Revisiting mTOR and Epithelial–Mesenchymal Transition

In the lungs, alveolar epithelial cells (AECs) are essential for the maintenance of alveolar structure and lung homeostasis. The alveolar epithelium is composed of flat type 1 cells (AT1), which are in close proximity to capillaries and mediate gas exchange, and cuboidal type 2 cells (AT2), which regulate surfactant production and secretion (1). In recent years, several lines of evidence suggest that lung diseases can result from failure of lung repair. For example, in the adult lung, AT2 cells can proliferate to self-renew and differentiate into AT1 (2, 3). Investigators have suggested that the self-renewal capacity in AT2 cells is limited, such that repeated injury or aging preclude normal epithelial regeneration, perhaps contributing to the pathobiology of fibrotic lung diseases, including idiopathic pulmonary fibrosis (IPF) (4). The disassembly of cell–cell junctions, loss of epithelial identity, and upregulation of the expression of mesenchymal markers has been termed epithelial–mesenchymal transition (EMT) and has been suggested to contribute to lung fibrosis (5, 6). However, direct evidence of EMT using genetic lineage tracing tools in murine models or humans with IPF is lacking (7, 8).

The serine/threonine kinase mTOR plays an essential role in cell proliferation, differentiation, growth, and survival. mTOR forms two different protein complexes: the mTOR complex 1 (mTORC1; rapamycin sensitive) and the mTOR complex 2 (mTORC2; rapamycin insensitive), both of which sense and integrate intracellular and environmental signals to mediate vital cell functions (9). Investigators have previously implicated mTOR signaling in fibroblasts in the pathogenesis of IPF. For example, the activation of mTOR is involved in the metabolic reprogramming of fibroblasts and exacerbates TGF-β–induced collagen biosynthesis (10). Moreover, IPF-derived lung fibroblasts show a persistent activation of this kinase, which is associated with apoptosis resistance (11). However, the role of mTOR activation in AT2 cells is incompletely understood.

In this issue of the *Journal*, Saito and colleagues (pp. 699–708) report findings from a transgenic mouse that has constitutive activation of mTORC1 in AT2 cells (Sftpc-mTOR^{SL1+IT} Tg) (12). In the uninjured lung, Sftpc-mTOR^{SL1+IT} Tg mice did not exhibit a detectable phenotype, but expression of the tight junctional proteins ZO-I and Cav-1 was reduced. When these mice were given bleomycin, the recruitment of inflammatory cells into the lungs and the resulting fibrosis were worse. The authors provide supportive data in cultured epithelial cells suggesting that activation of *Angptl4* (angiopoietin-like 4) acts downstream of mTOR signaling to alter the expression of tight junction proteins. They propose this as a mechanism for their observations but do not provide causal data *in vivo*. Their hypothesis is supported by findings in breast cancer cells in which TGF-β upregulates *Angptl4* to promote lung metastases (13). Moreover, deficiency of *Angptl4* reduces inflammatory cell recruitment in influenza and LPS-induced models of lung injury (14, 15).

The findings of Saito and colleagues support a link between mTOR signaling in the epithelium and the regulation of tight junctional integrity and inflammatory cell recruitment (12). This finding is consistent with recent reports by Wu and colleagues, who found that loss of a small GTPase *Cdc42* that regulates cell division and cell polarization promoted the development of lung fibrosis after pneumonectomy (16). However, their suggestion that *Angptl4* promotes EMT during pulmonary fibrosis is likely to be met with skepticism. To make such a claim, the investigators would need to show that labeled AT2 cells become fibroblasts during fibrosis when mTOR signaling is activated. Careful studies of this kind have been conducted by other laboratories and have failed to find evidence of EMT in the bleomycin model of pulmonary fibrosis (17). Several other limitations of these studies should be noted. Most importantly, the transgenic mouse used in this study has constitutive mTOR activation during development, raising the possibility that compensatory or alternative pathways may have compensated for the chronic activation of mTOR, contributing to the observed phenotypes. Although these studies suggest a potential new line of investigation, studies combining inducible systems with genetic lineage tracing and careful phenotyping of epithelial, immune, and mesenchymal populations during fibrosis will be required to understand the role of mTOR signaling in the lung epithelium during fibrosis. ■

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

Mariana M. Herrerias, Ph.D.
G. R. Scott Budinger, M.D.
Division of Pulmonary and Critical Care Medicine
Northwestern University
Chicago, Illinois

ORCID ID: 0000-0002-1791-818X (M.M.H.).

References

1. Raredon MSB, Adams TS, Suhail Y, Schupp JC, Poli S, Neumark N, *et al*. Single-cell connectomic analysis of adult mammalian lungs. *Sci Adv* 2019;5:eaaw3851.
2. Nabhan AN, Brownfield DG, Harbury PB, Krasnow MA, Desai TJ. Single-cell Wnt signaling niches maintain stemness of alveolar type 2 cells. *Science* 2018;359:1118–1123.
3. Barkauskas CE, Cronce MJ, Rackley CR, Bowie EJ, Keene DR, Stripp BR, *et al*. Type 2 alveolar cells are stem cells in adult lung. *J Clin Invest* 2013;123:3025–3036.

Originally Published in Press as DOI: 10.1165/rcmb.2020-0109ED on March 31, 2020

4. Martinez FJ, Collard HR, Pardo A, Raghu G, Richeldi L, Selman M, *et al*. Idiopathic pulmonary fibrosis. *Nat Rev Dis Primers* 2017;3: 17074.
5. Tanjore H, Xu XC, Polosukhin VV, Degryse AL, Li B, Han W, *et al*. Contribution of epithelial-derived fibroblasts to bleomycin-induced lung fibrosis. *Am J Respir Crit Care Med* 2009;180:657–665.
6. Tanjore H, Cheng DS, Degryse AL, Zoz DF, Abdolrasulnia R, Lawson WE, *et al*. Alveolar epithelial cells undergo epithelial-to-mesenchymal transition in response to endoplasmic reticulum stress. *J Biol Chem* 2015;290:3277.
7. Rock JR, Barkauskas CE, Cronce MJ, Xue Y, Harris JR, Liang J, *et al*. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. *Proc Natl Acad Sci USA* 2011;108:E1475–E1483.
8. Zepp JA, Zacharias WJ, Frank DB, Cavanaugh CA, Zhou S, Morley MP, *et al*. Distinct mesenchymal lineages and niches promote epithelial self-renewal and myofibrogenesis in the lung. *Cell* 2017;170: 1134–1148, e10.
9. Liu GY, Sabatini DM. mTOR at the nexus of nutrition, growth, ageing and disease. *Nat Rev Mol Cell Biol* 2020;21:183–203. [Published erratum appears in Nat Rev Mol Cell Biol 21:246.]
10. Selvarajah B, Azuelos I, Platé M, Guillotin D, Forty EJ, Contento G, *et al*. mTORC1 amplifies the ATF4-dependent de novo serine-glycine pathway to supply glycine during TGF-β1-induced collagen biosynthesis. *Sci Signal* 2019;12:eaav3048.
11. Romero Y, Bueno M, Ramirez R, Álvarez D, Sembrat JC, Goncharova EA, *et al*. mTORC1 activation decreases autophagy in aging and idiopathic pulmonary fibrosis and contributes to apoptosis resistance in IPF fibroblasts. *Aging Cell* 2016;15:1103–1112.
12. Saito M, Mitani A, Ishimori T, Miyashita N, Isago H, Mikami Y, *et al*. Active mTOR in lung epithelium promotes epithelial–mesenchymal transition and enhances lung fibrosis. *Am J Respir Cell Mol Biol* 2020;62:699–708.
13. Padua D, Zhang XH, Wang Q, Nadal C, Gerald WL, Gomis RR, *et al*. TGFbeta primes breast tumors for lung metastasis seeding through angiopoietin-like 4. *Cell* 2008;133:66–77.
14. Li L, Chong HC, Ng SY, Kwok KW, Teo Z, Tan EHP, *et al*. Angiopoietin-like 4 increases pulmonary tissue leakiness and damage during influenza pneumonia. *Cell Rep* 2015;10:654–663.
15. Guo L, Li S, Zhao Y, Qian P, Ji F, Qian L, *et al*. Silencing angiopoietin-like protein 4 (ANGPTL4) protects against lipopolysaccharide-induced acute lung injury via regulating SIRT1 /NF-kB pathway. *J Cell Physiol* 2015;230:2390–2402.
16. Wu H, Yu Y, Huang H, Hu Y, Fu S, Wang Z, *et al*. Progressive pulmonary fibrosis is caused by elevated mechanical tension on alveolar stem cells. *Cell* 2020;180:107–121, e17.
17. Chapman HA, Li X, Alexander JP, Brumwell A, Lorizio W, Tan K, *et al*. Integrin α6β4 identifies an adult distal lung epithelial population with regenerative potential in mice. *J Clin Invest* 2011;121:2855–2862. [Published erratum appears in *J Clin Invest* 121:3360.]