OFFICIAL ABSTRACT and CERTIFICATION

Identification of Novel Modulators of mTORC2 Activity

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Mammalian target of rapamycin (mTOR) is a serine/threonine kinase, central to many cellular processes, including cell metabolism, growth, proliferation, autophagy, and mobility. mTOR assembles two biochemically and functionally distinct complexes: mTORC1 and mTORC2. Because mTORC1 has been extensively studied, we focused on mTORC2, which remains poorly understood. It is known that mTORC2 becomes activated in response to growth factors, such as insulin; however, the mechanism underlying its activation remains largely unknown. In this study, we treated four common human cell lines with insulin for various times to determine the optimal conditions needed for robust mTORC2 activation. By monitoring AKT phosphorylation, we found that mTORC2 is rapidly activated in both HEK 293 and A549 cells; however, no measurable activation was observed in the HeLa or U-2OS cells. This optimal treatment was then used to activate mTORC2 in a cell line stably expressing FLAG-tagged mTOR, RICTOR, Sin1, and GβL. mTORC2 was immunoprecipitated from insulin-stimulated and untreated cells using anti-FLAG sepharose, and interacting proteins from each condition were identified via affinity enrichment mass-spectrometry (AE-MS). Over 1000 proteins were identified, and statistical analysis showed that only 20 were enriched in insulin treated cell lysates compared to untreated counterparts. Of these top 20 hits, 6 were part of the 14-3-3 phospho-protein binding family. For future advancements, these activators will be used in kinase assays to determine their potential to activate mTORC2. Positive hits may then be used to assemble an activated complex for cryo-EM structure determination.

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