Circular RNA Methylation: A New Twist in Lung Fibrosis

Pulmonary fibrosis is a devastating disease characterized by the pathological deposition of extracellular matrix proteins in the lung interstitium, leading to progressive tissue scarring and organ failure (1). Activated fibroblasts, also known as myofibroblasts, play a critical role in the pathogenesis of this lung condition, and their uncontrolled accumulation is ultimately responsible for the aberrant extracellular matrix deposition and persistent fibrosis (2). Numerous epigenetic abnormalities have been found to contribute to the accumulation of myofibroblasts during lung fibrosis, including altered DNA and histone modifications, as well as aberrant expression of noncoding RNAs (3).

With advances in RNA sequencing technologies, circular RNAs (circRNAs) have recently garnered increasing attention among the noncoding RNAs. circRNAs share the unique characteristic of forming covalently closed loops without 5′ caps or 3′ poly-A tails (4), which renders them more stable relative to linear RNAs. Originally considered to be "junk" byproducts of transcription generated by aberrant splicing events, circRNAs have been recently implicated in the regulation of multiple transcriptional and post-transcriptional mechanisms, including RNA splicing, RNA-mediated gene silencing, and microRNA sponging (5). Post-transcriptional modifications of circRNAs, such as methylation, play an essential role in the biogenesis, stability, and cellular localization of this class of RNAs (6). N^6-methyladenosine (m^6A) is the most abundant modification of circRNAs and is mediated by a heterodimeric complex consisting of METTL3 (methyltransferase-like-3) and METTL14 (methyltransferase-like-14), with METTL3 bearing the enzymatic activity (7, 8).

circRNAs have been previously implicated in the pathogenesis of pulmonary fibrosis, including idiopathic pulmonary fibrosis (IPF) and lung silicosis (9–11), two progressive diseases of the lung characterized by the pathological accumulation of myofibroblasts and lung scarring (12, 13). For example, the circRNA TADA2A was reported to alleviate lung fibroblast activation and fibrosis by acting as a sponge to sequester microRNAs, thereby affecting target gene expression (10). In another study using the mouse model of bleomycin-induced lung fibrosis, circRNAs were shown to be part of a coexpression network of diverse noncoding RNAs that are involved in the fibrotic process (14). Although circRNAs were found to be implicated in the pathogenesis of pulmonary fibrosis, the mechanisms regulating their post-transcriptional modifications and modality of action during lung fibroblast activation and fibrosis remain poorly understood. Dysregulated circRNA methylation, including N^6-methylation, has been reported in many diseases, such as cancer and pulmonary hypertension (15); however, no study has explored the role of this epigenetic modification in lung fibrosis.

In this issue of the *Journal*, Wang and colleagues (pp. 510–523) describe studies using a mouse model of silica-induced pulmonary fibrosis in combination with an epitranscriptomic screen (16). They discovered that mouse lungs exposed to silicon dioxide (SiO_2) exhibited elevated m^6A-circRNA concentrations, suggesting that circRNA methylation may play a role in the progression of lung silicosis. Consistent with this finding, human lung fibroblasts exposed to SiO_2 *in vitro* also exhibited elevated concentrations of m^6A-circRNA. Further analysis identified two circRNAs, hsa_circ_0000672 and hsa_circ_0005654, as the major targets of this methylation in both mouse lungs and in isolated human lung fibroblasts. By performing RNA pull-down experiments, the authors also found that RNA-binding protein eIF4A3, a key player in circRNA biogenesis (5), interacted with these circRNAs, and its inhibition in SiO_2-stimulated lung fibroblasts attenuated the expression of profibrotic genes in these cells, suggesting that circRNA biogenesis is needed to support fibroblast activation. Loss-of-function experiments *in vitro* confirmed the fibrogenic nature of hsa_circ_0000672 and hsa_circ_0005654 and revealed that their simultaneous silencing was necessary to inhibit SiO_2-induced lung fibroblast activation. This latter finding reveals a novel mechanism of action for these circRNAs and suggests that a synergistic function may underlie their lung fibrogenic activity. Intriguingly, Wang and colleagues further show that silica treatment *per se* did not alter the expression of these two circRNAs, suggesting that post-transcriptional modifications, such as methylation, may be required for the fibrogenic activity of these circRNAs.

The study by Wang and colleagues also unveils a new role for the fibroblast methyltransferase METTL3 in the methylation of the circRNAs hsa_circ_0000672 and hsa_circ_0005654 during SiO_2-induced lung fibroblast activation and in the pathogenesis of lung silicosis. Indeed, the authors found that, among the methyltransferases that are known to modify circRNAs, METTL3 was the only one exhibiting increased expression in lung fibroblasts exposed to SiO_2, and this observation was confirmed in silica-exposed mouse lungs and in lungs of patients with silicosis. Using a series of elegant experiments, including *in situ* RNA hybridization and circRNA *in vitro* mutagenesis, the authors further establish that the methyltransferase METTL3 binds to and methylates the circRNAs hsa_circ_0000672 and hsa_circ_0005654, and these activities are important to support the fibrogenic activation of lung fibroblasts after silica exposure. Notably, METTL3-mediated methylation of linear RNAs was recently found to regulate the expression of gene transcripts implicated in the transition of lung fibroblasts to myofibroblasts (16), and, consistent with these findings, m^6A

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methylation was found elevated in the lungs of mice exposed to bleomycin as well as in IPF lungs (17). These findings implicate a broad mechanism of action for METTL3-mediated RNA methylation in various fibrotic lung disorders.

Overall, the paper by Wang and colleagues, together with the existing body of literature, open up future research opportunities to study noncoding RNAs and their synergistic activity in diseased lung fibroblasts as well as to assess how their epigenetic modifications ultimately contribute to lung fibroblast activation and fibrosis progression. Epigenetic alterations have been implicated in the pathogenesis of numerous chronic diseases, including pulmonary fibrosis; however, whether these changes affect circRNAs and contribute to lung fibroblast activation is not fully understood. This study sheds new light onto the epitranscriptional regulation of two m^{6}A-modified circRNAs and demonstrates their contribution to silica-induced lung fibroblast activation and silicosis. However, many questions remain regarding the downstream targets and coordinated mechanisms of action. With the implication of circRNAs in the pathogenesis of fibrotic lung disorders, including IPF (10, 11, 17), it is important to better understand the molecular mechanisms involved in the regulation of these types of noncoding RNAs during the fibrogenic process. Future work is needed to fill these knowledge gaps and identify novel and advanced tools to target circRNAs and their epigenetic modifications in fibrogenic lung disorders. ■

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