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Investigating Matrix–Fibroblast Regulation of MicroRNAs A Dice(r)y Proposition

Wound repair is an orchestrated response involving an array of resident and recruited cells that are regulated by multiple soluble mediators in a microenvironment characterized by dynamic biochemical and biomechanical changes (1). Our understanding of the mechanisms by which cellular dysfunction leads to aberrant repair and fibrosis has expanded dramatically (2). Recently, research has increasingly focused on how the matrix itself serves to perpetuate the cycle of aberrant repair, primarily through regulation of myofibroblast behavior (3–6). However, “the matrix” represents a complex structure that can be organized, cross-linked, and remodeled into scaffolds with varying mechanical properties and biochemical compositions. How different aspects of matrix biology regulate fibroblast function remains an area of active investigation. Cells sense and respond to the matrix largely through mechanotransduction signaling proteins, including integrins, FAK (focal adhesion kinase), ROCK (Rho-kinase), myocardin-related transcription factor-A, and the Hippo-kinase–associated Yap and Taz. Each of these proteins is activated by exposure to stiff (rather than compliant) substrates, promotes a profibrotic myofibroblast phenotype *in vitro*, and contributes to lung fibrosis *in vivo* (1, 5, 7–9).

In a study presented in this issue of the *Journal*, Herrera and colleagues (pp. 486–496) sought to determine how decellularized idiopathic pulmonary fibrosis extracellular matrices (IPF-ECM) diminished the stromal gene inhibitor miR-29 in normal fibroblasts (10). Prior work showed miR-29 suppression in fibroblasts on IPF-ECM and linked decreased miR-29 to pulmonary fibrosis, but the mechanisms involved have not been explored (4, 6, 11). Other studies showed that Yap activation in fibroblasts on stiff substrates also drove profibrotic gene expression and contributed to lung fibrogenesis (8). Thus, the investigators postulated that that Yap activation in fibroblasts exposed to an IPF-ECM would suppress miR-29 and promote increased stromal gene expression. In a series of rigorous experiments using normal and IPF-ECM and polyacrylamide hydrogels to model normal or fibrotic lungs *in vitro* along with two *in vivo* xenograft models of fibrosis, they found that that neither Yap nor the other mechanotransduction-related proteins listed above were responsible for miR-29 suppression by the IPF-ECM. Instead, they identified a novel mechanism by which

the IPF-ECM alters microRNA (miRNA) processing machinery to prevent maturation of miR-29.

After confirming that miR-29 suppression was coupled with increased collagen synthesis in cells on IPF-ECM, they discovered that miR-29 was increased (not decreased, as expected) in cells on stiff hydrogel substrates, and that Yap nuclear localization was reduced (not increased) in cells on the IPF-ECM. They also observed increased miR-29 precursors in cells on the IPF-ECM, suggesting that defective miRNA processing might account for the decreased mature miR-29. Indeed, the miRNA processing enzymes Dicer1, Ago2, and Drosha were decreased by the IPF-ECM. However, they were not influenced by differences in the stiffness of polyacrylamide hydrogels. Among the three enzymes, Dicer1 was notably diminished within the myofibroblast-rich core of fibroblastic foci in IPF tissue. Consistently, Dicer1 knockdown was associated with decreased miR-29 and increased ECM protein expression in fibroblasts on control ECMs. Finally, the introduction of Dicer1-deficient fibroblasts into zebrafish embryos or into mice promoted the development of lesions with increased procollagen expression suggestive of fibrosis.

These studies define a novel layer of mechanistic regulation involving defective post-transcriptional miRNA processing and enhance our evolving understanding of fibrosis biology. Prior studies have examined miRNA expression and explored how individual miRNAs impact cell behavior (12), but this study prompts us to consider the role of miRNA processing in fibrosis. One prior study reported decreased Ago1 levels in lung tissue from patients with IPF and decreased Ago2 in a subset of patients with IPF and rapid disease progression (13). In contrast to the current study, however, there were no observed differences in Dicer1 (13). Of course, the prior study compared total RNA from lung biopsies and protein from explanted fibroblasts cultured on plastic, so cell-specific differences and effects of substrate mechanics/composition could not be assessed (11). In the current study, Ago2 and Drosha were decreased by IPF-ECM, but that finding did not consistently extend to *in situ* studies of IPF tissue. Viewed more broadly, it is important to note that defects in miRNA processing should impact global miRNA expression, and that suppression of miRNAs other than miR-29 may, collectively, have significant effects on an *in vivo* system. It will be important to understand the mechanisms by which the IPF-ECM regulates miRNA processing, to determine what other miRNAs are altered, to evaluate the specific mechanism of miR-29 miRNA suppression, and to understand how these mechanisms interact with mechanotransduction signaling.

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This study calls attention to the strengths and weaknesses of reductionist model systems in the study of fibrosis. Laudably, the investigators used complementary approaches to clearly show that the IPF-ECM regulates miR-29 through mechanisms that are distinct from the mechanotransduction hierarchies reported using stiffness-controlled hydrogels. However, the results require that we reconcile the discordant findings generated from the different model systems. Matrix factors are critical to fibroblast biology, and incorporation of different matrix conditions into experimental design can provide important mechanistic insights (1, 6). However, it also demands appreciation of different levels of complexity. Hydrogels allow control of stiffness and ECM ligands, but lack dimensionality, matrix–protein interactions, and cross-linking. Decellularized matrices incorporate dimensionality, preserve native protein biochemistry, and maintain regional matrix mechanical properties. Nevertheless, they can be labor-intensive to produce and difficult to predict (14). Moreover, the effects of tissue processing on proteoglycans and glycosaminoglycans, on protein conformation, on matrix protein interactions, and on the enzymes/growth factors harbored within the matrix are all poorly understood. Neither approach replicates the *in vivo* effects of stretch or recapitulates the effect of multiple resident and recruited cell types interacting in time and space in the context of a chemically and mechanically changing matrix. With increasing complexity, control of experimental variables diminishes, as does the ability to study specific mechanistic biology in depth. In time, tissue engineering may improve our ability to study intact systems (14). Nevertheless, the use of *in vivo* xenograft models in the current study provides confidence that the miRNA processing machinery can have a critical effect on fibrogenesis. Whether this represents “the” answer for IPF remains to be seen, but the evidence that it could represent “an” answer is indicative of ongoing progress in the field. ■

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Of Mice and Men . . . and Microbes: Conclusions and Cautions from a Murine Study of the Lung Microbiome and Microbiome–Immune Interactions

The culture-independent sequencing revolution has debunked the myth that healthy lungs are sterile (1–3). Evidence from human studies supports a model in which the lower respiratory tract microbiome is formed predominantly by microaspiration of

bacteria from the upper airway, primarily the oropharynx (1, 4). This microbiome has a low biomass and likely is regulated by entry versus clearance involving a combination of mechanical, innate, and adaptive host defenses. Although the nature of this type of microbiome differs from the robust microbial communities at some other body sites where locally replicating populations may dominate over immigration and emigration, two key questions addressed in the study by Dickson and colleagues (pp. 497–508) in this issue of the *Journal* (5) are 1) does this “normal” lower respiratory tract

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