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Integrins, Myofibroblasts, and Organ Fibrosis

Michel Fausther, PH.D. and Jonathan A. Dranoff, M.D.

¹University of Arkansas for Medical Sciences, Little Rock, AR

Abstract

Myofibroblasts are the major source of extracellular matrix components that accumulate during tissue fibrosis, and hepatic stellate cells (HSCs) are believed to be the major source of myofibroblasts in the liver. To date, robust systems to genetically manipulate these cells have not been developed. We report that Cre under control of the promoter of *Pdgfrb* (*Pdgfrb*-Cre) inactivates loxP-flanked genes in mouse HSCs with high efficiency. We used this system to delete the gene encoding α_V integrin subunit because various α_V -containing integrins have been suggested as central mediators of fibrosis in multiple organs. Such depletion protected mice from carbon tetrachloride–induced hepatic fibrosis, whereas global loss of β_3 , β_5 or β_6 integrin or conditional loss of β_8 integrins in HSCs did not. We also found that *Pdgfrb*-Cre effectively targeted myofibroblasts in multiple organs, and depletion of the α_V integrin subunit using this system was protective in other models of organ fibrosis, including pulmonary and renal fibrosis. Pharmacological blockade of α_V -containing integrins by a small molecule (CWHM 12) attenuated both liver and lung fibrosis, including in a therapeutic manner. These data identify a core pathway that regulates fibrosis and suggest that pharmacological targeting of all α_V integrins may have clinical utility in the treatment of patients with a broad range of fibrotic diseases.

Integrins are transmembrane receptors involved in cell-cell and cell-matrix signalling pathways. Each integrin is typically formed by the non-covalent pairing of one α subunit, of which, 18 types are known to exist, and one β subunit, of which 8 types are known to exist. Together, 24 distinct heterodimers have been identified to date (1). This observed high variability in dimeric integrin assembly translates into diversity in ligand-binding specificity (most integrin ligands being matrix components) and functional non-redundancy (2). It is well established that, in multiple organs, integrins can control the release and activation of the important profibrogenic cytokine transforming growth factor beta (TGF- β) (3-5). Importantly, this regulatory effect of integrins on TGF- β activity appears to involve α_V -containing integrins primarily. Tissue myofibroblasts, which are the dominant effector cells during fibrosis in multiple organs, express multiple α_V -containing integrins (6). Taken together, these findings suggest that α_V integrin itself may be essential for the development of fibrosis, regardless of its associated β subunit.

The goal of the present study by Henderson and colleagues was to examine the role of α_V integrin in liver fibrosis, and specifically to determine whether targeted deletion of this integrin in myofibroblasts prevents fibrosis. For that purpose, the authors used *Itgav^{lox/lox}*; *Pdgfrb*-Cre mice to perform experimental models of fibrosis in liver (carbon tetrachloride intoxication, CCl₄ for 6 weeks), lungs (bleomycin instillation for 14 and 28 days), and kidneys (unilateral ureteric obstruction, UUO for 14 days). The approach used here was to

induce targeted deletion of α_V integrin gene specifically in cells displaying *Pdgfrb* promoter activity – that is, myofibroblasts in the fibrosing organ. Without wanting to overstate our case, we suggest that this superbly clever methodology (along with the experimental designs applied) makes this work required reading in the liver fibrosis field for 2014.

The authors initially validated *Pdgfrb*-Cre mice as a model to study and track tissue pericytes or myofibroblasts by crossing them with dual mTmG-GFP (7) and/or single Ail4 (8) reporter mice. Using the well-characterized CCl₄-induced liver fibrosis model, they showed that *Pdgfrb* is a reliable marker for liver pericytes and hepatic stellate cells (HSCs), as most reporter-labeled cells expressed *Pdgfrb* and desmin in normal and fibrotic livers and α -SMA *de novo* in fibrotic livers. A similar expression pattern for these markers was observed in pericytes from lungs and kidneys, using the bleomycin and UUO fibrosis models, respectively. In aggregate, these findings suggest that the *Pdgfrb* promoter is an effective way to target myofibroblasts in a variety of organs. The constitutive expression of *Pdgfrb* in quiescent HSCs was confirmed in *Pdgfrb*-BAC-eGFP reporter mice. This is an interesting observation, since *Pdgfrb* had been previously characterized only as an HSC activation marker (9). Here, use of other myofibroblast markers such as, Lrat (10) or Cytoglobin (11) would have helped to better characterize liver *Pdgfrb*-expressing cells. Indeed, liver myofibroblasts are increasingly recognized as heterogeneous groupings of cells as liver fibrosis progresses (12, 13). In particular, α -SMA-expressing myofibroblasts and collagen-producing myofibroblasts are distinct, but overlapping, populations (14). Besides myofibroblasts, “vascular wall” cells, likely vascular smooth muscle cells, also express *Pdgfrb*, desmin, and α -SMA, meaning that vascular wall cells could also contribute functionally to any observed phenotype of *Pdgfrb*-Cre mice. It is also worth noting that there is no scientific consensus as to the ideal markers or set of markers defining hepatic stellate cells or liver myofibroblasts. That said, in all three fibrosis models, the authors showed increased expression of profibrogenic collagen and α -SMA genes among others in primary tdTomato⁺-sorted Ail4; *Pdgfrb*-Cre pericytes isolated from fibrotic tissues, when compared to controls.

The most exciting aspect of the study is the observation that α_V integrin is important in the survival and/or function of liver myofibroblasts. The authors demonstrated that α_V integrin expression, which gradually increases in primary liver *Pdgfrb*-labeled HSCs upon culture activation, can be efficiently suppressed in primary liver *Itgav*^{flox/flox}; *Pdgfrb*-Cre HSCs in similar conditions. Here, the authors did not describe whether (or to which extent) α_V integrin expression is reduced in quiescent *Itgav*^{flox/flox}; *Pdgfrb*-Cre HSCs, which harbor an active *Pdgfrb*. This is a critical point to address, because it is unknown whether this could induce potential phenotypic changes in HSCs, even prior to myofibroblastic differentiation. Next, the authors presented the main finding of the paper: targeted deletion of α_V integrin in myofibroblasts is protective against organ fibrosis *in vivo* and *in vitro*. Histologic analysis of fibrotic liver, lung and kidney tissues from *Itgav*^{flox/flox}; *Pdgfrb*-Cre mice showed significant (although partial) reduction in matrix deposition, myofibroblastic differentiation, and phosphorylation of the TGF- β downstream target Smad3 as compared to controls. Of note, while the chosen time course for bleomycin model is appropriate (15), the one chosen for

the CCl₄ liver model appears to be brief (16). Still, the findings of fibrosis reduction are highly noteworthy.

As noted above, integrins function only as obligate heterodimers, with α and β subunits necessary for function; interestingly, this heterodimer arrangement is preserved even in primitive organisms (17). Thus, the authors investigated whether β integrins known to be associated with α_V affected experimental liver fibrosis. Surprisingly, the answer was (more or less), no. Genetic deletion of the α_V -associated integrins β_3 , 5, 6, or 8 in mice did not confer protection against CCl₄-induced liver fibrosis (although deletion of β_1 integrin could not be tested in vivo due to a lack of mouse viability). It may be inferred that an array of β integrins may associate with α_V in a way necessary for liver myofibroblast function.

How does α_V integrin regulate liver myofibroblast functions? The authors provide in vitro evidence that the answer may be via regulation of the TGF- β signal transduction cascade. Specifically, α_V integrin deletion in culture-activated *Itgav*^{flox/flox}, *Pdgfrb*-Cre HSCs or antibody-mediated α_V integrin inhibition in culture-activated tdTomato⁺-sorted Ail4; *Pdgfrb*-Cre HSCs led to decreased expression of collagen and α -SMA, but not TGF- β . However, a TGF- β reporter assay demonstrated that suppression of α_V integrin induced defective TGF- β /p-Smad3 signalling in *Itgav*^{flox/flox}, *Pdgfrb*-Cre HSCs. This suggests that the effect observed did not result from reduced cytokine expression, but rather from diminished integrin-mediated capacity to activate TGF- β . This hypothesis is intriguing and needs to be explored in greater depth.

Is it possible that the findings of this work may be applied in a translational fashion? To address this, the authors tested the effectiveness of a small molecule inhibitor of α_V integrin, CWHM12, in liver and lung fibrosis models. In both models, CWHM12 treatment decreased organ fibrosis and impaired TGF- β /p-Smad3 signalling. Thus, it is indeed plausible that an anti- α_V integrin approach will be rational to investigate as a means to prevent or treat liver (and kidney and lung) fibrosis. However, caveats are important to consider. The experience of Crohn's disease treatment with the humanized α_4 integrin monoclonal blocking antibody Natalizumab provides a cautionary tale (18). Natalizumab presumably works by blocking α_4 integrin function(s) in white blood cells and is effective in Crohn's disease treatment (19). However, Natalizumab treatment is associated with a risk of progressive multifocal leukoencephalopathy, as these same white blood cells are known to cross the blood-brain barrier (which is why this drug is effective for treatment of multiple sclerosis) (20-22). Still, we are optimistic that α_V -mediated therapy is highly worth pursuing, and we look forward to laboratory refinements to better understand mechanisms of action.

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