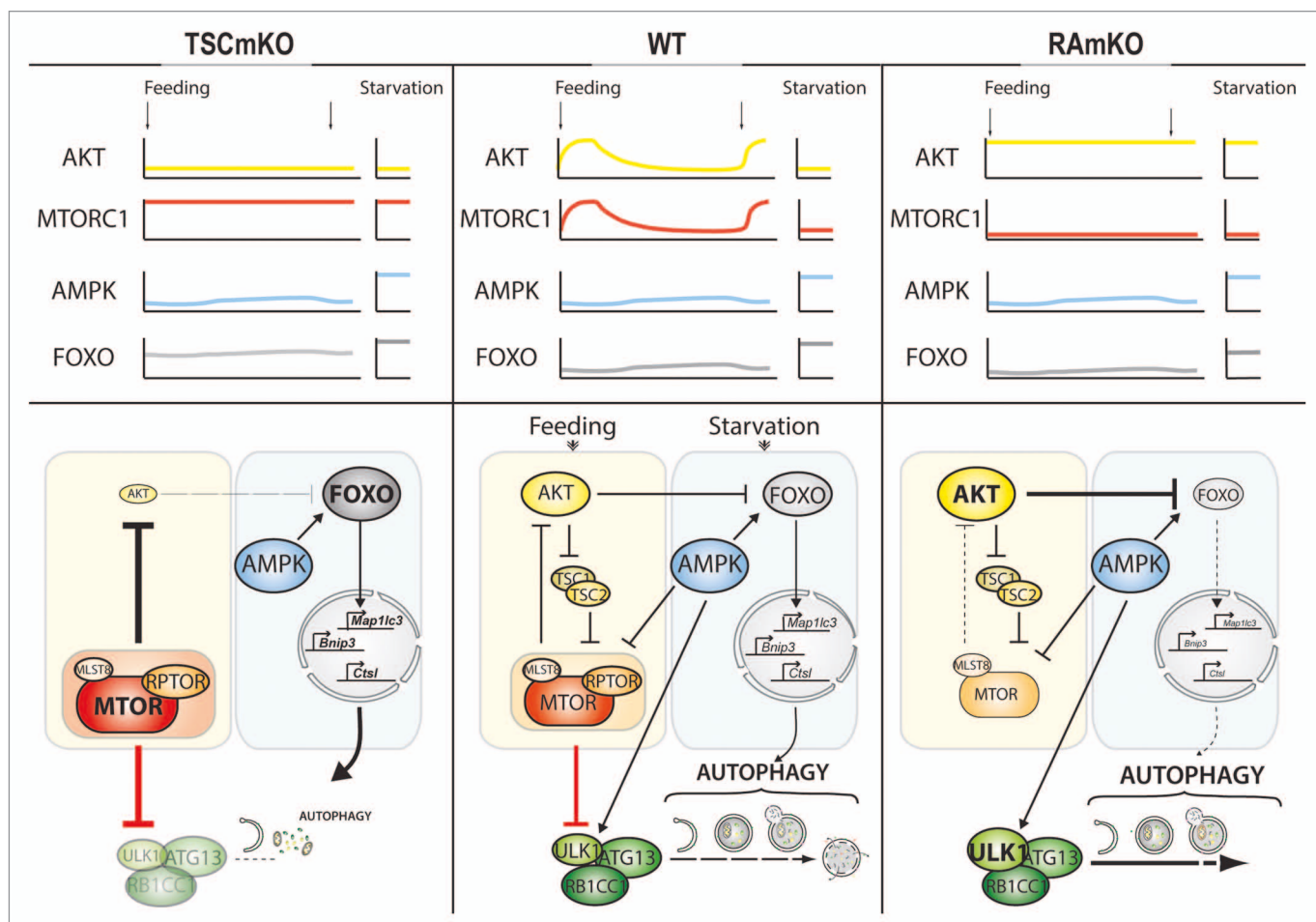


Figure 1. In physiological conditions, the activity of the AKT/MTORC1 axis depends on feeding behavior and varies within the day (top panel). In the fed state, MTORC1 blocks the induction of autophagy through phosphorylation of ULK1 at Ser757. Constitutive autophagy involved in basal turnover of organelles and proteins is induced upon the release of MTORC1-dependent inhibition of the ULK1 complex (lower panel). During starvation, activation of the AMPK/FOXO axis promotes transcription of autophagy genes and ensures higher, prolonged autophagy flux required to maintain cell homeostasis. In TSCmKO muscles, MTORC1 is active irrespective of other stimuli and constantly inhibits autophagy induction (left panel). Conversely, MTORC1 inactivation in RAmKO muscles facilitates permanent induction of the flux, whereas FOXO inactivation may limit the degradation steps (right panel). In these two mouse models, impaired autophagy flux is likely involved in the atrophy of most muscles and the onset of the myopathy.

observation was that TSCmKO mice show permanently active MTORC1 signaling irrespective of their feeding behavior, which was further confirmed in vitro by incubating TSCmKO fibers in amino acid- and glucose-depleted medium. Hence, TSC1 depletion is sufficient to promote MTORC1 activation in muscle cells, independently from other stimuli. In TSCmKO muscle, it remains to be determined whether MTORC1, known to be activated by RHEB at the lysosome membrane, is targeted to this organelle in an amino acid-independent mechanism or whether its activation occurs at a different subcellular compartment.

Importantly, induction of autophagy is blocked in TSCmKO muscles even after

prolonged starvation of the mice, as well as in vitro, when mutant muscle fibers are exposed to glucose- and amino acid-depleted medium. The observed effects are not due to long-term adaptation of the muscle, as transient hyperactivation of MTORC1 using shRNA-mediated knockdown of TSC1/2 is sufficient to impair the autophagy flux. Since previous reports showed that expression of autophagy genes, such as *Map1lc3b* (*Lc3b*) or *Bnip3*, is critical for autophagy, we verified that those genes are efficiently upregulated in mutant muscles, consistent with the activation of FOXO signaling. Instead, we established that this blockade was based on the strong increase in phospho-ULK1^{S757} as overexpression of

nonphosphorylated ULK1 by muscle electroporation is sufficient to activate autophagy and thus to reverse the inhibitory effect of MTORC1 (Fig. 1). It remains to be tested whether other phosphorylation sites of ULK1 physiologically modulate its interaction with AMPK and MTORC1, and interfere with the stabilization of the ULK1-ATG13-RB1CC1 complex in muscle cells.

Interestingly, treatment of TSCmKO mice with the MTORC1 inhibitor rapamycin restores autophagy, and normalizes phosphorylation of ULK1 at Ser757 becomes undetectable. Most importantly, 3 weeks treatment is also sufficient to reverse some of the myopathy defects and to re-establish force in

12-mo-old mutant mice. The possibility to alleviate the muscle phenotype of aged TSCmKO mice by inhibiting the MTORC1 pathway was unexpected to say the least and this observation raises the interesting question as to the mechanisms involved in the fast removal of those autophagy-related muscle alterations, including vacuoles and protein aggregates.

Last, to test the consequences of the simultaneous inactivation of MTORC1 and FOXO pathways on autophagy, we also examined RAmKO mice, which are depleted for RPTOR in adult skeletal muscle. In those mice, autophagy is permanently induced, even in fed conditions. Expression of autophagy-related genes, although slightly increased upon starvation, remains strongly reduced indicating that the increased autophagy induction occurs independently from FOXO inhibition (Fig. 1). Nonetheless, the scaffold molecule SQSTM1/p62, ubiquitinated

proteins and LAMP-positive structures accumulate in muscle from 6-mo-old RAmKO mice. These features are indicative of an impaired processing of the flux, which may be related to the inactivation of FOXO signaling: one possibility might be that degradation steps are impaired, as expression of the lysosomal protease CTSL/cathepsin L is barely detectable in RAmKO muscles. This aspect requires more detailed investigation to dissect the origin of the defects and the potential involvement of FOXO inhibition.

In conclusion, on the one hand the work establishes that MTORC1 plays a dominant role in controlling the induction of autophagy in skeletal muscle. Both exaggerated activation and inhibition of MTORC1 affect proper, balanced autophagy flux and are thus detrimental for muscle homeostasis. Hence, modulations of MTORC1 may be the trigger in altering autophagy flux during the day

in a circadian manner (Fig. 1). On the other hand, the data also indicate that prolonged, sustained autophagy may rely on correct activation of FOXO signaling. Based on these lines of evidence, one should reconsider the direct implication of MTORC1 in the pathogenesis of muscle disorders, such as collagen VI-myopathies, for which autophagy impairment has been related to AKT imbalance. Our study also opens new perspectives for the development of therapeutic strategies targeting MTORC1 as a determinant element for autophagy regulation and muscle wasting. However, it also points out that major vigilance has to be taken in the modulation of the pathway given that muscle homeostasis appears to depend on a dynamic and balanced regulation of autophagy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.