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Mammalian Target of Rapamycin: A Target for (Lung) Diseases and Aging

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Abstract

The mammalian target of rapamycin (mTOR) signaling pathway has been studied in the context of an impressive number of biological processes and disease states, including major diseases of the lung such as idiopathic pulmonary fibrosis and chronic obstructive pulmonary disease, as well as the rare condition lymphangioleiomyomatosis. The involvement of mTOR in so many disease states (in and out of the lung) raises the question how one signaling pathway can have overlapping but diverse roles seemingly everywhere. Findings in the last decade have placed the mTOR pathway in a new context as an important, conserved mediator of the aging process. This offers one explanation for the pleiotropic effects of mTOR: –that many chronic diseases are also diseases of aging and that pathways modulating aging will have widespread effects on associated disease. However, this may not be the entire story, because mTOR is also implicated in a large number of diseases not linked to aging. In this article, we discuss the current state of knowledge regarding mTOR, especially in the context of lung pathologies, and offer a potential explanation for its widespread involvement in human disease.

Keywords: mammalian target of rapamycin; idiopathic pulmonary fibrosis; lymphangioleiomyomatosis

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Rapamycin and related rapalogs have had many lives since the 1960s, when rapamycin was discovered from bacteria in a soil sample from Easter Island (1). Originally identified for their ability to kill eukaryotic cells, they have been studied by many pharmaceutical companies, evaluated in hundreds of clinical trials across the spectrum of diseases, and approved for use in a (disappointingly) limited number of disease indications. The limiting factor for these compounds is often their toxicity, which manifests as a range of side effects that reduce dosing and/or lead to discontinuation of use (2).

The mammalian target of rapamycin (mTOR) has since been studied extensively throughout the landscape of eukaryotic species (a recent PubMed search of "mTOR" revealed nearly 20,000 papers) (3–5). Authors of several recent reviews described the pathway in detail; in this article, we provide only a brief overview. There are two mTOR complexes (mTORC1 and mTORC2) with overlapping and distinct subunits, of which the mTORC1 complex is the most understood. It is linked to a myriad of cellular processes, serving as a signaling node to integrate upstream environmental sensors with downstream outputs, balancing growth and proliferation with stress resistance. Foremost among the upstream signals are nutrients with separate inputs from carbohydrates by way of insulin–insulin-like growth factor signaling and amino acids. Stress signals include mitogen-activated protein kinase signaling, hypoxia, activation of 5′ adenosine monophosphate–activated protein kinase, and others. In short, high nutrient and low stress levels promote cell growth and proliferation, whereas the reverse situation stimulates stress resistance pathways, of which autophagy is the most studied. Other outputs downstream of mTORC1 include mitochondrial function and lipid metabolism, although the role of mTORC1 in these cellular processes is less understood. Importantly, mTORC1 has overlapping but distinct cellular roles in different cell types (4), which will be elaborated below.

mTORC2 has an established role (mostly elaborated in yeast) regulating the cytoskeleton (6), but it is more studied in mammalian cells as an effector of insulin–insulin-like growth factor signaling (4). Among its substrates are protein kinase B/Akt and other signaling kinases, which have been described in recent reviews. Of note, mTORC2 can regulate mTORC1 through Akt phosphorylation, and mTORC1 can negatively regulate

mTORC2 through the activity of S6 kinase, a substrate that in turn phosphorylates insulin receptor substrate 1 (7, 8). In fact, there are likely a number of mechanisms by which the two complexes influence each other's activity.

mTOR in Disease and Aging

Rapalogs have been studied in the clinic for central nervous system, immunology, hematology, oncology, metabolic, cardiovascular, and other indications. The motivations underlying these indications are often disease-specific, but one cannot help but consider underlying reasons why mTOR signaling could be so widely implicated. A clue to the puzzle came from studies several years ago indicating that rapamycin could extend the lifespan of mice, even when administered late in life (9, 10). More recent studies have identified dosing regimens that result in up to 30% lifespan extension (11) and also that the drug can lead to lifespan extension even when administered only for a 3-month window starting around 20 months of age (10, 12). If mechanistic aspects of the aging process promote age-associated chronic diseases, as current research indicates, then a pathway linked to aging is likely to influence a wide range of diseases.

It is surprising that rapamycin can be administered late in life and still extend lifespan. One possible interpretation of this finding is that the levels of mTORC1 signaling are important late in life but not early. This is supported by evidence, still controversial, that mTORC1 signaling may be aberrantly upregulated during the aging process (4). Current studies are inconclusive, and it may be important to consider that mTORC1 signaling is not constant, but nutrient- and stress-responsive. The complex can be in either an induced state, for instance to activate adult stem cells during tissue repair, or at a basal level during normal tissue homeostasis. A likely interpretation of the current data is that the induced state may be unchanged with aging, but the basal state may be aberrantly high. Inflammatory signals are known to activate mTORC1, and the enhanced chronic inflammation (as well as other signals) that accompanies aging may lead to high basal mTORC1 activation, in turn driving pathologic processes.

Although no comprehensive list has been assembled, mTORC1 signaling is hyperactivated in a wide range of diseases, including many not directly linked to aging. This suggests that chronic activation of this pathway may be a common response to cellular metabolic dysregulation. Suppression of mTORC1 activation often reduces disease pathology. For instance, aberrant mTORC1 activation is observed in muscular dystrophy and dilated cardiomyopathy associated with mutation of A-type lamins, and rapamycin improves both cardiac and skeletal muscle function in mouse disease models (13, 14).

mTOR and Lung Diseases

mTOR has been studied in the context of two of the major lung diseases, idiopathic pulmonary fibrosis (IPF) and chronic obstructive pulmonary disease (COPD), with similar findings with regard to mTOR activity. Researchers in several studies have reported that basal levels of mTORC1 and mTORC2 signaling are elevated in either IPF fibroblasts or tissue biopsies (15–18). A notable feature of IPF is the presence of apoptosis-resistant lung fibroblasts. In a recent study, investigators found that normal aging promoted resistance to apoptosis through chronic basal activation of mTORC1 signaling and resultant suppression of autophagy (18). Fibroblasts from patients with IPF demonstrated a further increase in mTORC1 signaling. Either rapamycin or an active site mTOR inhibitor (PP242) resulted in autophagy activation and restored sensitivity to apoptosis in fibroblasts from patients with IPF. *In vivo* preclinical studies designed to determine whether rapamycin affects bleomycin-induced lung fibrosis have been performed in rats and mice, with mixed results. In rats, 1 month of rapamycin treatment demonstrated significant efficacy (19). In mice, however, rapamycin enhanced disease pathology (20). One notable difference is that rapamycin was initiated prior to bleomycin treatment in the rat study but after treatment in the mice, suggesting that the benefits may require prophylactic administration. One clinical trial has been performed with rapamycin, which worsened outcomes in patients with IPF (21). Other studies have been initiated, but it remains unclear whether rapamycin (or other rapalogs) is a viable candidate for IPF treatment.

A disease that dramatically increases in prevalence with age, COPD has been likened to accelerated aging of the lung (22). It is associated with several hallmarks of aging, including chronic inflammation, cell senescence, reduced stem cell function, and defective autophagy and mitochondrial function. In addition, elevated reactive oxygen species, derived from smoking, may be a major factor that drives these age-related changes. Moreover, preclinical studies suggest that small molecules that modulate aging pathways might be effective in COPD (23). Consistently, mTOR signaling is also elevated, with increased oxidative stress as a likely driver (22). Given that rapamycin affects several hallmarks of aging and has a robust effect on lifespan, it may be an interesting candidate for treatment of COPD. In one mouse genetic model of airway hyperactivity and remodeling, rapamycin was found to improve several parameters of lung function (24). Moreover, in a study of mononuclear cells from patients with COPD, rapamycin restored sensitivity to corticosteroids (25). Together, these findings suggest that mTOR inhibitors (and possibly other drugs that affect aging pathways) should be strongly considered as potential therapeutic approaches to COPD.

Another rare lung disease, lymphangioleiomyomatosis (LAM), has been linked to elevated mTOR signaling (26). This condition, virtually exclusive to women and often presenting in early adulthood, is characterized by the accumulation of lung cysts and the unusual hyperproliferation of smooth muscle cells throughout the lung. A major clue that this disease was linked to mTOR was the finding that many patients with LAM also have tuberous sclerosis complex (TSC), which is caused by mutations inactivating *TSC1* or *TSC2*, upstream repressors of mTORC1 (27). However, not all patients with LAM have TSC, and these cases are referred to as sporadic. LAM is generally fatal in the absence of lung transplant, but the use of rapalogs to reduce mTORC1 signaling has emerged as a new therapeutic approach. A number of clinical studies have been performed, the foremost being a double-blind, placebo-controlled study of sirolimus that showed clear efficacy in reducing the loss of FEV, a common feature of LAM (28). Studies have been

performed with other rapalogs, and it the optimal drug and dosing strategy remains unclear. Nevertheless, mTOR inhibition is rapidly becoming a standard treatment in patients with LAM (26).

Interestingly, patients with a variety of diseases, including those with breast cancer and renal cell carcinoma as well as transplant recipients, treated with the rapamycin or the rapamycin derivative everolimus have an increased tendency to develop noninfectious drug-induced pneumonitis (29). These symptoms, which occur in up to one-third of treated patients depending upon the clinical study, are usually mild, and only around 10% of patients require treatment. However, more severe cases have been noted. Mechanistically, little is known about this adverse event, although it is associated with macrophage infiltration and T-cell–mediated inflammatory responses. mTOR inhibition may increase production of inflammatory cytokines (30). Another histological study suggested that elevated mTOR signaling in pneumocytes and T cells may be associated with disease in patients treated with rapalogs (31), although studies on a wider range of mTOR substrates need to be performed.

Overview

How does elevated basal mTOR signaling contribute to pathology, and is it the same in every context? On the basis of our assessment of evidence from a wide variety of laboratories and diseases, it seems that the downstream effects of aberrant mTOR signaling are likely to be context-dependent, with the cell type, other disease manifestations, and aging state all serving as modifiers (Figure 1). Therefore, elevated mTOR can mean different things and can also be caused by different cellular insults. To identify and predict the efficacy of therapeutic strategies targeting mTOR, it will be essential to identify the specific upstream elements and downstream consequences of elevated mTOR in a specific disease state, or indeed in aging.

Examining chronic diseases through the lens of aging is relatively recent and looks to be an effective strategy for reexamining the causes of disease and possible therapeutic approaches. In this review, we have focused on the mTOR pathway, but other aging pathways, such as sirtuins and cellular senescence (to name a couple), are linked to a wide range of indications (32, 33). Stepping back, aging itself is likely the result of at least a handful of molecular causes, and individual causes may be more relevant in specific tissues and associated disease states. Although extensive work remains to be done to explore this hypothesis, the promise is great, and therapeutic approaches are already underway (34, 35). We predict this will lead to novel strategies that make chronic diseases of aging increasingly preventable and treatable. ■

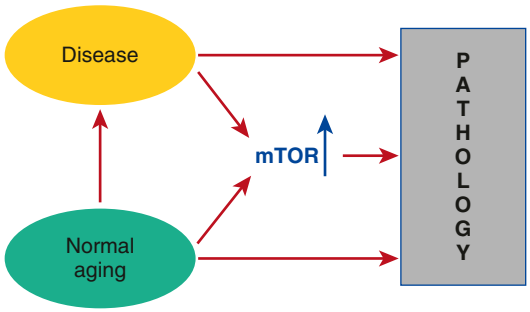

Figure 1. Mammalian target of rapamycin complex 1 (mTORC1), aging, and disease: a schematic linking mTORC1 signaling to aging. Both aging and a variety of disease states drive elevated mammalian target of rapamycin (mTOR) signaling, and this drives pathology in both contexts. Complicating the issue, aging increases risk of disease independently of mTOR. Furthermore, aberrant mTOR signaling does not explain the entire suite of pathologies associated with a specific disease or normal aging. Finally, the type of pathology in a particular tissue likely relates to a combined and interactive effect of the specific pathophysiology of a disease, the level of mTOR elevation, and the age of the individual. Testing and understanding this model may yield unexpected therapeutic avenues for the chronic diseases of aging.

Author disclosures are available with the text of this article at www.atsjournals.org.

References

1 Vézina C, Kudelski A, Sehgal SN. Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *J Antibiot (Tokyo)* 1975;28:721–726.
2 Soefje SA, Karnad A, Brenner AJ. Common toxicities of mammalian target of rapamycin inhibitors. *Target Oncol* 2011;6:125–129.
3 Liko D, Hall MN. mTOR in health and in sickness. *J Mol Med (Berl)* 2015;93:1061–1073.
4 Kennedy BK, Lamming DW. The mechanistic target of rapamycin: the grand conducTOR of metabolism and aging. *Cell Metab* 2016;23: 990–1003.
5 Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012;149:274–293.
6 Bartlett K, Kim K. Insight into Tor2, a budding yeast microdomain protein. *Eur J Cell Biol* 2014;93:87–97.
7 Tremblay F, Marette A. Amino acid and insulin signaling via the mTOR/p70 S6 kinase pathway: a negative feedback mechanism leading to insulin resistance in skeletal muscle cells. *J Biol Chem* 2001;276: 38052–38060.
8 Harrington LS, Findlay GM, Gray A, Tolkacheva T, Wigfield S, Rebholz H, Barnett J, Leslie NR, Cheng S, Shepherd PR, *et al*. The TSC1-2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins. *J Cell Biol* 2004;166:213–223.
9 Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, Nadon NL, Wilkinson JE, Frenkel K, Carter CS, *et al*. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* 2009;460:392–395.
10 Chen C, Liu Y, Zheng P. mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells. *Sci Signal* 2009;2:ra75.
11 Miller RA, Harrison DE, Astle CM, Fernandez E, Flurkey K, Han M, Javors MA, Li X, Nadon NL, Nelson JF, *et al*. Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction. *Aging Cell* 2014;13:468–477.
12 Bitto A, Ito TK, Pineda VV, LeTexier NJ, Huang HZ, Sutlief E, Tung H, Vizzini N, Chen B, Smith K, *et al*. Transient rapamycin treatment can increase lifespan and healthspan in middle-aged mice. *eLife* 2016; 5:e16351.
13 Ramos FJ, Chen SC, Garelick MG, Dai DF, Liao CY, Schreiber KH, MacKay VL, An EH, Strong R, Ladiges WC, *et al*. Rapamycin

reverses elevated mTORC1 signaling in lamin A/C-deficient mice, rescues cardiac and skeletal muscle function, and extends survival. *Sci Transl Med* 2012;4:144ra103.
14 Choi JC, Muchir A, Wu W, Iwata S, Homma S, Morrow JP, Worman HJ. Temsirolimus activates autophagy and ameliorates cardiomyopathy caused by lamin A/C gene mutation. *Sci Transl Med* 2012;4: 144ra102.
15 Chang W, Wei K, Ho L, Berry GJ, Jacobs SS, Chang CH, Rosen GD. A critical role for the mTORC2 pathway in lung fibrosis. *PLoS One* 2014;9:e106155.
16 Nho RS, Hergert P. IPF fibroblasts are desensitized to type I collagen matrix-induced cell death by suppressing low autophagy via aberrant Akt/mTOR kinases. *PLoS One* 2014;9:e94616.
17 Park JS, Park HJ, Park YS, Lee SM, Yim JJ, Yoo CG, Han SK, Kim YW. Clinical significance of mTOR, ZEB1, ROCK1 expression in lung tissues of pulmonary fibrosis patients. *BMC Pulm Med* 2014;14:168.
18 Romero Y, Bueno M, Ramirez R, Álvarez D, Sembrat JC, Goncharova EA, Rojas M, Selman M, Mora AL, Pardo A. mTORC1 activation decreases autophagy in aging and idiopathic pulmonary fibrosis and contributes to apoptosis resistance in IPF fibroblasts. *Aging Cell* [online ahead of print] 26 Aug 2016; DOI: 10.1111/acel.12514.
19 Jin X, Dai H, Ding K, Xu X, Pang B, Wang C. Rapamycin attenuates bleomycin-induced pulmonary fibrosis in rats and the expression of metalloproteinase-9 and tissue inhibitors of metalloproteinase-1 in lung tissue. *Chin Med J (Engl)* 2014;127:1304–1309.
20 Madala SK, Maxfield MD, Davidson CR, Schmidt SM, Garry D, Ikegami M, Hardie WD, Glasser SW. Rapamycin regulates bleomycin-induced lung damage in SP-C-deficient mice. *Pulm Med* 2011;2011: 653524.
21 Malouf MA, Hopkins P, Snell G, Glanville AR; Everolimus in IPF Study Investigators. An investigator-driven study of everolimus in surgical lung biopsy confirmed idiopathic pulmonary fibrosis. *Respirology* 2011;16:776–783.
22 Mercado N, Ito K, Barnes PJ. Accelerated ageing of the lung in COPD: new concepts. *Thorax* 2015;70:482–489.
23 Ito K, Colley T, Mercado N. Geroprotectors as a novel therapeutic strategy for COPD, an accelerating aging disease. *Int J Chron Obstruct Pulmon Dis* 2012;7:641–652.
24 Kramer EL, Hardie WD, Mushaben EM, Acciani TH, Pastura PA, Korfhagen TR, Hershey GK, Whitsett JA, Le Cras TD. Rapamycin decreases airway remodeling and hyperreactivity in a transgenic model of noninflammatory lung disease. *J Appl Physiol (1985)* 2011;111:1760–1767.
25 Mitani A, Ito K, Vuppusetty C, Barnes PJ, Mercado N. Restoration of corticosteroid sensitivity in chronic obstructive pulmonary disease by inhibition of mammalian target of rapamycin. *Am J Respir Crit Care Med* 2016;193:143–153.
26 Yates DH. mTOR treatment in lymphangioleiomyomatosis: the role of everolimus. *Expert Rev Respir Med* 2016;10:249–260.
27 Orlova KA, Crino PB. The tuberous sclerosis complex. *Ann N Y Acad Sci* 2010;1184:87–105.
28 McCormack FX, Inoue Y, Moss J, Singer LG, Strange C, Nakata K, Barker AF, Chapman JT, Brantly ML, Stocks JM, *et al*.; National Institutes of Health Rare Lung Diseases Consortium; MILES Trial Group. Efficacy and safety of sirolimus in lymphangioleiomyomatosis. *N Engl J Med* 2011;364:1595–1606.
29 Duran I, Goebell PJ, Papazisis K, Ravaud A, Weichhart T, Rodriguez-Portal JA, Budde K. Drug-induced pneumonitis in cancer patients treated with mTOR inhibitors: management and insights into possible mechanisms. *Expert Opin Drug Saf* 2014;13:361–372.
30 Buron F, Malvezzi P, Villar E, Chauvet C, Janbon B, Denis L, Brunet M, Daoud S, Cahen R, Pouteil-Noble C, *et al*. Profiling sirolimus-induced inflammatory syndrome: a prospective tricentric observational study. *PLoS One* 2013;8:e53078.
31 Saito R, Yanai M, Miki Y, Fukasawa Y, Tominaga J, Ebina M, Sasano H. Immunohistochemical evidence for the association between attenuated mTOR signaling and diffuse alveolar damage, a fatal lung complication. *Tohoku J Exp Med* 2014;234:67–75.
32 Kennedy BK, Berger SL, Brunet A, Campisi J, Cuervo AM, Epel ES, Franceschi C, Lithgow GJ, Morimoto RI, Pessin JE, *et al*. Geroscience: linking aging to chronic disease. *Cell* 2014;159: 709–713.
33 López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell* 2013;153:1194–1217.
34 Mannick JB, Del Giudice G, Lattanzi M, Valiante NM, Praestgaard J, Huang B, Lonetto MA, Maecker HT, Kovarik J, Carson S, *et al*. mTOR inhibition improves immune function in the elderly. *Sci Transl Med* 2014;6:268ra179.
35 Barzilai N, Crandall JP, Kritchevsky SB, Espeland MA. Metformin as a tool to target aging. *Cell Metab* 2016;23:1060–1065.