TACSM Abstract

Selective Inhibition of Ribosomal Biogenesis Over Cap-Dependent Translation by Rapamycin in L6 Myotubes

SELINA URANGA & JAMES D. FLUCKEY

Muscle Biology Laboratory; Kinesiology and Sport Management; Texas A&M University; College Station, TX

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Advisor / Mentor: Fluckey, James D. (jfluckey@tamu.edu)

ABSTRACT

The mechanistic target of rapamycin, or mTOR, lies at the center of skeletal muscle anabolic regulation. The mTOR kinase has at least two complexes that aide in carrying out responses to anabolic signals. Complex 1 (mTORC 1) activation leads to cap-dependent translation and ribosomal biogenesis via phosphorylation of its two downstream targets 4EBP1 and P70S6K respectively. mTORC1 is inhibited by rapamycin while mTORC2 is not; however, cap-dependent translation via mTORC1 may still occur under rapamycin inhibition. **PURPOSE**: To identify timing and mechanistic pathway by which selective skeletal muscle anabolism continues in the presence of rapamycin inhibition. **METHODS**: Murine L6 myoblasts were cultured in $10cm^3$ plates in standard culture medium supplemented with 1% pen/strep and 10% FBS. Differentiation was induced at 70% confluence by switching to a low serum medium. Myoblasts were differentiated until myotubes were visible at which point plates were treated with 100nM of rapamycin for 1 hour or 12 hours. Control plates were treated in the same way with vehicle control, DMSO. Myotubes were harvested immediately after treatment and protein content of key anabolic markers was measured using Western blotting techniques. **RESULTS**: Treatment with rapamycin significantly decreased P70S6K phosphorylation, and phosphorylated to total ratio after 12 hours of treatment compared to its respective DMSO control ($p<0.05$ for both). Twelve hours of treatment with rapamycin resulted in significantly lower phosphorylated, and total 4EBP1 ($p< 0.05$ for both) resulting in no significant change in ratio. Interestingly, eIF4E protein content remained unchanged with rapamycin treatment resulting in an increased ratio of eIF4E to 4EBP1. **CONCLUSION**: Treatment with rapamycin ablates P70S6K phosphorylation and subsequently inhibits mTORC1-dependent ribosomal biogenesis. Although there was no apparent reduction of the p4EBP1/4EBP1 ratio, acute cap-dependent translation is likely uninhibited by 1 or 12 hours of treatment with rapamycin. Total 4EBP1 protein content was decreased while its binding partner, eIF4E remained unchanged. Thus, the ratio of eIF4E to 4EBP1 was elevated after treatment with rapamycin making it likely that unbound eIF4E was free to initiate cap-dependent translation even in the presence of rapamycin.