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Review

Integrin-mediated regulation of TGFβ in fibrosis ☆

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ABSTRACT

Fibrosis is a major cause of morbidity and mortality worldwide. Currently, therapeutic options for tissue fibrosis are severely limited, and organ transplantation is the only effective treatment for end-stage fibrotic disease. However, demand for donor organs greatly outstrips supply, and so effective anti-fibrotic treatments are urgently required. In recent years, the integrin family of cell adhesion receptors has gained prominence as key regulators of chronic inflammation and fibrosis. Fibrosis models in multiple organs have demonstrated that integrins have profound effects on the fibrotic process. There is now abundant *in vivo* data demonstrating critical regulatory roles for integrins expressed on different cell types during tissue fibrogenesis. In this review, we will examine the ways in which integrins regulate these processes and discuss how the manipulation of integrins using function blocking antibodies and small molecule inhibitors may have clinical utility in the treatment of patients with a broad range of fibrotic diseases. This article is part of a Special Issue entitled: Fibrosis: Translation of basic research to human disease.

Contents

1. Introduction

Fibrosis represents a massive health care burden worldwide. Chronic tissue injury with fibrogenesis results in disruption of tissue architecture, organ dysfunction and eventually organ failure. Our therapeutic repertoire for the treatment of tissue fibrosis is severely limited and organ transplantation is currently the only effective treatment in end-stage fibrotic disease. However, organ transplantation has several disadvantages including limited donor organ availability, high cost, co-morbidities in potential recipients and on a global scale, organ transplantation can only be offered to a small percentage of the patients suffering from the complications of fibrosis. Therefore there is an urgent imperative to develop effective anti-fibrotic therapies.

A universal feature of tissue fibrogenesis is the complex interplay between the inflammatory, epithelial, myofibroblast and extracellular matrix components of the wound healing response [1–3]. Furthermore, the pericellular extracellular matrix is a highly dynamic environment known to exert profound influences on cell behavior. Many of the key cell–cell and cell–matrix interactions which regulate fibrosis are mediated

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by members of the integrin family of cell adhesion molecules, of which there are 24 known members in humans (noncovalent α/β heterodimers composed from 18 different α subunits and 8 β subunits). Integrins represent a major mode of communication between the extracellular matrix, inflammatory cells, fibroblasts and parenchymal cells, and hence are intimately involved in the processes that govern the initiation, maintenance and resolution of tissue fibrosis. Integrins are transmembrane proteins and are major receptors for cell adhesion to extracellular matrix proteins and cell–cell adhesion [4]. These molecules can therefore mediate the translation of spatially fixed extracellular signals into a wide variety of changes in cell behavior including cell adhesion, migration, proliferation, differentiation and apoptosis [4,5]. In addition to their direct effects on cellular proliferation and survival, integrins can also potentiate signals from soluble growth and survival factors. For example, nearly all of the pro-fibrogenic cytokine transforming growth factor beta 1 (TGFβ1) is secreted and bound to the extracellular matrix in a latent form, and therefore conversion to an active form is an important step in the regulation of TGFβ1 activity. In recent years it has become clear that a subset of the integrin family (αv integrins) play a key role in the activation of latent TGFβ1. Specifically, the integrins αvβ3, αvβ5, αvβ6 and αvβ8 have been shown to bind the RGD sequence in the latency associated peptide (LAP) of TGF-β1 and -β3, and have the potential to activate latent TGF-β [6–10]. In this review we will highlight recent data demonstrating the profound effects of integrins in modulating the fibrotic process *via* activation of TGFβ, and how pharmacologic manipulation of specific integrins may lead to the development of new antifibrotic treatments.

2. Lung fibrosis

2.1. αv integrin-mediated activation of latent TGFβ

Secreted transforming growth factor beta 1 (TGFβ1) is a major pro-fibrogenic cytokine and a key regulator of fibrosis in multiple organs [11–13]. Therefore, the molecular pathways that regulate TGFβ1 activity and signaling are attractive targets for novel anti-fibrotic therapies. There are three mammalian isoforms of TGFβ, and all are synthesized as precursor proteins that are processed by proteolytic cleavage in the endoplasmic reticulum and 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 a larger amino terminal fragment called the latency associated peptide (LAP), forming the "small latent complex". In this form the associated LAP homodimer prevents the mature C-terminal fragment from binding to its receptors and inducing TGFβ's known effects. This "small latent complex" is further modified in the endoplasmic reticulum by disulfide linkage to another family of gene proteins called latent TGFβ binding proteins, which, upon secretion, are themselves chemically cross-linked to the extracellular matrix, to store and tether TGFβ in a latent form in the extracellular space. Much of the regulation of TGFβ biology thus occurs at the level of extracellular activation of this stored latent complex [14,15].

Because the active form of TGFβ is non-covalently linked to the latency associated peptide and easily dissociates upon changes in temperature or pH [15], *in vitro* examination of TGFβ activation has been difficult. Therefore, the *in vivo* mechanisms of matrix-bound latent TGFβ conversion into an active cytokine are the subject of intense research. Two of the three mammalian TGFβ isoforms (TGFβ1 and 3) can be activated by members of the integrin family that interact with a linear arginine–glycine–aspartic acid (RGD) motif present in the latency associated peptide [6,7,16]. Inhibition and blockade of two of these integrins (αvβ6 and αvβ8) phenocopies all of the developmental effects of loss of TGFβ1 and 3 [17], suggesting that these two integrins are required for most or all important roles of these TGFβ isoforms during development. However, the mechanisms of TGFβ activation that contribute to tissue pathology in adults are less well understood.

In the lung, the αvβ6 integrin is minimally expressed in alveolar epithelial cells at baseline but is rapidly induced in this cell type following lung injury [18]. Evidence supporting an important role for the αvβ6 integrin in TGFβ1 activation came from observation of the phenotype of β6 integrin subunit knockout mice. These mice develop exaggerated inflammatory responses in the lungs and skin, reminiscent of, but less severe than the exaggerated inflammation seen in mice homozygous for a null mutation of TGFβ1 [19]. Furthermore, following treatment with bleomycin (a widely used inducer of pulmonary fibrosis), β6 null mice develop exaggerated inflammation but are dramatically protected from subsequent pulmonary fibrosis [6]. β6 inhibition (both by genetic knockout and blockade by anti-αvβ6 antibodies) was also protective in radiation-induced pulmonary fibrosis [20]. The αvβ6 integrin can bind directly to the LAP of TGFβ1 and TGFβ3 [16] and cells expressing αvβ6 generate TGFβ1 activity *in vitro* that can be completely inhibited by β6 blocking antibodies. In addition, microarray analysis of the lungs of wild type or β6 null mice following intratracheal instillation of bleomycin identified a large group of TGFβ-inducible genes that were induced at substantially lower levels in β6 knockout mice [21]. Taken together, these data demonstrate that αvβ6 integrin expression on lung epithelial cells is a major regulator of TGFβ1 activation during lung fibrosis.

Activation of TGFβ1 was inhibited by blockade of actin polymerization [6] and by Rho kinase inhibition [22], suggesting a role for force generation by the actin cytoskeleton. Indeed, the recently solved crystal structure of the small latent complex of TGFβ1 demonstrated that mechanical force generated by integrins is a common mechanism for activating latent TGFβ1 [23]. Shi and colleagues found that crystals of dimeric porcine proTGF-β1 revealed a ring-shaped complex, a novel fold for the prodomain (LAP) of TGFβ1, and demonstrated that the prodomain shields the growth factor from recognition by receptors and alters its conformation. Furthermore, complex formation between αvβ6 integrin and the prodomain of TGFβ1 was insufficient for TGFβ1 release, and force-dependent activation of TGFβ1 required unfastening of a "straitjacket" that encircles each growth factor monomer.

Myofibroblasts are a further cell type intrinsically involved in the fibrotic process, as they are the major source of extracellular matrix proteins during organ scarring. These contractile cells express several αv integrins and force generated by the actomyosin cytoskeleton can be transmitted to the extracellular matrix by αv integrins. Elegant *in vitro* studies of myofibroblasts have shown that these cells can utilize alternative αv integrins to activate TGFβ1, and demonstrates that myofibroblasts can liberate and activate TGFβ1 from pre-existing and self-generated deposits in the extracellular matrix by transmitting their high contractile force to the large latent complex through αvβ5 integrin and as yet unidentified β1 and 3 integrins [10].

The integrin αvβ8 is also capable of binding to and activating TGFβ1 [7]. This was an unexpected finding, as αvβ6-mediated activation was found to depend critically on sequences within the β6 cytoplasmic domain [6], however the β8 cytoplasmic domain and the β6 cytoplasmic domain are completely divergent. In addition, even deletion of the β8 cytoplasmic domain did not diminish αvβ8-mediated TGFβ1 activation, suggesting that these integrins (which both bind to the same RGD sequence in the TGFβ1 and TGFβ3 latency associated peptides) might activate the TGFβ1 latent complex by differing mechanisms. Further work demonstrated this to be the case. In contrast to αvβ6 mediated activation of TGFβ1, which depends on direct cell–cell contact, αvβ8-mediated activation releases active TGFβ1 into the culture medium of αvβ8 expressing cells. In addition, whereas αvβ6-mediated activation is completely resistant to inhibition by a variety of protease inhibitors, metalloprotease inhibitors abolish αvβ8-mediated TGFβ1 activation, and transfection studies in cells demonstrated a role for the protease MT1-MMP (MMP14) in this process. Therefore αvβ8 appears to activate TGFβ1 by presenting latent complexes to cell–surface metaloproteases which degrade the latency associated peptide and release free TGFβ1 into the extracellular milieu. An important role for αvβ8-mediated TGFβ1 activation *in vivo* is supported by studies of β8 knockout mice. Some of these mice die in mid-gestation from a defect in vascular development reminiscent of that seen in some TGFβ1 null mice [24]. Mice that survive to

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