

$\alpha\text{v}\beta\text{1}$ integrin as a novel therapeutic target for tissue fibrosis

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Abstract: Chronic tissue injury with fibrosis results in disruption of tissue architecture, organ dysfunction and eventually organ failure. Currently, therapeutic options for tissue fibrosis are severely limited and organ transplantation including high cost and co-morbidities is the only effective treatment for end-stage fibrotic disease. Therefore, it is imperative to develop effective anti-fibrotic agents. Integrins are transmembrane proteins and are major receptors for cell-extracellular matrix (ECM) and cell-cell adhesion. Modulation of these molecules, particularly αv integrin family, has exhibited profound effects on fibrosis in multiple organ and disease state. Based on the several studies, the integrins $\alpha\text{v}\beta\text{3}$, $\alpha\text{v}\beta\text{5}$, $\alpha\text{v}\beta\text{6}$, and $\alpha\text{v}\beta\text{8}$ have been known to modulate the fibrotic process via activation of latent transforming growth factor (TGF)- β in pre-clinical models of fibrosis. In this perspective, we reviewed the functions of $\alpha\text{v}\beta\text{1}$ integrin as a potentially useful target molecule for antifibrotic agent and introduced novel specific small-molecule inhibitors targeting this integrin.

Keywords: Integrin; transforming growth factor-beta; fibrosis; small-molecule inhibitor

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Regulation of TGF- β activation during tissue fibrosis

A general feature of fibrosis is the complex interplay between the inflammatory/epithelial myofibroblast and extracellular matrix components of the wound healing response (1-4). TGF- β is a most pleiotropic cytokine and a major pro-fibrogenic cytokine. Thus, to avoid uncontrolled TGF- β activated processes, the modulation of its activity is attractive targets for novel anti-fibrotic therapies (5,6). A pivotal role of TGF- β in fibrogenesis was first revealed when subcutaneous injection of purified TGF- β1 was seen to induced fibrosis lesions at the injection site (5). This potent effect was further confirmed by the fibrotic response in lungs of rodents following intratracheal administration of adenovirus expressing active TGF- β1 (7). Conversely, neutralization of TGF- β and the inhibition of the TGF- β /Smad pathway were shown to prevent fibrosis in mice (8-11).

The mammalian TGF- β family forms a group of three isoforms (TGF- β1 , TGF- β2 , and TGF- β3). TGF- β

proteins are initially synthesized as precursor proteins containing growth factor and latency associated peptide (LAP) in endoplasmic reticulum. After proteolytic process, the peptides are assembled as a non-covalent complex of a disulfide linked homodimer of the mature cytokine (a short C-terminal fragment) and a disulfide linked homodimer of LAP. The complex of TGF- β dimer and LAP homodimer is referred to as the small latent complex (SLC). In this complex, the associated LAP chains by conformational change and non-covalent bonds form a specific type of protection, maintaining TGF- β in its inactive form and preventing its interaction with a receptor. SLC is then connected with a latent TGF- β binding protein (LTBP), which is usually linked to ECM, through LAP; the resultant protein complex is referred to as the large latent TGF- β complex (LLC). The fact that the association of LTBP with SLC is also important for proper TGF- β function was proven by experiment that mice expressing a mutant TGF- β1 precursor with a pro-segment that is unable to bind LTBP, showed the decreased levels of active TGF- β1 and the attenuated TGF- β signaling (12).

SLC containing the latent TGF- β complex can be considered as a sensor that remains in the ECM by chemical cross linking until triggered by a specific signal to release TGF- β as an active cytokine; this process is referred to as activation (13,14). There are two distinct mechanisms to explain the release of the active TGF- β from LLC. The one mechanism is an integrin-dependent TGF- β activation. Latent TGF- β complex contains arginine-glycine-aspartate (RGD) sequence in C-terminal end of the LAP chain, which shows high affinity to integrin molecules, is required for interaction with integrin. Once integrin interacts with latent TGF- β complex via RGD sequence, mature TGF- β can be released (activated) by conformational change of the whole complex without a need for proteolytic digestion. As an alternative mechanism, mature TGF- β can be released from the LLC in the presence of numerous molecules, mainly including proteases such as plasmin, matrix metalloproteinase 2 (MMP2, gelatinase A), matrix metalloproteinases 9 (MMP9, gelatinase B), BMP-1, or non-protease molecules such as thrombospondin 1 (THBS1), retinoic acid, and fibroblast growth factor 2 (FGF2), as well as reactive oxygen species (ROS); low ECM pH can also influence TGF- β activity (13-20).

Integrin heterodimers as a regulator of fibrosis

In the last decade, it has become clear that a subset of the integrin family plays a key role in the activation of latent TGF- β . Integrins are transmembrane receptors involved in cell-cell and cell-matrix signaling pathways during fibrosis (21,22). Thus, integrins represent a major node of communication between the ECM, inflammatory cells, fibroblasts and parenchymal cells and are intimately involved in progression of tissue fibrosis. In addition, it also can translate extracellular signals, resulting in a wide range of downstream effects on cell adhesion, migration, proliferation, differentiation, and apoptosis (23,24). 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 (25). The αv subunit can form heterodimers with the $\beta 1$, $\beta 3$, $\beta 5$, $\beta 6$ or $\beta 8$ subunits and $\beta 1$ can associate with many different α subunits from $\alpha 1$ to $\alpha 11$, and αv , indicating that not all theoretically possible α and subunit pairs form. Interestingly, the activation of TGF- β appears to be a common function of multiple αv integrins. Deletion

of αv integrin from myofibroblast significantly inhibited fibrosis in the liver, lung and kidney, demonstrating that myofibroblast αv integrins are major components of a core pathway widely shared by pathological fibrosis in multiple solid organs (26).

Integrins $\alpha v \beta 6$ and $\alpha v \beta 8$ are well-characterized activators for TGF- β (23,27-32). In addition, other αv integrins, such as $\alpha v \beta 1$, $\alpha v \beta 3$, and $\alpha v \beta 5$, have also been implicated as interacting with LAP and activating latent TGF- β (33-35). For example, $\alpha v \beta 6$ is required to promote lung fibrosis in response to bleomycin (22) and kidney fibrosis in response to deficiency of collagen subunit Col4A3 (36). Wipff *et al.* reported that thrombin-activated contraction of myofibroblasts activates latent TGF- $\beta 1$ predominantly via $\alpha v \beta 5$ and, less so, via $\alpha v \beta 3$ or an unidentified $\beta 1$ integrin (37). In addition, inhibition and blockade of $\alpha v \beta 6$ and $\alpha v \beta 8$ phenotypic concordance have the developmental effects of loss of TGF- $\beta 1$ and 3, suggesting that the relative importance of these two integrins mediates TGF- β activation during development (38). In recent study by Henderson *et al.*, they showed that specific silencing of αv subunit expression in activated fibroblasts achieves robust suppression of TGF- $\beta 1$ activation *in vitro* and protection from carbon tetrachloride (CCl₄)-induced organ fibrosis *in vivo* (26). However, they were unable to effectively inhibit liver fibrosis by individual deletion of three of four β subunits known as partners for αv in fibroblast ($\beta 3$, $\beta 5$, and $\beta 8$). Notably, the $\alpha v \beta 1$ is difficult to challenge because $\beta 1$ subunit can form dimer with multiple α subunits and, therefore, investigation for the role of $\alpha v \beta 1$ itself has not been possible by using standard transgenic mouse approaches. Moreover, there are no proper experimental tools such as specific function blocking antibodies or small molecule antagonists against $\alpha v \beta 1$.

Recently, Reed *et al.* has been firstly developed a small molecule inhibitor of $\alpha v \beta 1$, which is highly specific and has potential activity in liver and lung fibrotic disease (34). This study has highlighted the points that $\alpha v \beta 1$ integrin binds to and activates latent TGF- β complex and this activity is mediated in tissue fibrosis. They designed a computational model of the $\alpha v \beta 1$ integrin to predict crystal structure of ligand-binding site and synthesized a small set of compounds, including the αv binding base compound and the $\beta 1$ binding sulfonamidoproline moiety amide linker. The most encouraging compound, C8, effectively inhibited $\alpha v \beta 1$ integrin-mediated cell adhesion to the ligand fibronectin, with a sub-nanomolar median inhibitory concentration.

These studies showed that systemic administration of C8 to mice attenuated bleomycin-induced pulmonary fibrosis and CCl₄-induced liver fibrosis by suppressing TGF- β signaling through reduced phosphorylation of Smad3, suggesting that this anti-fibrotic effect is attributable to the inhibition of α v β 1 integrin-mediated TGF- β activation. Although Reed *et al.* found that α v β 1 integrin could bind to TGF- β LAP and activate TGF- β function, the insight into detailed mechanisms still remains unclear. However, it is worth that the possibility of clinical approaches is able to identify additional roles of integrin family by using C8 inhibitor as a potent tool.

Therapeutic targeting of α v β 1 integrin

Today, the integrins are known to have vital roles in both health and disease, and their potential as therapeutic target is now widely recognized. However, therapeutically successful inhibition of integrins is still left to be elusive despite the discovery of highly potent inhibitors. It is due to several reasons including diversity and redundancy of the integrins, the importance of integrins in key physiological systems.

According to the great success of Reed and Sheppard, α v β 1 integrin is an attractive target for multiple chronic fibrotic diseases and C8 could be a useful tool to investigate the functional role of this integrin in disease model. However, they haven't provided detailed insight of mechanistic role of the inhibitors in integrin/TGF- β receptor signaling pathway. Therefore, the expanding knowledge for the integrin signaling pathways make it possible to allow a more refined and targeted approach to integrin-TGF- β signaling with fewer undesirable side effects and to reduce the failures of many candidates in clinical trial. In addition, it should be considered whether other TGF- β ligands or activators are somewhat involved in these fibrotic processes or not, how TGF- β differently acts as a pleiotropic growth factor in physiological or pathological systems, as well as how it is connected and cross-talked among the redundant integrin families. Collectively, a therapeutically specific targeting to α v β 1 integrin is expected to yield higher efficiency for targeting toward new anti-fibrotic inhibitor or TGF- β inhibitor and it must be pursued enthusiastically.

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