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DEPTOR Is an mTOR Inhibitor Frequently Overexpressed in Multiple Myeloma Cells and Required for Their Survival

Timothy R. Peterson,[1,2] Mathieu Laplante,[1,2] Carson C. Thoreen,[1,2] Yasemin Sancak,[1,2] Seong A. Kang,[1,2] W. Michael Kuehl,[4] Nathanael S. Gray,[5,6] and David M. Sabatini[1,2,3,*]

[1]Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142, USA
[2]Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[3]Koch Center for Integrative Cancer Research at MIT, 77 Massachusetts Avenue, Cambridge, MA 02139, USA
[4]National Cancer Institute, 8901 Rockville Pike, Bethesda, MD 20814, USA
[5]Department of Cancer Biology, Dana Farber Cancer Institute
[6]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School
250 Longwood Avenue, Boston, MA 02115, USA
*Correspondence: sabatini@wi.mit.edu
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SUMMARY

The mTORC1 and mTORC2 pathways regulate cell growth, proliferation, and survival. We identify DEPTOR as an mTOR-interacting protein whose expression is negatively regulated by mTORC1 and mTORC2. Loss of DEPTOR activates S6K1, Akt, and SGK1, promotes cell growth and survival, and activates mTORC1 and mTORC2 kinase activities. DEPTOR overexpression suppresses S6K1 but, by relieving feedback inhibition from mTORC1 to PI3K signaling, activates Akt. Consistent with many human cancers having activated mTORC1 and mTORC2 pathways, DEPTOR expression is low in most cancers. Surprisingly, DEPTOR is highly overexpressed in a subset of multiple myelomas harboring cyclin D1/D3 or c-MAF/MAFB translocations. In these cells, high DEPTOR expression is necessary to maintain PI3K and Akt activation and a reduction in DEPTOR levels leads to apoptosis. Thus, we identify a novel mTOR-interacting protein whose deregulated overexpression in multiple myeloma cells represents a mechanism for activating PI3K/Akt signaling and promoting cell survival.

INTRODUCTION

Mammalian TOR (mTOR) is an evolutionarily conserved serine/threonine kinase that integrates signals from growth factors, nutrients, and stresses to regulate multiple processes, including mRNA translation, cell-cycle progression, autophagy, and cell survival (reviewed in Sarbassov et al., 2005). It is increasingly apparent that deregulation of the mTOR pathway occurs in common diseases, including cancer and diabetes, emphasizing the importance of identifying and understanding the function of the components of the mTOR signaling network. mTOR resides in two distinct multiprotein complexes referred to as mTOR complex 1 (mTORC1) and 2 (mTORC2) (reviewed in Guertin and Sabatini, 2007). mTORC1 is composed of the mTOR catalytic subunit and three associated proteins, raptor, PRAS40, and mLST8/GβL. mTORC2 also contains mTOR and mLST8/GβL, but instead of raptor and PRAS40, contains the proteins rictor, mSin1, and protor.

mTORC1 controls cell growth in part by phosphorylating S6 kinase 1 (S6K1) and the eIF-4E-binding protein 1 (4E-BP1), key regulators of protein synthesis. mTORC2 modulates cell survival in response to growth factors by phosphorylating its downstream effectors Akt/PKB and serum/glucocorticoid regulated kinase 1 (SGK1) (reviewed in Guertin and Sabatini, 2007).

In addition to directly activating Akt as part of mTORC2, mTOR, as part of mTORC1, also negatively regulates Akt by suppressing the growth factor-driven pathways upstream of it. Specifically, mTORC1 impairs PI3K activation in response to growth factors by downregulating the expression of insulin receptor substrate 1 and 2 (IRS-1/2) and platelet-derived growth factor receptor-beta (PDGFR-β) (reviewed in Sabatini, 2006). The activation of Akt that results from treating cells with the mTORC1 inhibitor rapamycin may contribute to the limited success to date of this drug and its analogs as cancer therapies.

While most information concerning the involvement of the mTOR pathway in human cancers is consistent with a role for mTOR in directly promoting tumor growth, there are also indications in the literature that mTOR possesses tumor suppressor-like properties. Thus, the tumors that develop in patients with tuberous sclerosis complex (TSC), a syndrome characterized by mTORC1 hyperactivation, are thought to have a limited growth potential due to the PI3K inactivation caused by the aforementioned feedback loop (Manning et al., 2005; Zhang et al., 2007). In addition, partial loss-of-function alleles of mTOR confer susceptibility to plasmacytomas in mice, though the mechanism for this effect has not been clarified (Bliskovsky et al., 2003).

Here, we identify DEPTOR as an mTOR binding protein that normally functions to inhibit the mTORC1 and mTORC2

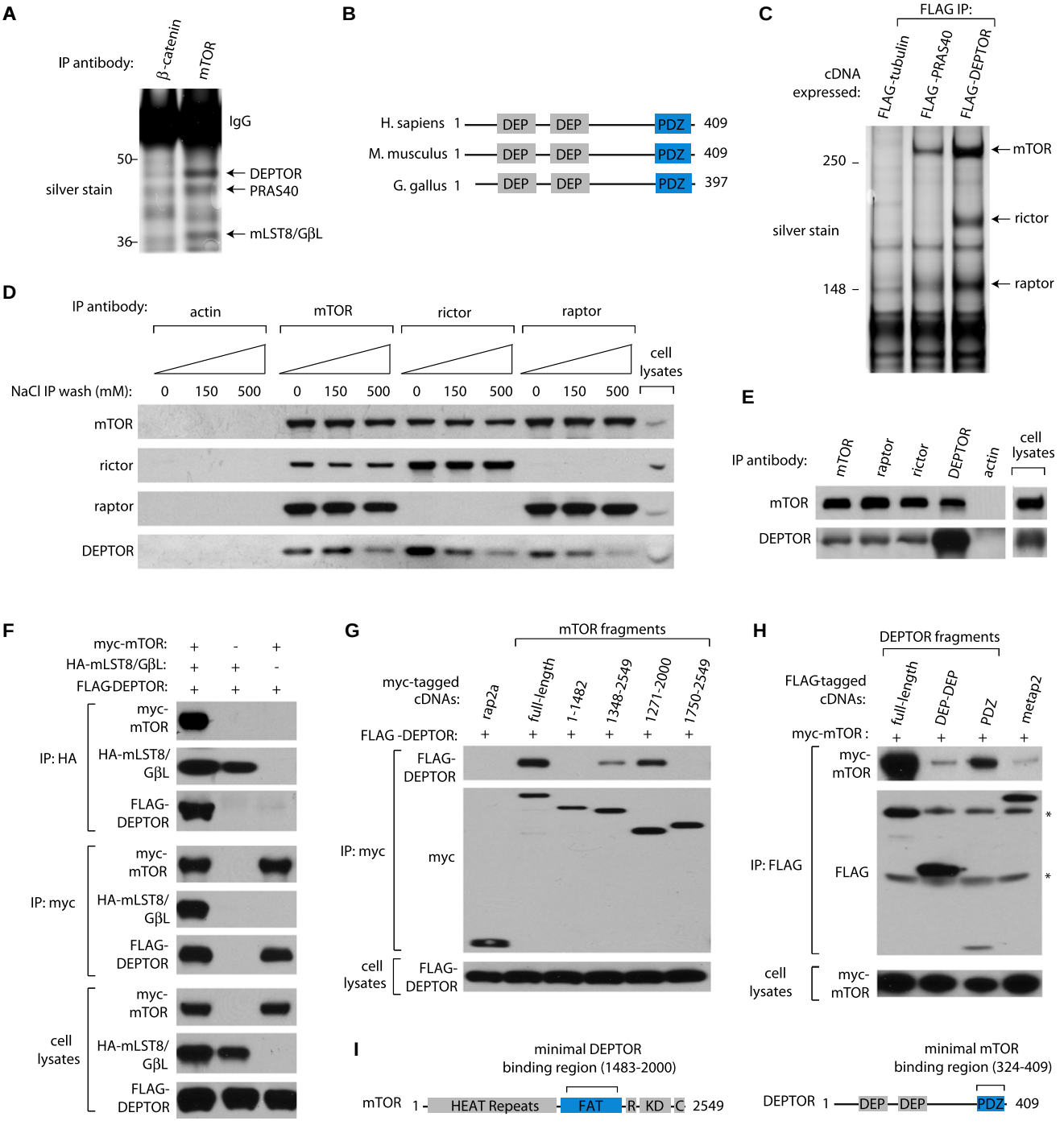

Figure 1. DEPTOR Is an mTOR-Interacting Protein

(A) Silver stain of SDS-PAGE analysis of mTOR immunoprecipitates prepared from HEK293E cells.

(B) Schematic representation of structural features of human DEPTOR and its mouse and chicken orthologs.

(C) Endogenous mTOR, raptor, and rictor coimmunoprecipitate with epitope-tagged DEPTOR. FLAG immunoprecipitates from HEK293E cells expressing the indicated proteins were analyzed by SDS-PAGE and silver staining.

(D) Interaction of endogenous DEPTOR with endogenous mTORC1 and mTORC2 is sensitive to high salt-containing buffers. Indicated immunoprecipitates were prepared from HEK293E cells, washed with buffers containing the indicated amounts of NaCl, and analyzed by SDS-PAGE and immunoblotting for the indicated proteins.

(E) Endogenous DEPTOR coimmunoprecipitates endogenous mTOR. DEPTOR immunoprecipitates were prepared from HeLa cells, washed in a buffer containing 150 mM NaCl, and analyzed as in (D).