

mTOR/PRAS40 interaction

Hypertrophy or proliferation

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Signals from growth factors, nutrients, energy status, as well as many stressors impinge upon the mechanistic target of rapamycin (mTOR), which exists in 2 distinct complexes, mTORC1 and mTORC2. mTORC1 is rapamycin-sensitive and controls cell size, cell cycle, and metabolism, whereas mTORC2 mediates survival and cytoskeletal organization. Thousands of publications document the key role played by mTOR as a central controller of cellular growth and tissue homeostasis, with alteration of mTOR signaling associated with several disease states, including cancer or heart diseases.¹ Indeed, chronic elevated mTOR signaling associated with altered growth kinetics and metabolic changes are characteristics of dysfunctional cancer cells and cardiomyocytes. The mTOR-dependent stimulation of cellular growth and proliferation in human diseases highlights its importance as a clinically important drug target, and mTORC1 inhibition with rapamycin has been shown to reduce cancer growth, improve cardiac function after pressure overload, and prolong lifespan.^{1,2} Novel approaches are needed to specifically target mTOR in cells, since off-target and systemic effects limit clinical use of rapamycin. Our recent work published in *PNAS*³ uncovers a unique way to treat mTORC1-dependent pathological growth in cardiomyocytes using clinically relevant cardiac gene therapy with the mTORC1 inhibitor PRAS40.

Proline-rich AKT substrate 40 kDa (PRAS40) was identified as a component and negative regulator of the mTOR complex as well as cell growth.⁴ Studies showed that PRAS40 binds to Raptor and the kinase region of mTORC1.⁵ As specified by the given name, PRAS40

contains 2 proline-enriched stretches at the N terminus and an Akt consensus phosphorylation site located at Thr246. Phosphorylated PRAS40 dissociates from mTORC1 in response to growth factors, insulin, as well as glucose and nutrients, and thereby releases the inhibitory function of PRAS40 on mTORC1.

Cellular growth (hypertrophic growth) is the main mechanism of adult cardiomyocytes in response to growth stimulation, as the majority of cardiomyocytes are considered to be post-mitotic in the adult heart. Many stimuli that provoke hypertrophic growth in cardiomyocytes involve the same signaling molecules known to be involved in proliferation and oncogenic transformation. Furthermore, chronic cardiovascular stresses, such as arterial hypertension, result in pathological growth associated with decreased cardiac function, ventricular remodeling, and, ultimately, heart failure.

Pathological growth in myocytes in vitro as well as the molecular remodeling of myocytes was blocked by PRAS40 overexpression by inhibition of mTORC1.³ PRAS40-overexpressing mice were protected against pathological hypertrophy and heart failure associated with decreased fibrotic remodeling and ventricular dilatation. PRAS40 protects heart function, even when the treatment started after initiation of pathological hypertrophy, highlighting the important role of increased mTORC1 activity in the process of cardiac remodeling and opening up unique possibilities for therapeutic regulation to mitigate pathologic myocardial hypertrophy by PRAS40. An even stronger inhibition of cellular growth could be achieved by using a phospho-dead mutant (that

cannot be phosphorylated and therefore results in stronger mTORC1 inhibition), supporting the idea that phosphorylation of PRAS40 is necessary during growth in cardiomyocytes.

These results raise a number of fascinating questions in proliferating cells, as human cancers frequently show a robust activation of the PTEN/PI3K/Akt and mTORC1 signaling. Does inactivation of PRAS40 after phosphorylation by Akt causally contribute to increased cancer cell growth or proliferation? Indeed, elevated PRAS40 phosphorylation has been reported in cancer and Thr²⁴⁶ phosphorylation of PRAS40 has been used as a biomarker for the effects of novel inhibitors targeting the PI3K/Akt and mTORC1 pathway.⁵ Accordingly, increased levels of phosphorylated PRAS40 has been reported to promote cellular survival and growth, whereas reduced PRAS40 levels increased apoptosis and decreased cellular proliferation in melanoma cells.⁶ Consequently, overexpression of PRAS40 reduced cell size in cancer cells and ubiquitous overexpression of PRAS40 in *Drosophila* reduced the size of the entire animal and caused pupal lethality.⁷ However, only few studies addressed the effects of PRAS40 on the regulation of the cell cycle, but silencing of PRAS40 reduced proliferation in C2C12 cells due to a cell cycle arrest in the G₁ phase.⁸

Phosphorylation of PRAS40 by AKT results in nuclear localization.³ Existence of a nuclear export sequence in the C terminus implies shuttling of PRAS40 between the nucleus and the cytosol, supported by observation of PRAS40 in nuclear extracts and nuclear localization of phosphorylated PRAS40 on Thr246

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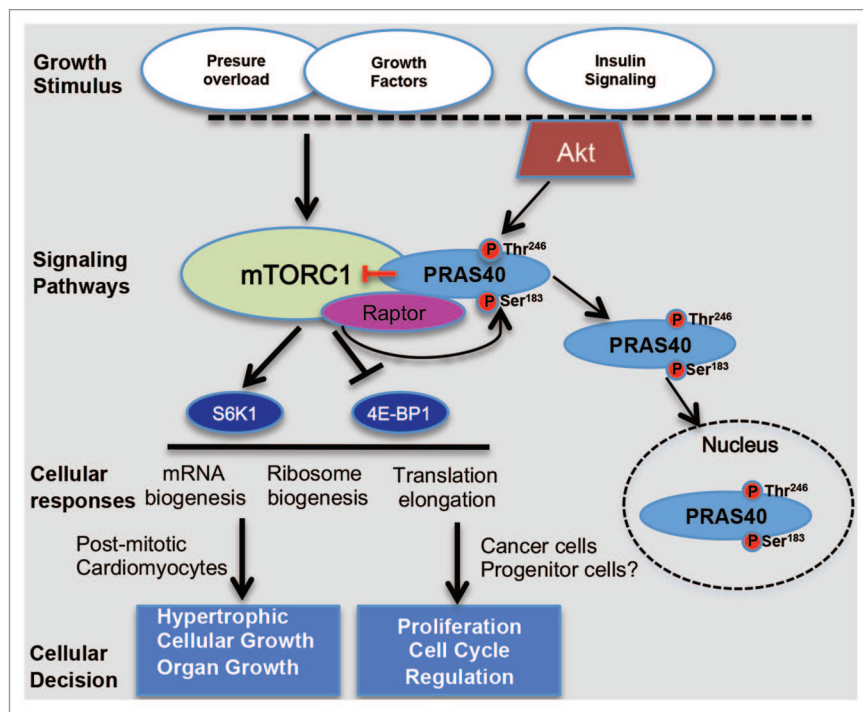


Figure 1. Schematic model of PRAS40/mTOR signaling. Stimulation of cellular growth following cardiac pressure overload or growth factor signaling activates mTORC1. Full activation of mTORC1 requires the dissociation of PRAS40 from mTORC1, which requires phosphorylation of PRAS40 by Akt and mTORC1. Increased mTORC1 activity regulates protein synthesis and cellular growth through phosphorylation of downstream targets (S6K1 and 4EBP1). Overexpression of PRAS40 blocks mTORC1 activation and reduces cellular growth or proliferation.

promoted by growth stimuli. What is the role of PRAS40 in the nucleus, and how does it contribute to the regulation of cellular growth/proliferation? Studies employing overexpression of the wild-type

or mutant proteins in proliferation cells could be helpful to identify the function of PRAS40 regarding cell cycle regulation in further detail. Tissue-specific overexpression or deficiency of PRAS40 will be

another important tool to dissect the role of this important regulatory protein of the Akt-mTOR pathway. Further insights will not only help to understand the regulatory role of PRAS40 of cell cycle/growth in cancer, but also in the heart. Many proliferating cells (fibroblast, inflammatory cells, cardiac progenitor cells) influence disease progression after chronic pressure overload or myocardial infarction and the role of PRAS40 in these cells is completely unknown. Thus, PRAS40 represents a new approach in our ongoing struggle to direct cells in pathological conditions toward making good decisions (Fig. 1).

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