

Targeting αv Integrins in Pancreatic Fibrosis: Progress in Resolving the Scar



Integrins are the glue that maintain cell-to-cell adhesion and are found in nearly all cell types and function as heterodimeric cell surface receptors. Although not regarded as active signaling receptors in the basal state, integrins transduce signals that regulate fibrosis, angiogenesis, cellular migration, and other wound-healing processes during pathologic conditions.^{1,2} In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Ulmasov et al³ report that a synthetic peptide (CWHM-12) containing a conserved Arg-Gly-Asp (RGD) integrin-recognition domain ameliorated cerulein-induced pancreatic fibrosis and pancreatic stellate cell (PSC) activation by inhibiting transforming growth factor (TGF)- β activation.

In a previous study, 2 of the current authors identified a decisive role for the αv integrin subunit on hepatic stellate cells in mediating tissue fibrosis by activating TGF- β .⁴ In that study, CWHM-12 reduced liver and pulmonary fibrosis by blocking integrin heterodimers containing the essential αv subunit. In the present translational study of chronic pancreatitis, CWHM-12 was tested prophylactically and therapeutically during the course of cerulein-induced pancreatitis. Prophylactic CWHM-12 administration reduced pancreatic fibrosis by 80%, as assessed by Sirius red staining compared with control-treated mice. These findings were accompanied by markedly reduced PSC activation as measured by α -smooth muscle actin labeling and protein levels, decreased SMAD3 activation, and decreased collagen I expression. Therapeutic administration of CWHM-12 also significantly lessened Sirius red staining and α -smooth muscle actin expression compared with control and CWHM-96, the inactive enantiomer of the active peptide drug.

A central finding of this study was identification of the αv subunit as a critical integrin in mediating fibrotic responses in the murine pancreas and in primary PSCs. The αv integrin subunit was expressed abundantly by quantitative polymerase chain reaction and induced with cerulein stimulation. Labeling of αv in pancreatic sections was minimal in the normal pancreas, markedly induced after cerulein, and diminished with CWHM-12 pretreatment. A concentration-response curve showed a robust 50% inhibition of TGF- β luciferase reporter activity with 2.5 nmol/L CWHM-12. In contrast, the CWHM-12 dose used in mice (100 mg/kg/day) achieved serum levels (6–9 μ g/mL) that exceeded concentrations necessary to inhibit TGF- β luciferase activity in vitro. Whether a lower amount of CWHM-12 would yield similar inhibitory effects on fibrosis in vivo is unclear from their studies.

The authors excluded other possibilities by which CWHM-12 may have affected fibrotic responses seen in

their model. The severity of acute pancreatitis responses was not different between control and CWHM-12-treated mice, as assessed by histology, pancreatic weights, and amylase measurements. Surprisingly, serum white blood cell counts seen in the acute pancreatitis were not increased with cerulein alone, but addition of CWHM-12 more than doubled serum white blood cell and neutrophil counts. This may reflect an important off-target effect of blocking αv integrins, which are present at notably higher levels in endothelial cell populations compared with stellate cells.⁴ The authors further discovered that CWHM-12 decreased (matrix metallo-proteinase-2) activity, thereby implicating a blockade of matrix production rather than protease induction as the mechanism for reduced fibrosis with CWHM-12. The dynamic nature of protease activity and the multitude of proteases that cannot be accounted for by zymography, however, make this conclusion receptive for further testing.

This study follows several notable successes in translating integrin-based therapeutics to the clinic.⁵ Drugs targeting integrins on platelets and lymphocytes are already in clinical use, including the recent clinical approval for vedolizumab, a humanized monoclonal antibody that targets the $\alpha 4\beta 7$ heterodimer in patients with inflammatory bowel disease who have failed standard therapies.^{6,7} Tackling fibrotic diseases using integrin-based therapeutics has not yet been successful. However, studies by this group and others have noted that lung and liver fibrosis could be reduced through CWHM-12 or a small-molecule inhibitor of the $\alpha v\beta 1$ integrin.^{4,8} These integrin subunits were expressed at high levels in the total murine pancreas and primary PSCs, and induced by cerulein-induced pancreatitis or serum-induced activation, respectively. Whether targeting a single heterodimer containing the αv subunit is enough to block pancreatic fibrosis is unclear.

Several limitations of the current study warrant comment. The first is that a true irreversible and progressive fibrotic phenotype that replicates human chronic pancreatitis (CP) is difficult to model.⁹ The 3-day course of repetitive cerulein-induced fibrosis used in this study induces a certain degree of fibrosis, but a wash-out period alone would have resulted in fibrosis resolution. Previous studies of CP typically have used longer periods of repetitive cerulein injections for up to 10 weeks to induce persistent fibrosis.¹⁰ Moreover, the authors of this study who are experts in experimental chronic pancreatitis have noted the varying fibrotic responses found in different genetic strains of mice.¹¹ Related to this point is the diversity, chronicity, and, hence, unpredictability of human CP. This genetic diversity may make applications with

integrin-based therapeutics for CP potentially far more challenging.

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