Role of prostaglandins in fibroblast activation and fibrosis

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Abstract Fibroblasts release prostaglandins and express a range of prostanoid receptors. However the importance of prostaglandins in fibroblast biology have not been fully explored. Our studies showed that the prostaglandin metabolite PGI₂ blocks the activation of fibroblasts, antagonising the induction of Ras/MEK/ERK signalling by TGFβ. Endogenous PGI₂ acts so as to limit the activation of fibroblasts following tissue injury. By contrast PGE₂ induced in injured tissues or disease states may promote recruitment of inflammatory cells and lead to secondary activation of fibroblasts. The effects of PGI₂ on cell signaling could be manipulated to inhibit fibrosis in patients.

Keywords Prostaglandin · Prostacyclin · Fibrosis · Inflammation · Tissue repair

Introduction

Prostaglandins are ubiquitously expressed 20 carbon molecules derived from plasma membrane phospholipids (Moncada and Vane 1978). The role of prostaglandins in promoting human disease has been widely studied particularly in inflammatory disorders, because of the role of PGE₂ for example in causing inflammation and pain (Narumiya 2009). Fibroblasts release prostaglandins including prostaglandin E₂ (PGE₂) and prostacyclin (PGI₂)

(Stratton et al. 2001), but the importance of PGs in fibroblast biology is not fully understood.

Prostaglandin synthesis is dependent on 3 enzymatic conversions beginning with the conversion of membrane derived phospholipids to arachidonic acid by phospholipase (for review see Smyth et al. 2009). Arachidonic acid derived from this step is metabolised further in the cytoplasm by cyclooxygenase I (COX I), which is widely expressed in a number of cell types under basal conditions, or cyclooxygenase II (COX II), induced in inflammatory cells by cytokines but basal in endothelial cells. COX enzymes catalyse the conversion of arachidonic acid to PGH₂ (Smith et al. 1996). The third level enzymes in prostanoid synthesis are a range of cell and tissue specific enzymes which further metabolise PGH₂ to specific active prostanoids, for example PGE synthase in inflammatory cells resulting in PGE₂ release, or prostacyclin synthase in endothelial cells leading to PGI₂ synthesis. Prostaglandins exert their effects via prostanoid specific Gs coupled receptors (Kobayashi and Narumiya 2002).

Thus far, three PGE synthases, namely cytosolic PGE synthase (cPGES), microsomal PGE synthases mPGES-1 and mPGES-2, have been identified (Jakobsson et al. 1999; Tanikawa et al. 2002; Tanioka et al. 2000). cPGES is localized in the cytoplasm of cells under basal conditions and appears to be involved in the homeostatic production of PGE₂. mPGES-2 is also constitutively expressed in wide variety of tissues and cell types and synthesized as a Golgi membrane associated protein. In contrast, mPGES-1 is induced in response to inflammation, so that in inflammatory cells COX II and downstream mPGES-1 induction are coupled (Kamei et al. 2004). mPGES-1 plays a key role in inflammation, pain and arthritis; however, the role of mPGES-1 in fibrosis has not been extensively studied. More recently we went on to measure the activity of

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mPGES-1 in fibroblasts from patients with the severe fibrosing condition systemic sclerosis (SSc) (experiments performed by Xu Shiwen, Royal Free Hospital, and Leask, University of Western Ontario, article under review, McCann et al. 2010). Levels of mPGES were greatly elevated in SSc fibroblasts compared to healthy control cells. Mice in which the mPGES-1 is knocked out are protected from bleomycin induced fibrosis, with diminished inflammatory response and reduced macrophage infiltration in bleomycin injured tissues (Kapoor et al. 2009). One possibility is that following injury fibroblast derived PGE₂ promotes inflammatory cell infiltration and this in turn enhances extracellular matrix gene induction for example via release of TGFβ or PDGF by inflammatory cells.

Consistent with the induction of mPGES-1 in SSc fibroblasts, levels of PGE₂ release were much higher in fibroblasts from patients with (SSc) than control fibroblasts (Stratton et al. 2001). Both in control and disease fibroblasts, exposure to TNFα alone or in combination with TGFβ, prostanoid release was enhanced. Basal and cytokine induced levels of COX I and II appeared similar in control and SSc fibroblasts (Stratton et al. 2001). Therefore it seems that in both tissue repair and fibrosis PGE₂ production by fibroblasts is enhanced via induction of mPGES-1, and that release of PGE₂ by wound fibroblasts promotes inflammatory cell infiltration.

PGI₂ is also released under basal conditions by fibroblasts and is induced during wound healing. We became interested in the idea that PGI₂ might modify the activation of fibroblasts because SSc patients report an improvement in skin tightness following infusion of the PGI₂ agonist Iloprost, given for vascular dysfunction. PGI₂ agonists were found to block the induction of CTGF and type I collagen in fibroblasts activated in tissue culture by TGFβ (Stratton et al. 2002). These effects were prostacyclin (IP) receptor mediated and due to elevation of cAMP. We found that in fibroblasts exposure to IP receptor agonists blocks signal transduction via the Ras/MEK/ERK signal transduction pathway, and this effect inhibits the fibrotic response to TGFβ (Stratton et al. 2002) (Fig. 1). Fibroblasts were exquisitely sensitive to prostacyclin agonists, and inhibition of CTGF induction was seen with picomolar amounts of PGI₂. Suppression of CTGF induction can also be seen with PGE₂ but this requires higher levels in the nanomolar range (Ricupero et al. 1999).

Also studies using the bleomycin model of lung injury followed by fibrosis, show that the fibrotic response is enhanced in the prostacyclin receptor (IP) knockout whereas prostaglandin receptor knockouts (EP) do not show enhancement of fibrosis, supporting the idea that IP receptor agonists rather than EP receptor agonists have

Fig. 1 Effect of prostanoids on fibroblast activation

endogenous anti-fibrotic effects (Lovgren et al. 2006). Mice which are IP receptor knockout have also been shown to develop cardiac fibrosis (Francois et al. 2005).

Prostanoids released by inflammatory and local mesenchymal cells influence fibroblast biology during wound healing and tissue repair. PGE₂ released early after injury via mPGES-1 induction promotes the early inflammatory phase of wound healing and may promote secondary fibroblast activation. Prostacyclin PGI₂ released late after wounding inhibits fibroblast activation via antagonism of Ras/MEK/ERK.

We found rapid induction of PGE₂ in the early inflammatory phase of wound healing, whereas PGI₂ induction was seen in the late resolution phase of wound healing when extracellular matrix gene induction declines. Taking these results together our model is that endogenous PGE₂ is released early following tissue injury inducing inflammatory cell infiltrate and may cause secondary enhancement of fibroblast activation in some situations, whereas PGI₂ induced at a late stage after injury acts so as to suppress and terminate the profibrotic/extracellular matrix response. This model is analogous to one proposed by Derek Willoughby in which cyclopentone prostanoids induced at a late stage of inflammation terminate the inflammatory response by promoting apoptosis of inflammatory cells (Gilroy et al. 2003). Exogenous PGI₂ derivatives such as Iloprost may have some clinically useful anti-fibrotic effects, or at least the mechanism of action of PGI₂ in fibroblasts could be exploited to suppress fibrosis. Blanket suppression of inflammatory responses by corticosteroid or cytokine inhibitors may fail to prevent secondary fibrosis because some of the beneficial effects of prostaglandin induction are lost.

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