Outsourcing Invasion, a Novel Function for Extracellular Vesicles in the Lung

Activated fibroblasts in fibrotic lungs demonstrate increased adhesion, chemotaxis, matrix metalloproteinase secretion, and collagen deposition, comprising a matrix-invasive phenotype (1). However, the molecular mechanisms that drive this phenotype are not well understood. Signals from the matrix itself, be they biophysical through mechanosensing (2) or via changes in extracellular matrix (ECM) composition, seem to regulate the aberrant invasive phenotype (3). Cell-to-cell communication via the secretome, however, is increasingly appreciated as a driver of pathologic fibroblast differentiation. A recent study demonstrated that WNT-5a on extracellular vesicles (EVs) secreted from lung fibroblasts contributes to progression of lung fibrogenesis (4), establishing a role for EVs in pulmonary fibrosis through cell–cell communication. EVs, which can deliver genetic information (largely noncoding RNA), bioactive lipids, and proteins, are increasingly appreciated as important messengers conveying phenotype-altering signals between cells (5, 6). In this issue of the *Journal* (pp. 279–288), Chanda and colleagues (7) bring paracrine signaling and matrix signaling together by demonstrating that EV-associated fibronectin (FN) changes fibroblast phenotype by engaging cellular integrin $\alpha_5\beta_1$ signaling.

Chanda and colleagues undertook complementary approaches to assess the central hypothesis that surface expression of FN on EVs contributes to fibroblast migration and invasion. The authors used a cellular model of replicative senescence in IMR-90 fibroblasts to begin their studies. Cellular senescence is an important paradigm in pulmonary fibrosis that is associated with changes in the secretome that drive remodeling (8). Fibroblasts with high population-doubling levels (45 to 55), abbreviated "HPDL," represented senescence in this study, and they were compared with low population doubling (LPDL; $<$30). This simple *in vitro* model of senescence was compared in cultured normal versus idiopathic pulmonary fibrosis fibroblasts and also with fibroblasts treated with transforming growth factor-β1. Although the latter groups had increased EV production, HPDL cells had the highest levels of EV production by an order of magnitude, suggesting that EV secretion was strongly correlated with the degree of cellular senescence. The EVs in these different conditions were of a similar size range. Proteomics revealed abundant ECM proteins, particularly FN, in the EV; comparison of senescence-associated versus normal EV proteomes demonstrated enrichment for cellular adhesion and ECM remodeling. Interestingly, the majority of the transforming growth factor-β1–induced and HPDL-derived EVs were located not in the conditioned media but in the ECM.

Next, Chanda and colleagues considered whether EVs could alter fibroblast phenotype, specifically whether EVs could impact fibroblast invasion. They found that EVs added to Matrigel amplified the invasive phenotype of LPDL nonsenescent fibroblasts. This invasive phenotype induction was found to be dependent on FN on vesicles and could also be induced by direct incubation of EV with fibroblasts and subsequent removal. Invasion was blocked by incubation with antibody to either FN or $\alpha_5\beta_1$ integrin.

To explore the underlying mechanisms involved, the authors focused on integrin-regulated focal adhesion kinase (FAK) and Src signaling activation. Fibroblasts exposed to EVs displayed elevated FAK activity at tyrosine 397 as well as elevated Src kinase activity on tyrosine 416, illustrating that the exosomal FN initiated integrin-mediated FAK and Src signaling. Inhibition of Src kinase using AG1879 inhibitor abrogated fibroblast invasion.

The observations of Chanda and colleagues add to the growing number of functions of EVs in the context of tissue remodeling, encouraging further detailed and comprehensive studies. Additional studies are needed to further define the molecular mechanisms. For instance, does exosomal FN promote further cellular ECM assembly (9)? Integrin binding is known to stimulate FN self-assembly and actin cytoskeletal rearrangement to promote cell contractility and ECM remodeling. Subsequent conformational changes upon binding expose additional FN-binding sites associated with rearrangement of cellular FN dimers to insoluble fibrils. EV-associated FN may itself present as excessive soluble dimers, tethering $\alpha_5\beta_1$ integrin and controlling the downstream integrin signaling cascades and FN fibrillogenesis (10). The interconnected FN matrix impacts tissue organization by contributing to assembly of other ECM proteins, such as collagen. Alternatively, exosomal FN may compete with cellular secreted FN binding to integrin, disrupting cellular homeostasis as a result of altered signaling. Exosomal FN could also alter integrin-β1–dependent FN matrix turnover. Localized EV deposition with additional localized FN could alter the avidity of integrin binding and engage caveolin 1–dependent FN endocytosis (11). EV endocytosis via clathrin, caveolin, or lipid raft mechanisms could further alter FN-integrin signaling (12). It would be interesting to understand whether exosomal FN-integrin signaling is dependent on cellular uptake via caveolin 1 or other routes. It would also be useful to determine whether integrin internalization upon exosomal FN binding/endocytosis could regulate matrix remodeling by generating new adhesion sites (13). Similar mechanisms have been demonstrated for cancer cell exosomes regulating metastatic invasion on two-dimensional (2D) matrices (14).

It remains to be seen how the interesting novel observations in this study manifest in the setting of fibrotic remodeling. Does localized deposition of EVs in provisional matrix drive fibroblast invasion and activation *in vivo*? Localized EV deposition with excessive exosomal FN was shown to alter the behavior of less invasive fibroblasts (LPDL) on 2D Matrigel by mediating FAK-Src phosphorylation, propagating a transformed-like phenotype with acquired invasive ability. Is the FAK-Src signaling network initiating a genetic or epigenetic process fundamentally changing cellular phenotype? It remains to be seen how such ECM-deposited signals *in vivo* alter overall fibrotic susceptibility within tissues. Future studies will likely elucidate the contexts in which such

"outsourcing" of invasive signaling takes place, as well as the consequences for both functional and pathologic tissue remodeling. ■

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Tzu-Pin Shentu, Ph.D.
Section of Pulmonary and Critical Care, Department of Medicine
University of Chicago
Chicago, Illinois

James S. Hagood, M.D.
Division of Pulmonology, Department of Pediatrics
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina

References

1. Li Y, Jiang D, Liang J, Meltzer EB, Gray A, Miura R, *et al*. Severe lung fibrosis requires an invasive fibroblast phenotype regulated by hyaluronan and CD44. *J Exp Med* 2011;208:1459–1471.
2. Chen H, Qu J, Huang X, Kurundkar A, Zhu L, Yang N, *et al*. Mechanosensing by the α6-integrin confers an invasive fibroblast phenotype and mediates lung fibrosis. *Nat Commun* 2016;7:12564.
3. Thannickal VJ, Henke CA, Horowitz JC, Noble PW, Roman J, Sime PJ, *et al*. Matrix biology of idiopathic pulmonary fibrosis: a workshop report of the National Heart, Lung, and Blood Institute. *Am J Pathol* 2014;184:1643–1651.
4. Martin-Medina A, Lehmann M, Burgy O, Hermann S, Baarsma HA, Wagner DE, *et al*. Increased extracellular vesicles mediate WNT-5A signaling in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2018; 198:1527–1538.
5. Shentu TP, Huang TS, Cernelc-Kohan M, Chan J, Wong SS, Espinoza CR, *et al*. Thy-1 dependent uptake of mesenchymal stem cell-derived extracellular vesicles blocks myofibroblastic differentiation. *Sci Rep* 2017;7:18052.
6. Tkach M, Théry C. Communication by extracellular vesicles: where we are and where we need to go. *Cell* 2016;164:1226–1232.
7. Chanda D, Otoupalova E, Hough KP, Locy ML, Bernard K, Deshane JS, *et al*. Fibronectin on the surface of extracellular vesicles mediates fibroblast invasion. *Am J Respir Cell Mol Biol* 2019;60:279–288.
8. Schafer MJ, White TA, Iijima K, Haak AJ, Ligresti G, Atkinson EJ, *et al*. Cellular senescence mediates fibrotic pulmonary disease. *Nat Commun* 2017;8:14532.
9. Singh P, Carraher C, Schwarzbauer JE. Assembly of fibronectin extracellular matrix. *Annu Rev Cell Dev Biol* 2010;26:397–419.
10. Huveneers S, Truong H, Fässler R, Sonnenberg A, Danen EH. Binding of soluble fibronectin to integrin $\alpha_5\beta_1$ – link to focal adhesion redistribution and contractile shape. *J Cell Sci* 2008;121:2452–2462.
11. Shi F, Sottile J. Caveolin-1-dependent beta1 integrin endocytosis is a critical regulator of fibronectin turnover. *J Cell Sci* 2008;121:2360–2371.
12. Mulcahy LA, Pink RC, Carter DR. Routes and mechanisms of extracellular vesicle uptake. *J Extracell Vesicles* 2014;3:24641.
13. De Franceschi N, Hamidi H, Alanko J, Sahgal P, Ivaska J. Integrin traffic – the update. *J Cell Sci* 2015;128:839–852.
14. Wang Z, von Au A, Schnölzer M, Hackert T, Zöller M. CD44v6-competent tumor exosomes promote motility, invasion and cancer-initiating cell marker expression in pancreatic and colorectal cancer cells. *Oncotarget* 2016;7:55409–55436.