Perspective

Rapamycin in fibrotic diseases: beneficial or detrimental agent?

XU Xue-feng and DAI Hua-ping

**Keywords**: fibrotic disease; rapamycin; mammalian target of rapamycin; negative feedback loop; antifibrogenesis

Rapamycin was first isolated from a strain of *Streptomyces hygroscopicus* in the early 1970s from a soil sample taken on Easter Island. Because the antiproliferative effects of rapamycin on yeast cell, as well as B and T lymphocytes, it was first identified as an antimicrobial agent with potent immunosuppressive activity and has been used in antirejection therapy.

The discovery of rapamycin led to the identification and clone of the mammalian target of rapamycin (mTOR). mTOR is a highly conserved serine-threonine kinase shown to integrate and coordinate diverse signaling pathway mediated by growth factors, nutrient availability and energy status to play a critical role in cell growth, proliferation, division, migration, and survival. Structurally, mTOR is a part of two signaling complexes, mTOR complex 1 (mTORC1) and mTORC2. Rapamycin is a potent, highly specific inhibitor of mTORC1 and does not attenuate any kinase other than mTOR. After diffusing into cells, rapamycin forms a complex with FK506-binding protein 12 (FKBP-12) and then binds and inhibits mTOR phosphorylation and inactivates ribosomal S6 kinase 1 (S6K1) and the eukaryotic initiation factor 4E (eIF4E) inhibitory binding protein (4E-BP). Via inhibiting the mTOR signal axis, rapamycin modulates malignant cellular growth, progression of fibrotic disorders. The current review will give a brief overview on the rapamycin/mTOR network relevant to fibrotic diseases and will mainly focus on recent scientific debates on rapamycin’s regulatory role in fibrosis models both in vitro and in vivo (Table 1).

**RAPAMYCIN INHIBITS TYPE I COLLAGEN, FIBRONECTIN, α-SMA, TIMP-1 AND AUGMENT MMP-1/9 EXPRESSION: IN VITRO EVIDENCES FOR ANTIFIBROTIC EFFECTS**

Dysregulated wound healing response to a variety of acute and/or chronic stimuli contributes to fibrotic disorders. Fibrosis will ultimately occur due to an over production of extracellular matrix components (Figure 1), including type I collagen. These fibrillar collagen synthesis and deposition will disrupt normal organ architecture, induce abnormal tissue remodeling and organ malfunction. (Myo)fibroblasts are the main effector cells for matrix production (Figure 1). They recruited from a variety of sources such as resident mesenchymal cells, bone marrow progenitors (circulating fibrocytes) and epithelial or endothelial cells via a process called epithelial or endothelial mesenchymal transition (EMT or EndMT).

In addition to the increase in collagen production, the accumulation of extracellular matrix (ECM) is also due to an insufficient matrix turnover (Figure 1). Physiologically, the metabolism of ECM proteins is delicately regulated by proteases and their corresponding inhibitors, including the matrix metalloproteases (MMPs) and plasminogen activators (PAs). Pathologically, if the balance becomes in favor of TIMPs/PAIs, ECM resolution will be difficult and may cause insufficient fibrillar matrix destruction and fibrotic lesions. So agents targeting to modulate ECM production and degradation would have potential benefits for the treatment of fibrotic diseases.

Poulalhon et al. found that rapamycin attenuated expression of both types I and III collagens in human lung fibroblast (WI-26), while significantly enhanced the expression of interstitial collagenase (MMP-1) at the protein and mRNA levels. Another study found that rapamycin significantly increased intracellular fibronectin expression in rabbit lens epithelium cells (rLECs). Simultaneously, it was found that α-SMA expression in NIH3T3 cells was remarkably reduced by rapamycin treatment. In line with these results, Shegogue and Trojanowska also showed inhibition of mTOR activity using rapamycin remarkably reduced collagen mRNA levels of human fibroblast in vitro. These results drew the conclusion that rapamycin had direct antifibrotic activities in vitro through modulating ECM synthesis and degradation.

**REFERENCES**

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A series of animal or human models were used in vivo to examine the pathogenesis and treatment of fibrotic disorders, including bleomycin induced mouse models of Systemic Sclerosis, transforming growth factor-β (TGF-β) induced pulmonary fibrosis, bile duct ligation induced hepatic fibrosis and chronic allograft nephropathy (CAN). In their remarkable work, Yoshizaki et al. showed that rapamycin treatment reduced skin and lung fibrosis models of fibrosis via attenuating multiple profibrogenic pathways (Figure 2B).

Studies on the modulatory effects of rapamycin in other organs in vivo seem rather insufficient. Some case reports showed that rapamycin may be effective on the treatment for chronic intestinal disease and scleroderma.

RAPAMYCIN AUGMENTS CTGF, TIMP-1, PAI-1 AND INHIBIT MMP-1/9 EXPRESSION: IN VITRO EVIDENCES AGAINST ANTIFIBROTIC EFFECTS

The antifibrotic effects hypothesis of rapamycin was challenged by some other in vitro studies which showed that rapamycin has a profibrotic property. In their significant studies, Osman et al. demonstrated that rapamycin treatment caused an increase in profibrotic gene expression as exemplified by the connective tissue growth factor (CTGF) and PAI-1 in rat mesangial cells (MC). These profibrotic effects were exerted by activation of the TGFβ1/Smad signaling cascade. CTGF plays an important role in fibrogenic responses via promoting fibroblasts proliferation and extracellular matrix synthesis. Concomitantly, Osman et al. also found that rapamycin promoted cytokine-triggered TIMP-1 expression in rat MC. On the contrary, the expression of MMP-9 was remarkably reduced. The differential modulation on protease/antiprotease system indicates that rapamycin may be a potent drug to promote fibrotic process. In another study, Ma and fellows showed that incubation of human umbilical vein endothelial cells (HUVECs) with rapamycin strongly inhibited eNOS expression of mouse mesangial cells. These profibrotic effects were exerted by reduced expression of INF-γ.

RAPAMYCIN DOES NOT ATTENUATE PROGRESSION OF LUNG, LIVER AND KIDNEY FIBROSIS: IN VIVO EVIDENCES AGAINST ANTIFIBROTIC EFFECTS

Contrary to these studies arguing for antifibrotic activity of rapamycin in vivo, Madala et al. showed that rapamycin increased weight loss and decreased survival of bleomycin-treated Sftpc−/− mice. Furthermore, rapamycin did not reduce the fibrotic disease in the prophylactic or rescue experiments and even augmented airway resistance and reduced lung compliance. Rapamycin also caused a significantly increased expression of pro-fibrotic TH2 cytokines and reduced expression of INF-γ. All of these results showed that rapamycin treatment could not attenuate the onset or progression of lung fibrosis, rather it may promote the fibrotic process through different ways.
the major ECM accumulation in vitro. 2B: Anti-fibrotic hypothesis of rapamycin in vivo. Rapamycin decreased total collagen deposition, fibrosis score and increased body weights and organ functions. Rapamycin exerted its modulatory effects via multiple pathways, including inhibiting expressions of fibrotic cytokines, promoting ECM resolution, and etc.

2C: Pro-fibrotic hypothesis of rapamycin in vitro. Inhibition of the mTOR activity with rapamycin results in a hyperactive receptor tyrosine kinase (RTK)/(PI3K) and TGF-β/Smad pathway. These secondarily activated pathways may contribute to the accumulation of profibrotic genes produced by fibroblasts and/or epithelial cells. Finally, rapamycin showed pro-fibrotic in vitro.

2D: Pro-fibrotic hypothesis of rapamycin in vivo. Rapamycin may upregulated Th2 cytokines, TGF-β1, PAI-1 in vivo. These pro-fibrotic genes may counteract other beneficial effects of rapamycin and result in a pro-fibrotic function.

Figure 3. mTOR related signal pathways. mTOR is a part of two distinct complexes, mTORC1 and mTORC2. mTORC1 phosphorylates and activates S6K1 resulting in the initiation of transcription and translation. mTORC2 phosphorylates and activates Akt. Rapamycin is a specific inhibitor of mTORC1 and does not affect mTORC2’s function. Emerging evidence has shown that inhibiting mTOR would promote a negative feedback loop which results in the activation of proliferative signals such as Akt or MAPK that in turn counteract the inhibitory properties of rapamycin. Rapamycin also induces TGF-β1/Smad cascade in some cell type. These different signal pathways activated due to inhibition of mTOR may involve in the profibrotic effects of mTOR inhibitors. Combination treatment with inhibitors of PI3K-AKT (Wortmannin), MAPK (U1026, SB202474, SB203580), TGF-β1-Smad (SB431542) cascade may augment the antifibrotic activity of rapamycin.

et al also showed that rapamycin treatment failed to attenuate progression of kidney and liver fibrosis in PCK rats. In line with these data, many other studies showed that rapamycin induced TGF-β1 production in vivo. Also, some recent case reports showed that rapamycin would induce interstitial pneumonia and even fibrotic lesions when used in renal transplant or renal cell carcinoma patients. Collectively, these data showed that rapamycin has profibrotic abilities in vitro (Figure 2D).

**IN VITRO AND IN VIVO EVIDENCES FOR AND AGAINST ANTIFIBROTIC ACTIVITY OF RAPAMYCIN: HOW TO RECONCILE?**

As is mentioned above, we have listed a lot of evidence for or against rapamycin’s antifibrotic effects in vitro and in vivo. We seem rather confused with the real modulation effects of rapamycin on fibrotic disorders because all the researches with the opposite data seem reasonable. So it’s a high time to debate now. Issues discussed below may serve to reconcile the discrepancy: (1) Various doses of rapamycin and different animal models. Lower or higher doses of rapamycin with different length of treatment time would be involved in the paradox about rapamycin’s modulatory effects on fibrotic disorders. Studies with lower dose (such as 2.0 mg/kg, or 2.5 mg/kg) of rapamycin administration showed a significant antifibrotic effects of this agent in bleomycin-induced lung fibrosis. However, larger dose of rapamycin (such as 4 mg/kg) would exert no antifibrotic or even profibrotic effects in bleomycin-
Table 1. Studies of pro- or anti-fibrotic effects of rapamycin on fibrotic disorders in vitro and in vivo

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induced lung fibrosis of SP-C-deficient mice model.24 In a cytokine (TGF-α) induced lung fibrosis model, even large dose of rapamycin (4 mg/kg) could prevent fibrosis as well as prevent progression of fibrosis when rapamycin was administered after extensive fibrosis had already developed.14 So varying doses of rapamycin and animal models should be concerned in the different remarkable data. (2) Intrinsic or acquired ‘rapamycin resistance’ may serve to explain rapamycin’s failure to exhibit antifibrotic effects.25,32,34 Rapamycin is a specific but not a complete inhibitor of mTORC1. S6K activity has been shown to be completely inhibited by rapamycin, however, the phosphorylation of 4E-BP1 is not affected by it.35 Therefore after rapamycin treatment, some mTOR activities can still be executed by activated eIF-4E. Any rapamycin resistant activity of mTORC1 would induce to a more complex regulatory process in fibrotic disorders after rapamycin treatment. However, whether this “resistance” phenomenon really exists in fibrotic disorders should be further elucidated. (3) The complexity and widely crosslink between mTOR system and other signaling pathways activated secondarily to the mTOR inhibition by rapamycin. It was already known that there exist negative feedback loops in mTOR/S6K1/4EBP1 signal system.36-38 Activation of mTOR would lead to phosphatidylinositol 3kinase (PI3K) and mitogen-activated protein kinase (MAPK) inhibition through the negative feedback loop stemming from S6K1. While inhibition mTOR activity with rapamycin and its analogue results in a hyperactive receptor tyrosine kinase (RTK) / (PI3K) pathway, increasing the signal toward the Ras-Raf1-MEK1/2-EXTRACELLULAR SIGNAL REGULATED KINASE (ERK) pathway.39 In addition, rapamycin administration also could induce TGF-β1/Smad signaling cascade.20 Beyond this, additional interactions between mTORC1 (target of rapamycin) and mTORC2-mediated signalling are likely to exist. So alternative activation of signal pathways induced by mTOR inhibition may contribute to profibrotic effects of rapamycin both in vitro and in vivo and combined inhibition of PI3K-AKT/MAPK/TGF-β1 and mTOR signal pathways may have additive anti-anfibrotic effects (Figure 3).

CONCLUSION

In this review, we listed evidences for and against rapamycin’s effects on fibrotic disorders and aimed to explain the remarkable debates. We believe rapamycin
has a more sophisticated modulatory effect on fibrotic diseases in vitro or in vivo than we used to think. The real or final effects of rapamycin depend on the balance of changes in various pro- or anti-fibrotic gene expressions as well as on the synergy of mTOR signal cascade and other signal pathways activated by mTOR inhibition. Actually, rapamycin is “a coin of two sides”, how to amplify anti-fibrogenic and avoid pro-fibrotic effects is a great challenge for investigators. Careful consideration and evaluation of in vitro or in vivo studies can promote the possibility of identifying the effects and safeness of this agent.

REFERENCES


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