The Contribution of Peroxisome Proliferator-Activated Receptor Gamma to Cutaneous Wound Healing

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Significance: Cutaneous tissue repair involves an initial inflammatory phase, followed by a fibroproliferative phase and finally by a resolution phase. Failure to initiate fibroblast recruitment during the fibroproliferative phase results in chronic wounds, whereas failure to terminate the fibroproliferative phase results in fibroproliferative disorders. Thus, understanding how to regulate the fibroproliferative phase of tissue repair is, therefore, of high clinical relevance. Controlling the rate of the fibroproliferative response is essential to promote proper wound repair.

Recent Advances: (1) The myofibroblast is essential for mediating the fibroproliferative phase of tissue repair. (2) The potent profibrotic cytokine transforming growth factor beta (TGF- β) is a major *in vivo* contributor to myofibroblast differentiation and activity *in vivo*.

Critical Issues: An increasing body of evidence indicates that the transcription factor peroxisome proliferator-activated receptor gamma (PPAR- γ) plays a key *in vivo* role in suppressing the fibrogenic response by antagonizing TGF- β signaling. Excessive scarring and/or chronic wounds, caused by a dysregulated fibroproliferative phase, are major clinical problems in response to tissue injury.

Future Directions: The development of drugs to control the rate of the fibroproliferative response are clinically relevant. Controlling PPAR- γ activity may be useful for prevention of scarring as well as for promoting the closure of chronic wounds.

SCOPE

THE DERMIS, comprised of fibroblasts and a highly organized meshwork of extracellular matrix (ECM), absorbs mechanical stress and, hence, serves as a protective barrier to the external environment. When the connective tissue network is disrupted due to injury, the dermis can no longer absorb mechanical tension and, as a result, resident fibroblasts become subjected to mechanical stress. This tension, together with the action of growth factors/cytokines [e.g., transforming growth fac-

tor beta (TGF- β)] that are released by immune cells/platelets at the wound site, causes fibroblasts to migrate from the dermis of the intact adjacent skin into the site of injury and to differentiate into myofibroblasts. These myofibroblasts, characterized by alpha-smooth muscle actin (α -SMA)-containing stress fibers, are widely believed to be responsible for the formation and remodeling of new connective tissue.^{1,2} Myofibroblasts are generally considered to be the cell type essential for executing connective tissue repair; excessive

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Abbreviations and Acronyms

α -SMA = alpha-smooth muscle actin

ECM = extracellular matrix

PGJ₂ = prostaglandin 15-deoxy- Δ 12,14-prostaglandin J₂

PPAR- γ = peroxisome proliferator-activated receptor gamma

RXR = retinoid X receptor

TGF- β = transforming growth factor β

T β RI and T β RII = type I and type II TGF- β receptors

myofibroblast activity is thought to be responsible for fibroproliferative disorders.¹⁻³

TARGET ARTICLES

1. Ghosh AK, Bhattacharyya S, Wei J, Kim S, Barak Y, Mori Y, and Varga J: Peroxisome proliferator-activated receptor-gamma abrogates Smad-dependent collagen stimulation by targeting the p300 transcriptional coactivator. *FASEB J* 2009; **23**: 2968.

2. Wu M, Melichian DS, Chang E, Warner-Blankenship M, Ghosh AK, and Varga J: Rosiglitazone abrogates bleomycin-induced scleroderma and blocks profibrotic responses through peroxisome proliferator-activated receptor-gamma. *Am J Pathol* 2009; **174**: 519.

3. Sha W, Thompson K, South J, Baron M, and Leask A: Loss of PPAR γ expression by fibroblasts enhances dermal wound closure. *Fibrogenesis Tissue Repair* 2012; **5**: 5.

TRANSLATIONAL RELEVANCE

In response to tissue injury, (myo)fibroblasts synthesize and remodel new ECMs. Several transcription factors have been identified that control the tissue repair program. For example, the Smad family of transcription factors mediates the ability of TGF- β to induce ECM production and remodeling.³⁻⁵ Recently, evidence has emerged that peroxisome proliferator-activated receptor gamma (PPAR- γ), a member of the PPAR family of nuclear hormone receptor transcription factors, may play a key role suppressing TGF- β activity.⁶ PPAR- γ has been studied extensively in diabetes and obesity due to its role in regulating glucose metabolism, adipocyte differentiation, and lipid metabolism, and has been extensively reviewed elsewhere (for example, see ref 7 for a comprehensive discussion of the role that PPAR- γ plays *in vivo*). However, the role of PPAR- γ is only beginning to be elucidated, and is the subject of this commentary. PPAR- γ expression is induced in the skin in response to wounding, notably in the resolution phase.⁸ Collectively, these data suggest the intriguing notion that PPAR- γ may play a physiological role in suppressing fibrogenic responses *in vivo* by blocking TGF- β signaling.⁶

PPAR- γ is normally bound to the retinoid X receptor (RXR) and its corepressors and, hence, cannot bind DNA. Upon receptor ligation, the corepressors are displaced from the PPAR- γ /RXR

complex and, as a result, PPAR- γ can bind PPAR- γ response elements target gene promoters.⁹ Natural PPAR- γ ligands include free fatty acids and eicosanoids, including the prostaglandin 15-deoxy- Δ 12, 14-prostaglandin J₂ (PGJ₂).^{10,11} Synthetic PPAR- γ agonists are in clinical use; for example, thiazolidinediones (*e.g.*, troglitazone, pioglitazone, and rosiglitazone) are potent insulin-sensitizers, are used to treat type 2 diabetes.¹² Thus, it is theoretically possible that, in the future, tissue repair or fibrotic responses might be controlled by thiazolidinediones or similar drugs that modulate PPAR- γ activity.

CLINICAL RELEVANCE

Dysregulated tissue repair causes pathological conditions. In response to injury, scarring of connective tissue can result. Excessive scarring (*e.g.*, in response to surgery or burns) can result in keloids or hypertrophic scars, or in diseases, such as scleroderma. Conversely, failure to properly initiate repair results in chronic wounds, which are often seen in diabetic patients. There is no therapy for these conditions. Drugs controlling the rate and progression of cutaneous tissue repair are therefore likely to be of clinical benefit.

EXPERIMENTAL MODEL AND MATERIAL: ADVANTAGES AND LIMITATIONS

The dermal punch wound is a well-established model of normal, cutaneous tissue repair. The bleomycin-induced model is a well-established model of inflammatory-driven fibrosis and is mediated by excessive canonical TGF- β signaling.^{13,14} These models would be considered good predictive tools to assess the whether or not particular pathways might, in principle, be involved with normal tissue repair or scarring. However, as animal models are imperfect representations of human condition, data derived from these animal models ultimately need to be confirmed in humans or human-derived material. Additional models are required to assess effects on chronic wounds, hypertrophic scars, and keloids. Moreover, it is important to differentiate between the effects that are caused by loss or overexpression of PPAR- γ itself and those caused by synthetic PPAR- γ ligands, which have effects that are both dependent and independent of PPAR- γ .

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

Of the three TGF- β isoforms, TGF- β 1 is the principal isoform implicated in fibrosis.¹⁵ TGF- β 1

is normally stored as an inactivated form bound to a latency-associated protein. Following injury, TGF- β 1 cleaved from the latency-associated protein in response to mechanical loading. Free active TGF- β 1 can then bind to type I and type II TGF- β receptors (T β RI and T β RII). T β RI is a kinase, and phosphorylates Smad2 and Smad3, which then associates with Smad4, and translocates into the nucleus to trigger target gene transcription. Extensive studies have indicated that Smad3 is essential for fibrogenesis.^{16–18} PPAR- γ blocks the ability of the potent profibrotic cytokine TGF- β to signal. Both *in vivo* and *in vitro*, PPAR- γ agonists inhibit basal and TGF- β -induced collagen deposition and myofibroblast differentiation.^{19–21} Moreover, PPAR- γ agonists block bleomycin-induced fibrosis²² and reduce the overexpression of profibrotic genes in cultured scleroderma fibroblasts.²³ Mice harboring a fibroblast-specific deletion for PPAR- γ displayed an enhanced susceptibility to bleomycin-induced skin scleroderma, as well as an increased rate of cutaneous tissue repair.^{24,25} Dermal homeostasis, however, is unaffected in PPAR- γ knockout mice²⁵ suggesting that loss of endogenous PPAR- γ is insufficient to induce fibroproliferative responses *in vivo*; instead the role endogenous PPAR- γ is to properly suppress a fibrogenic program. In this regard, it is interesting to note that although PPAR- γ expression is reduced in scleroderma fibroblasts²³ and PPAR- γ is induced in skin keratinocytes in response to tissue injury, PPAR- γ expression is unaltered in the dermis of injured mice.⁸

Although PPAR- γ knockout mouse embryonic fibroblasts display elevated constitutive Smad3 phosphorylation and collagen production,²⁶ PPAR- γ knockout mouse adult dermal fibroblasts *in vivo* and *in vitro* show neither elevated Smad3 phosphorylation nor collagen production.^{24,25} However, PPAR- γ knockout mouse adult dermal fibroblasts instead show enhanced sensitivity to exogenously added TGF- β , bleomycin, and injury in terms of both enhanced phosphorylation of Smad3 and expression of collagen/ α -SMA mRNAs.^{24,25} Collectively, these data strongly suggest that, in adult mice, endogenous PPAR- γ suppresses fibrogenic responses by impairing cellular responses to TGF- β (Fig. 1).

Synthetic PPAR- γ ligands abrogate TGF- β /Smad stimulation of transcriptional responses; however, they affect neither the expression nor phosphorylation of the Smad2/3 protein. Instead PPAR- γ ligands seem to act by suppressing the transcriptional coactivator p300, a histone acetyltransferase required for TGF- β -induced gene expression.²⁷ In particular, in response to TGF- β , the PPAR- γ ligand PGJ₂ suppresses the interaction between activated Smad2/3 and p300, the accumulation of p300 on consensus Smad-binding DNA sequences, and the hyperacetylation of histone H4 at the COL1A2 locus. These effects were not observed if expression of PPAR- γ was depleted.²⁷ Wild-type p300, but not a mutant form of p300 lacking functional histone acetyltransferase, restored TGF- β -induced stimulation of COL1A2 in the presence of PPAR- γ ligands. Conversely, the PPAR- γ agonist

Figure 1. Control of profibrotic gene expression by peroxisome proliferator-activated receptor- γ (PPAR- γ). In normal skin, endogenous PPAR- γ puts a brake on fibroproliferative responses by competing with Smad2/3 for the cofactor p300. In PPAR- γ knockout or fibrotic skin, endogenous PPAR- γ is absent resulting in increased p300 recruitment to Smad binding elements in promoters and, hence, an elevated expression of profibrotic proteins.

pioglitazone activated collagen and CCN2 expression in both wild-type and PPAR- γ knockout fibroblasts,²⁵ supporting that this class of PPAR- γ agonists also have PPAR- γ -independent effects.^{20,28,29} This latter point is relevant since synthetic PPAR- γ agonists are known to have substantial side effects *in vivo*, which may therefore not be mediated by PPAR- γ *per se*.^{30,31} In this regard, it is interesting to note that, for example, that overexpressing PPAR- γ itself suppressed fibrotic responses in the cornea in response to alkali burning.³² Thus, data derived from the used PPAR- γ knockout animals and cells showing that PPAR- γ directly and specifically suppresses fibrogenic activity *in vivo*, by putting a brake on the fibroproliferative phase of repair by suppressing TGF- β signaling, are essential.

INNOVATION

PPAR- γ ligands can have both PPAR- γ -dependent and PPAR- γ -independent effects. Recent data, using fibroblast-specific knockout mice, showing that endogenous PPAR γ blocks TGF- β signaling and plays a specific and direct role in controlling fibrogenic responses by putting a brake on the fibroproliferative phase tissue repair program are therefore important. These observations suggest that further exploring the idea that using direct PPAR- γ agonists or antagonists to control tissue repair *in vivo* is warranted.

CAUTION, CLINICAL REMARKS, AND RECOMMENDATIONS

It remains unclear if PPAR- γ agonists actually have effects on cutaneous tissue repair or fibrotic responses in humans. To date, experimental observations implicating PPAR γ in skin connective tissue biology are few. Experiments that specifically test this hypothesis, or that PPAR- γ plays a role in hypertrophic scars or keloids, are necessary. Nonetheless, compelling *in vitro* and *in vivo* data have been accumulated that suggest a role for PPAR- γ in controlling fibrotic responses in the skin. There are concerns that available PPAR- γ agonists have side effects, although these effects may not be attributable to PPAR- γ itself given that these agonists are known to have PPAR- γ -independent effects.^{30,31}

FUTURE DEVELOPMENT OF INTEREST

Direct evidence suggesting that currently available PPAR- γ agonists control fibroproliferative responses in human skin is required. Moreover, development drugs of a different mechanism of action than glitazones that specifically target PPAR- γ itself would be useful.

AUTHOR DISCLOSURE AND GHOSTWRITING

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REFERENCES

- Hinz B, Phan SH, Thannickal VJ, Prunotto M, Desmoulière A, Varga J, De Wever O, Mareel M, and Gabbiani G: Recent developments in myofibroblast biology: paradigms for connective tissue remodeling. *Am J Pathol* 2012; **180**: 1340.
- Desmoulière A, Chaponnier C, and Gabbiani G: Tissue repair, contraction, and the myofibroblast. *Wound Repair Regen* 2005; **13**: 7.
- Leask A: Possible strategies for anti-fibrotic drug intervention in scleroderma. *J Cell Commun Signal* 2011; **5**: 125.
- Verrecchia F, and Mauviel A: Transforming growth factor-beta and fibrosis. *World J Gastroenterol* 2007; **13**: 3056.
- Ghosh AK, and Varga J: The transcriptional coactivator and acetyltransferase p300 in fibroblast biology and fibrosis. *J Cell Physiol* 2007; **213**: 663.
- Wei J, Bhattacharyya S, and Varga J: Peroxisome proliferator-activated receptor γ : innate protection from excessive fibrogenesis and potential therapeutic target in systemic sclerosis. *Curr Opin Rheumatol* 2010; **22**: 671.
- Yang J, Zhou Y, and Guan Y: PPAR γ as a therapeutic target in diabetic nephropathy and other renal diseases. *Curr Opin Nephrol Hypertens* 2012; **21**: 97.
- Kapoor M, Kojima F, Yang L, and Crofford LJ: Sequential induction of pro- and anti-inflammatory prostaglandins and peroxisome proliferator-activated receptor-gamma during normal wound healing: a time course study. *Prostaglandins Leukot Essent Fatty Acids* 2007; **76**: 103.
- Collino M, Patel NS, and Thiemermann C: PPARs as new therapeutic targets for the treatment of cerebral ischemia/reperfusion injury. *Ther Adv Cardiovasc Dis* 2008; **2**: 179.
- Kapadia R, Yi JH, and Vemuganti R: Mechanisms of anti-inflammatory and neuroprotective actions of PPAR-gamma agonists. *Front Biosci* 2008; **13**: 1813.
- Villacorta L, Schopfer FJ, Zhang J, Freeman BA, and Chen YE: PPARgamma and its ligands: therapeutic implications in cardiovascular disease. *Clin Sci (Lond)* 2009; **116**: 205.
- Leahy JL: Thiazolidinediones in prediabetes and early type 2 diabetes: what can be learned about that disease's pathogenesis. *Curr Diab Rep* 2009; **9**: 215.
- Takagawa S, Lakos G, Mori Y, Yamamoto T, Nishioka K, and Varga J: Sustained activation of fibroblast transforming growth factor-beta/Smad signaling in a murine model of scleroderma. *J Invest Dermatol* 2003; **121**: 41.
- Cutroneo KR, and Phan SH: TGF-beta1-induced Smad 3 binding to the Smad 7 gene: knockout of

- Smad 7 gene transcription by sense phosphorothioate oligos, autoregulation, and effect on TGF-beta1 secretion: bleomycin acts through TGF-beta1. *J Cell Biochem* 2003; **89**: 474.
15. Leask A, and Abraham DJ: TGF-beta signaling and the fibrotic response. *FASEB J* 2004; **18**: 816.
 16. Zhao J, Shi W, Wang YL, Chen H, Bringas P Jr, Datto MB, Frederick JP, Wang XF, and Warburton D: Smad3 deficiency attenuates bleomycin-induced pulmonary fibrosis in mice. *Am J Physiol Lung Cell Mol Physiol* 2002; **282**: L585.
 17. Flanders KC, Sullivan CD, Fujii M, Sowers A, Anzano MA, Arabshahi A, Major C, Deng C, Russo A, Mitchell JB, and Roberts AB: Mice lacking Smad3 are protected against cutaneous injury induced by ionizing radiation. *Am J Pathol* 2002; **160**: 1057.
 18. Lakos G, Takagawa S, Chen SJ, Ferreira AM, Han G, Masuda K, Wang XJ, DiPietro LA, and Varga J: Targeted disruption of TGF-beta/Smad3 signaling modulates skin fibrosis in a mouse model of scleroderma. *Am J Pathol* 2004; **165**: 203.
 19. Ghosh AK, Bhattacharyya S, Lakos G, Chen SJ, Mori Y, and Varga J: Disruption of transforming growth factor beta signaling and profibrotic responses in normal skin fibroblasts by peroxisome proliferator-activated receptor gamma. *Arthritis Rheum* 2004; **50**: 1305.
 20. Burgess HA, Daugherty LE, Thatcher TH, Lakatos HF, Ray DM, Redonnet M, Phipps RP, and Sime PJ: PPARgamma agonists inhibit TGF-beta induced pulmonary myofibroblast differentiation and collagen production: implications for therapy of lung fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2005; **288**: L1146.
 21. Zhang GY, Cheng T, Zheng MH, Yi CG, Pan H, Li ZJ, Chen XL, Yu Q, Jiang LF, Zhou FY, Li XY, Yang JQ, Chu TG, and Gao WY: Peroxisome proliferator-activated receptor-gamma (PPAR-gamma) agonist inhibits transforming growth factor-beta1 and matrix production in human dermal fibroblasts. *J Plast Reconstr Aesthet Surg* 2010; **63**: 1209.
 22. Wu M, Melichian DS, Chang E, Warner-Blankenship M, Ghosh AK, and Varga J: Rosiglitazone abrogates bleomycin-induced scleroderma and blocks profibrotic responses through peroxisome proliferator-activated receptor-gamma. *Am J Pathol* 2009; **174**: 519.
 23. Shi-wen X, Eastwood M, Stratton RJ, Denton CP, Leask A, and Abraham DJ: Rosiglitazone alleviates the persistent fibrotic phenotype of lesional skin scleroderma fibroblasts. *Rheumatology (Oxford)* 2010; **49**: 259.
 24. Kapoor M, McCann M, Liu S, Huh K, Denton CP, Abraham DJ, and Leask A: Loss of peroxisome proliferator-activated receptor gamma in mouse fibroblasts results in increased susceptibility to bleomycin-induced skin fibrosis. *Arthritis Rheum* 2009; **60**: 2822.
 25. Sha W, Thompson K, South J, Baron M, and Leask A: Loss of PPAR γ expression by fibroblasts enhances dermal wound closure. *Fibrogenesis Tissue Repair* 2012; **5**: 5.
 26. Ghosh AK, Wei J, Wu M, and Varga J: Constitutive Smad signaling and Smad-dependent collagen gene expression in mouse embryonic fibroblasts lacking peroxisome proliferator-activated receptor-gamma. *Biochem Biophys Res Commun* 2008; **374**: 231.
 27. Ghosh AK, Bhattacharyya S, Wei J, Kim S, Barak Y, Mori Y, and Varga J: Peroxisome proliferator-activated receptor-gamma abrogates Smad-dependent collagen stimulation by targeting the p300 transcriptional coactivator. *FASEB J* 2009; **23**: 2968.
 28. Ferguson HE, Kulkarni A, Lehmann GM, Garcia-Bates TM, Thatcher TH, Huxlin KR, Phipps RP, and Sime PJ: Electrophilic peroxisome proliferator-activated receptor-gamma ligands have potent antifibrotic effects in human lung fibroblasts. *Am J Respir Cell Mol Biol* 2009; **41**: 722.
 29. Mughal RS, Warburton P, O'Regan DJ, Ball SG, Turner NA, and Porter KE: Peroxisome proliferator-activated receptor gamma-independent effects of thiazolidinediones on human cardiac myofibroblast function. *Clin Exp Pharmacol Physiol* 2009; **36**: 478.
 30. Amato AA, and de Assis Rocha Neves F: Idealized PPAR γ -based therapies: lessons from bench and bedside. *PPAR Res* 2012; **2012**: 978687.
 31. Fiévet C, Fruchart JC, and Staels B: PPARalpha and PPARgamma dual agonists for the treatment of type 2 diabetes and the metabolic syndrome. *Curr Opin Pharmacol* 2006; **6**: 606.
 32. Saika S, Yamanaka O, Okada Y, Miyamoto T, Kitano A, Flanders KC, Ohnishi Y, Nakajima Y, Kao WW, and Ikeda K: Effect of over expression of PPARgamma on the healing process of corneal alkali burn in mice. *Am J Physiol Cell Physiol* 2007; **293**: C75.