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COMMENTARY

CURRENT APPROACHES TO THE THERAPY OF FIBROTIC DISEASES

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Progressive fibrosis of organs and tissues, including the liver, lungs, kidneys, heart, blood vessels and skin, comprises a constellation of mechanistically related disorders that are major causes of morbidity and mortality [1]. Additionally, keloids and hypertrophic dermal scarring can result in disfigurement and disability, whilst scarring in the central nervous system following traumatic injury is a major obstacle to neural regeneration and the successful reconnection of disrupted pathways [2]. Each of these disorders shares the common feature of a progressive and inappropriate accumulation of connective tissue, dominated by collagen, which leads to the disorganization of normal tissue architecture and consequent loss of function. The currently available pharmacologically based treatments are generally unsatisfactory [1, 3], and, in the case of major organ failure, organ replacement may be the only alternative. However, the uncertainties and high costs associated with organ replacement highlight the need for more effective, pharmacologically based treatments. Fortunately, recent rapid progress in our understanding of the cellular and biochemical phenomena that underlie fibrotic disease reveals some promising opportunities for developing novel therapies.

Primary causes of fibrosis

These are very diverse: for example, alcohol and viral infections are major causes of liver fibrosis. Glomerular sclerosis and tubulointerstitial fibrosis in the kidney result from glomerular nephritis, diabetes mellitus or hypertension [4]. Hypertension is also recognized as a major factor in the development of the diffuse cardiac fibrosis associated with progressive heart failure [5]. Whilst toxic vapours, inorganic dusts and paraquat induce pulmonary fibrosis, the cause of cryptogenic fibrosing aveolitis (otherwise known as idiopathic pulmonary fibrosis) remains unknown. Similarly, the abnormalities that give rise to keloids, hypertrophic dermal scars and scleroderma are obscure [6].

Clearly, where the initiating cause of a particular fibrosis is recognized, the first course of action must be to eliminate or minimize it. Abstention from alcohol in the early, fibrogenic phase of alcoholic liver disease slows the advance of the fibrosis [7], as does the use of interferon-α in liver fibrosis associated with viral infections [8, 9]. The antiviral activity of interferon, which reduces the fibrogenic challenge to the liver, may also be accompanied by a useful direct anti-fibrogenic action [10]. Measures to prevent the inhalation of harmful dusts and vapours dramatically reduced the incidence of industrially related pulmonary fibroses. The recognition that the left ventricular hypertrophy associated with chronic hypertension is an important prognostic indicator for heart failure led to a major emphasis on pharmacological and life-style measures to reduce blood pressure [5]. Antihypertensive treatment with inhibitors of ACE† ameliorates left ventricular hypertrophy not only by minimizing the mechanical stimulus of the pressure afterload but also by a direct anti-fibrotic action on cardiac fibroblasts [5]. Finally, in kidney disease, adequate antihypertensive therapy or careful management of diabetes mellitus may retard the slide towards progressive fibrosis and renal failure [11, 12].

Cellular and biochemical basis of fibrosis

Despite the undoubted benefits that stem from dealing with the initiating causes of fibrotic disease, many cases progress inexorably to the impairment of organ function, increasing disability, and eventual death. A chilling example is provided by cryptogenic fibrosing alveolitis, the frequency of which is increasing in the United Kingdom [13]. Despite all forms of treatment, short of heart–lung transplantation, the survival rate 5 years after diagnosis is only about 50% [3], and the outlook for patients with other forms of major organ fibrosis is often little better. Therefore, the opportunities for more successful treatments must be sought in the accumulating wealth of information concerning the

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† Abbreviations: ACE, angiotensin converting enzyme; TNF, tumor necrosis factor; FGFs, fibroblast growth factors; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; ECM, extracellular matrix; IGF, insulin-like growth factor; and RSTKs, receptor-specific tyrosine kinases.

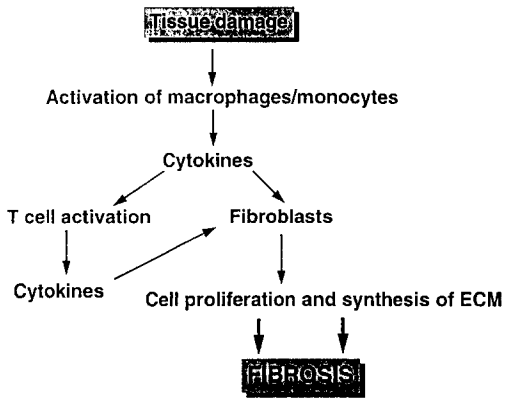

Fig. 1. Summary of the cellular and humoral events leading from the primary tissue damage to the initiation of fibrosis. The various cytokines and cell types involved are described in the text.

cellular and biochemical pathology of fibrosis. This hope is encouraged by the finding that the various forms of fibrosis share several common features in the sequence of events subsequent to the primary cause, which lead to the pathological expansion of connective tissue and extracellular matrix (Fig. 1) [14, 15]. The primary damaging insult to the tissue elicits an inflammatory reaction in which substances released from dead and dying cells activate monocyte/macrophage-type cells that may be acutely extravasated from the circulation or be normally resident in the organ, as in the case of Kupffer cells in the liver [16] and pulmonary macrophages in the lungs [17]. Notable amongst the many cytokines and inflammatory mediators that are released from activated macrophages are TNF-α and interleukins 1 and 6 (IL-1 and IL-6), which recruit further inflammatory cells into the local environment, acidic and basic FGFs, EGF and possibly the two most important fibrogenic cytokines that we are currently aware of, PDGF and TGF-β [15]. Other cells contribute to this cytokine pool, including platelets, lymphocytes and fibroblasts, and, in the case of liver fibrosis, modified lipocytes or Ito cells [18, 19].

In conditions where the primary tissue-damaging cause persists, the inflammatory response becomes chronic, thus perpetuating the drive to fibrogenesis. In these circumstances, it would seem logical to apply anti-inflammatory therapy. However, whilst there is no evidence to indicate that non-steroidal anti-inflammatory drugs exert any worthwhile benefits in chronic fibrosis, treatment with steroids is beneficial in some cases of liver and pulmonary fibrosis [7, 20], and topically applied steroids have a reasonable record of success in reducing the severity of hypertrophic dermal scarring [6]. Because of the multifarious effects of steroids, the basis of their anti-fibrotic action is unclear, but may depend upon a generalized suppression of the activities of many cell types including inflammatory cells and fibroblasts. The incidence of unacceptable side-effects of systemic therapy severely limits the use of steroids in chronic disease [21].

The inflammatory cascade results in the recruitment, activation and proliferation of fibroblasts or fibroblast-like cells and a marked increase in their production of ECM. PDGF is a major mitogen for fibroblasts, although EGF and the structurally related cytokine TGF-α may also contribute to this activity [22, 23]. TGF-β appears primarily to enhance the capacity of the fibroblast for the synthesis of components of the ECM, including the major fibrillar collagens, Types I and III, proteoglycans, fibronectin, laminin, and tenascin [24]. TGF-β also antagonizes the degradation of the ECM by repressing the synthesis of collagenases and inducing the synthesis of tissue inhibitors of metalloproteases (TIMPS) and α_2-macroglobulin [25]. Finally, TGF-β creates an autostimulatory loop by inducing both its own synthesis and that of TGF-β receptors in target cells [4].

Antagonism of cytokines as a route to anti-fibrotic therapy

TGF-β. The involvement of TGF-β in experimental and clinical fibroses is now well-established. Border and Noble [26] demonstrated increased expression of the TGF-β1 isoform at both the mRNA and protein levels in models of autoimmune glomerulonephritis and diabetic nephropathy in rats and in patients with diabetic kidney disease. Increased TGF-β has also been seen in experimental liver fibrosis in rats, in active liver fibrogenesis in humans [16], in pulmonary fibrosis in animals and humans [3], and in experimental cerebral scarring caused by penetrating injury to rat brains [2]. Finally, TGF-β is directly fibrogenic when injected subcutaneously [27].

In short, there can be little doubt that TGF-β is a significant mediator of fibrogenesis and that inhibition of its activity is a valid therapeutic target. Antibodies to TGF-β have already been shown to reduce scarring in incisional dermal injury in rats without impairing normal wound healing [28], to depress the accumulation of ECM in experimental acute glomerulonephritis [29], and to suppress intimal hyperplasia in a rat model of arterial restenosis following balloon injury [30]. However, whilst it might be possible to use antibodies to TGF-β to counter acute fibrosis in humans, as, for example, in paraquat-induced pulmonary fibrosis, it is difficult to see how antibodies could be given over extended periods in the more common, slowly progressive forms of fibrosis unless the antibodies could be "humanized" extensively to minimize their immunogenicity. Decorin, a proteoglycan that binds to and inactivates TGF-β, protects against TGF-β1-mediated renal scarring and dysfunction in experimental glomerulonephritis [31], raising the possibility that a recombinant form of human decorin could be used in chronic fibrotic disease.

However, the prospect of treating patients on a long-term basis with injectable proteins is not ideal. Chronic oral therapy with a low molecular weight antagonist or inhibitor of TGF-β is more attractive, both in terms of cost and convenience to the patient. Such a drug could emerge from various approaches including: (1) blockade of the interaction of TGF-β with its receptor(s), and (2) inhibition of the

signal transduction mechanism for TGF-β. After considerable initial uncertainty, much progress has been achieved recently towards defining the receptors for TGF-β. Three types of receptor have been identified. Types I and II are believed to mediate the biological actions of the cytokine, although which of these determines the fibrogenic activity of the cytokine is unknown [32, 33]. Type III receptors, on the other hand, are apparently not directly involved in signal transduction but may sequester TGF-β on the cell surface for presentation to the other receptors. Types I and II have much higher affinity for the TGF-β1 and -β2 isoforms than for TGF-β3. There are also subclasses of the Type II receptor with much higher affinities for the β2 isoform. The affinity of Type III receptors for the three isoforms varies in different cell lines, suggesting that there may also be more than one class of this receptor type. Numerous other proteins that bind TGF-β have been identified, but their physiological significance is uncertain [32].

The uncertainty as to which class of receptor determines fibrogenic activity, and also whether one of the cytokine isoforms has a dominant role in this activity or whether all three are involved depending upon the local circumstances, hinders a rational approach to the discovery of an antagonist to the ligand–receptor interaction. Nevertheless, the recently described three-dimensional X-ray structure for TGF-β2 and its implications for the structures of the other two isoforms [33, 34], together with the anticipated definition of the ligand binding domains in the receptors, should provide a major impetus for the design of TGF-β antagonists.

Progress towards defining the signal transduction mechanism that mediates the biological actions of the TGF-β family has been much slower than for some other cytokines. Recent cDNA cloning studies indicate that although there is little sequence homology between the extracellular domains of the Type I and Type II receptors, the cytoplasmic domains of both have sequences that are characteristic of serine/threonine kinases [35]. The cytoplasmic domain of the Type III receptor has no such region but, intriguingly, overall has a high content (42%) of serine and threonine residues [33]. Since the Type I and II receptors clearly do not belong to the tyrosine kinase receptor family, it seems quite likely that the serine/threonine kinase domains may participate in signal transduction, although there is no experimental evidence for this at present. Confirmation of the involvement of serine/threonine kinase activity in Type I and II receptor function would no doubt provoke a search for specific inhibitors of these kinases. Recent reports of cytokine receptor-specific inhibitors of tyrosine kinases [36] provide encouragement that a similar success might be achieved for the TGF-β receptor system for fibrogenesis.

Inhibition of RSTKs. Although a great deal of interest has focused on the role of TGF-β in fibrosis, other cytokines released at sites of fibrogenesis also contribute to this process. PDGF appears to be a major player since it is released by a variety of cells found at sites of tissue damage, including platelets, activated macrophages, fibroblasts, smooth muscle cells and endothelial cells. PDGF provides perhaps the crucial stimulus for the mitosis of fibroblasts and vascular smooth muscle cells. EGF and the related cytokine, TGF-α, may also contribute to fibrosis since both are mitogenic for fibroblasts, and exogenously administered EGF is a potent stimulant of fibrogenesis in a model of tendon injury repair in rats [37].

The signal transduction mechanisms of PDGF and EGF/TGF-α both involve activation of specific receptor-associated tyrosine kinases following the dimerization of neighbouring ligand–receptor complexes [38]. There is ample evidence that cytokine-induced activation of RSTKs is essential for the mitogenic action of both PDGF and EGF [38]. Since RSTKs participate in the biological activities of a wide range of cytokines and hormones, the prospects of designing agents targetted against the kinases associated with specific receptors initially seemed remote. However, the pioneering studies of Levitzki [36] have shown that such inhibitors can indeed be devised. Collectively known as "tyrphostins", several of these low molecular weight compounds preferentially inhibit either PDGF–receptor- or EGF–receptor-associated tyrosine kinases. The compounds also have antimitotic effects on cultured cells stimulated by PDGF or EGF. Very recently, a novel series of 4-anilinoquinazolines has been described, which include highly potent inhibitors of the EGF–receptor tyrosine kinase (IC_{50}: ~20 nM) and which inhibit EGF-stimulated proliferation of cultured KB cells [39]. These compounds do not inhibit IGF-stimulated growth of the same cell line. There is a wide range of potential therapeutic applications for receptor-specific inhibitors of tyrosine kinases including fibrotic disorders, although there is still some way to go before the interesting *in vitro* activities of the tyrphostins and 4-anilinoquinazolines are translated into safe and effective long-term treatments for chronic diseases.

Angiotensin II and endothelins in myocardial fibrosis. In recent years, it has been recognized that an important contributor to the pathology of left ventricular hypertrophy, which is associated with chronic hypertension and the resulting progressive heart failure, is an extensive interstitial fibrosis that adversely affects the elasticity and contractility of the myocardium [5]. Weber and his colleagues [5] identified an abnormal, diffuse accumulation of fibrillar collagen (mainly Type I) in hypertrophied hearts obtained at *post mortem* from hypertensive animals and human subjects. In addition, localized scarring is found in regions of the myocardium damaged by ischaemic events. The myocardial fibrosis associated with hypertension differs from fibrogenesis triggered by acute tissue injury in that the inflammatory components of the initiating cascade are absent and the humoral stimuli to fibrogenesis may not be the same. Studies in spontaneously hypertensive rats with left ventricular hypertrophy have shown that the diffuse fibrosis is prevented by continuous treatment with lisinopril or captopril, antihypertensive ACE inhibitors that block the formation of angiotensin II [5]. Antihypertensive treatment with a non-ACE inhibitor, hydralazine, failed to prevent interstitial fibrosis and consequent

diastolic stiffness, although it did prevent gross hypertrophy [5]. These results suggest that, in addition to its hypertensive effects, angiotensin II is directly fibrogenic in the myocardium, and there is some preliminary evidence that angiotensin II stimulates collagen synthesis in cultured human myocardial fibroblasts [40]. Angiotensin II has cytokine-like activity in the kidney where it stimulates mitogenesis and collagen synthesis in proximal tubular cells, apparently by enhancing the synthesis and release of TGF-β [41]. ACE inhibitors substantially reduce renal fibrosis in various models of kidney disease [41]. There may, therefore, be a case for a wider exploration of the potential usefulness of ACE inhibitors and angiotensin II–receptor antagonists in human fibrotic diseases.

Endothelins may also play a role in fibrosis. Thus, endothelins (ETs) 1 and 3 are reported to enhance the synthesis of collagens I and III in cultured rat cardiac fibroblasts [42]. ET 1 also inhibited collagenase production by these cells. The use of receptor-specific endothelin receptor antagonists indicated that the stimulation of collagen synthesis was effected at both ET(A) and ET(B) receptor subtypes, whilst the inhibition of collagenase synthesis by ET 1 was mediated via the ET(A) receptors. Other workers have implicated endothelins in an enhancement of collagen synthesis by fibroblasts isolated from the rat pulmonary artery [43], possibly suggesting a wider role for endothelins in fibrogenesis. The eventual availability of endothelin antagonists with *in vivo* activity should permit the investigation of the contribution of endothelins to a range of experimental fibroses.

Inhibition of collagen biosynthesis and processing

Histological examination of fibrotic tissue, using a collagen-specific stain such as Picrosirius Red, shows that the predominant component of the ECM is collagen. As we have seen, much attention is currently directed at modulating the cytokine activities that drive the accumulation of connective tissue, including the over-production of collagen. There is also considerable interest in more direct approaches to preventing the deposition of mature, cross-linked collagen into the ECM. Whilst the predominant collagens of fibrotic tissue are Types I and III, it should be remembered that there are least thirteen other members of the collagen family in addition to a number of other proteins with triple helical domains, including complement C1q, the acetylcholinesterase associated with nerve muscle endplates and lung surfactant protein [1]. All of these proteins share some common steps in the post-translational modifications that are characteristic of collagenous proteins. Therefore, specificity of attack on newly synthesized collagens Types I and III should optimally be at the level of gene transcription and translation. The application of the emerging technology of antisense oligonucleotides offers a reasonable hope of blocking the translation of specific mRNAs [44], whereas gene-specific control of transcription seems a more distant prospect.

Attempts to control the deposition of collagen into the ECM at the post-translational level have concentrated largely on the design of inhibitors of two key enzymes involved in the post-translational processing of collagen: prolyl 4-hydroxylase and lysyl oxidase.

Prolyl 4-hydroxylase (EC 1.14.11.2). The 4-hydroxylation of prolyl residues in the sequence context . . . *X-pro-gly* . . . in collagen chains is essential to the thermal stability of the triple helix that is characteristic of mature collagen. Even a small reduction (19%) in the 4-hydroxyproline content of Type I collagen is sufficient to depress the "melting" temperature of the triple helix below normal body temperature [45]. Suppression of collagen hydroxylation in cultured cells leads to a visible accumulation of procollagen in the endoplasmic reticulum [46, 47], indicating that the triple helical conformation is essential for the secretion of collagen into the external medium. The proposed use of inhibitors of prolyl 4-hydroxylase in fibrotic diseases rests on the assumption that the underhydroxylated procollagen chains are subject to rapid intracellular degradation. However, the accumulation of non-helical procollagen within cultured cells in which prolyl 4-hydroxylase is inactivated, either by the depletion of ascorbate (an essential co-factor) or by inhibitors of the enzyme, indicates that collagen synthesis outstrips its intracellular proteolysis. Indeed, there is evidence from other systems that rapid intracellular degradation of misfolded proteins is by no means a general phenomenon [48]. Furthermore, the accumulated pro-collagen is rapidly hydroxylated and secreted from cells when the activity of prolyl 4-hydroxylase is restored [46].

The implications of these observations for the proposed clinical application of inhibitors of prolyl 4-hydroxylase in fibrotic diseases are considerable. The apparently low rate of proteolysis of underhydroxylated collagen and its rapid hydroxylation upon the restoration of prolyl 4-hydroxylase activity suggest that the clinical efficacy of an inhibitor in slowing the rate of collagen deposition into the ECM may depend upon maintaining inhibition of hydroxylase activity to avert the escape into hydroxylation that could result from falling tissue drug levels. The need to sustain inhibition of hydroxylation for effective therapy raises concerns of the possible consequences on the processing and function of collagens not involved in pathological fibrosis and of other proteins with collagenous domains. Despite these concerns, several laboratories, including our own, have pursued the goal of finding novel, potent inhibitors of prolyl 4-hydroxylase as potential antifibrotic agents.

HOE 077 [Lufironil, pyridine-2,4-dicarboxylic-di(2-methoxyamide)] is a prodrug of the known inhibitor of prolyl 4-hydroxylase, 2,4-pyridyl-dicarboxylic acid (PDCA), and has been in Phase II clinical development as a potential treatment for various forms of liver fibrosis [49]. The compound is believed to be converted rapidly to PDCA within hepatocytes, although there is no experimental evidence for this [50]. There is also no evidence that prolyl 4-hydroxylase is inhibited *in vivo* following administration of HOE 077. Nevertheless, HOE 077 does protect against the development of carbon tetrachloride-induced liver fibrosis in rats [51],

although the mechanism involved is uncertain and may not depend upon inhibition of prolyl 4-hydroxylase. The outcome of the clinical studies in patients with HOE 077 is awaited with interest.

In our laboratory, we concentrated upon the discovery of inhibitors with proven activity against prolyl 4-hydroxylase *in vivo*. The seminal paper of Hanauske-Abel and Gunzler [52] on the probable catalytic mechanism of prolyl 4-hydroxylase provided a number of guiding principles for the design of potent inhibitors of the enzyme based upon the competitive antagonism of the co-substrate 2-oxoglutarate:

prolyl 4-hydroxylase, Fe^{2+}, ascorbate
. . . X-pro-gly . . . + 2-oxoglutarate → →
. . . X-4-hydroxypro-gly . . . + succinate

We previously described a novel series of heterocyclic carbonyl glycines, which were the first compounds with demonstrable inhibitory activity against prolyl 4-hydroxylase *in vivo* [53]. Unfortunately, the toxicological profile of this series prevented a detailed investigation of the consequences of the inhibition of prolyl 4-hydroxylase on the turnover and accumulation of collagen *in vivo*. However, a chemically distinct series of polycyclic heteroaromatic carboxylic acids (PHACs), which are also effective inhibitors of the enzyme *in vitro* and *in vivo*, proved sufficiently benign to enable us to carry out this evaluation.* We found that newly synthesized collagen in the uteri of sexually immature rats treated with estradiol was substantially underhydroxylated (25% 4-hydroxyproline content compared with 42% in untreated animals) for a minimum of 6 hr following a single oral dose of a PHCA. The underhydroxylated collagen was turned over more rapidly than normal collagen, as indicated by the rate of loss of radiolabel associated with prolyl and 4-hydroxyprolyl residues in uterine collagen from animals given [^{14}C]-proline. Subsequently, the underhydroxylation was progressively reversed as the tissue concentration of the inhibitor declined. The increasing hydroxylation was accompanied by a reduced rate of loss of radioactivity from the uterine collagen. It appears, therefore, that the prospect of a clinically useful reduction in the deposition of collagen in fibrosing tissues may depend on achieving sustained inhibition of prolyl 4-hydroxylase so as to maintain the accumulated procollagen in the non-helical state, which is vulnerable to progressive intracellular degradation. The possible long-term toxicological consequences of sustained inhibition of prolyl 4-hydroxylase remain to be established.

Lysyl oxidase (EC 1.4.3.13). Following the secretion of triple helical collagen into the extracellular environment, the protein is subject to two final steps of post-translational processing. First, specific amino- and carboxypeptidases remove the non-helical "extension" peptides from the amino and carboxyl termini [54]. The collagen molecules formed by the cleavage process then spontaneously assemble into fibrils that act as substrate for the extracellular enzyme lysyl oxidase [55] that oxidatively deaminates the ε-amino groups of certain lysyl and hydroxylysyl residues in the collagen chains. The resulting aldehyde groups react spontaneously with neighbouring aldehyde and ε-amino groups to form intra- and interchain covalent cross-links that impart to the collagen fibers high tensile strength and also resistance to collagenolytic degradation.

The association of enhanced lysyl oxidase activity with the progressive deposition of tough, non-degradable collagen in fibrotic conditions [56–58] has highlighted lysyl oxidase as a valid target for drug therapy. The naturally occurring compound, β-aminoproprionitrile, is an effective, irreversible inhibitor of lysyl oxidase [59] and has been reported to reduce the accumulation of collagen in experimental fibrotic states [57]. However, its toxicity precludes its application in chronic fibrotic disease in humans [60]. Vicinal diamines, such as *cis*-1,2-diaminocyclohexane and ethylenediamine, are also potent, irreversible inhibitors of lysyl oxidase [55, 61]. Representatives of this series of compounds are apparently showing encouraging activity against experimental fibrotic states in animals [62]. The recent successful cloning and sequencing of the cDNA for lysyl oxidase [63] may provide an opportunity to control the activity of the enzyme by the use of antisense oligonucleotides to block translation of its mRNA. As with prolyl 4-hydroxylase, it will probably be necessary to maintain constant suppression of lysyl oxidase to minimize cross-linking of the accumulated extracellular collagen fibrils so as to enhance their susceptibility to proteolytic attack.

Conclusion

Fibrotic diseases are major causes of human morbidity and mortality, and their chronic nature places considerable financial burdens on both individual patients and society. The current treatments, with the exception of organ replacement, are largely unsatisfactory, and there is, therefore, a considerable opportunity for effective new pharmacologically based treatments. It has been suggested that there is a potential market of more than $6 billion for antifibrotic drugs in the U.S.A. alone [62]. Research interest at the present time is centered largely around the antagonism of the major fibrogenic cytokines, including TGF-β, PDGF and EGF/TGF-α. The recently discovered fibrogenic activities of angiotensin II and the endothelins may offer additional opportunities for developing antifibrotic agents. Finally, inhibitors of two key enzymes involved in the post-translational processing of collagen, prolyl 4-hydroxylase and lysyl oxidase, continue to attract interest for their largely unexplored potential for controlling human fibrotic diseases.

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REFERENCES

1. Hahn EG and Gunzler V, Introduction. *J Hepatol* **13** (Suppl 13): S1, 1991.
2. Logan A, Frautschy SA, Gonzalez A-M, Sporn MB and Baird A, Enhanced expression of transforming growth factor β1 in the rat brain after a localized cerebral injury. *Brain Res* **587**: 216–225, 1992.
3. Laurent GJ, Coker RK and McAnulty RJ, TGF-β

antibodies: A novel treatment for pulmonary fibrosis? *Thorax* **48**: 953–954, 1993.
4. Yamamoto T, Nakamura T, Noble NA, Ruoslahti E and Border WA, Expression of transforming growth factor β is elevated in human and experimental diabetic nephropathy. *Proc Natl Acad Sci USA* **90**: 1814–1818, 1993.
5. Weber KT, Brilla CG and Janicki JS, Myocardial fibrosis: Functional significance and regulatory factors. *Cardiovasc Res* **27**: 341–348, 1993.
6. Rockwell AB, Cohen IK and Ehrlich HP, Keloid and hypertrophic scars: a comprehensive review. *Plast Reconstr Surg* **84**: 827–837, 1989.
7. Friedman SL, The cellular basis of hepatic fibrosis. *N Engl J Med* **328**: 1828–1835, 1993.
8. Schiff ER, Hepatic fibrosis—New therapeutic approaches. *N Engl J Med* **324**: 987–989, 1991.
9. Manaber N, Chevallier M, Chossegros P, Causse X, Guerret S, Trepo C and Grimaud J-A, Interferon-α_{2b} therapy reduces liver fibrosis in chronic non-A, non-B hepatitis: A quantitative histological evaluation. *Hepatology* **18**: 1344–1349, 1993.
10. Gutterman JU, Cytokine therapeutics: Lessons from interferon α. *Proc Natl Acad Sci USA* **91**: 1198–1205, 1994.
11. Anderson S, Rennke HG and Brenner BM, Therapeutic advantage of converting enzyme inhibitors in arresting progressive renal disease associated with systemic hypertension. *J Clin Invest* **77**: 1993–2000, 1986.
12. Bojestig M, Arnqvist HJ, Hermansson G, Karlberg BE and Ludvigsson J, Declining incidence of nephropathy in insulin-dependent diabetes mellitus. *N Engl J Med* **330**: 15–24, 1994.
13. Jonston I, Britton J, Kinnear W and Logan R, Rising mortality from cryptogenic fibrosing alveolitis. *Br Med J* **301**: 1017–1021, 1990.
14. Sporn MB and Harris ED, Proliferative diseases. *Am J Med* **70**: 1231–1236, 1981.
15. Kovacs EJ, Fibrogenic cytokines: The role of immune mediators in the development of scar tissue. *Immunol Today* **12**: 17–23, 1991.
16. Winwood PJ and Arthur MJP, Kupffer cells: Their activation and role in animal models of liver injury and human liver disease. *Semin Liver Dis* **13**: 50–59, 1993.
17. Crystal RG, Bitterman PB, Rennard SI, Hance AJ and Keogh BA, Interstitial lung diseases of unknown cause. (Part 1). *N Engl J Med* **310**: 154–166, 1984.
18. Friedman SL and Arthur MJP, Activation of cultured rat hepatic lipocytes by Kupffer cell conditioned medium. Direct enhancement of matrix synthesis and stimulation of platelet-derived growth factor receptors. *J Clin Invest* **84**: 1780–1785, 1989.
19. Casini A, Pinzani M, Milani S, Grappone C, Galli G, Jezequel AM, Schuppan D, Rotella CM and Surrenti C, Regulation of extracellular matrix synthesis by transforming growth factor β_1 in human fat-storing cells. *Gastroenterology* **105**: 245–253, 1993.
20. Crystal RG, Bitterman PB, Rennard SI, Hance AJ and Keogh BA, Interstitial lung diseases of unknown cause. (Part 2). *N Engl J Med* **310**: 235–244, 1984.
21. Haynes RC and Murad F, Adrenocorticotrophic hormone; adrenocortical steroids and their synthetic analogs; inhibitors of adrenocortical steroid biosynthesis. In: *The Pharmacological Basis of Therapeutics* (Eds. Gilman AG, Goodman LS, Rall TW and Murad F), pp. 1459–1489. Macmillan, New York, 1985.
22. El Nahas AM, Growth factors and glomerular sclerosis. *Kidney Int* **41** (Suppl 36): S15–S20, 1992.
23. Absher M, Sjostrand M, Baldor LC, Hemenway DR and Kelley J, Patterns of secretion of transforming growth factor-α (TGF-α) in experimental silicosis. Acute and subacute effects of cristobalite exposure in the rat. *Reg Immunol* **5**: 225–231, 1993.
24. Border WA and Noble NA, Transforming growth factor-beta in glomerular injury. *Exp Nephrol* **2**: 13–17, 1994.
25. Edwards DR, Murphy G, Reynolds JJ, Whitham SE, Docherty AJP, Angel P and Heath JK, Transforming growth factor beta modulates the expression of collagenase and metalloproteinase inhibitor. *EMBO J* **6**: 1899–1904, 1987.
26. Border WA and Noble NA, Cytokines in kidney disease: The role of transforming growth factor-β. *Am J Kidney Dis* **22**: 105–113, 1993.
27. Roberts AB, Sporn MB, Assoian RK, Smith KM, Roche NS, Wakefield LM, Heine UI, Liotta LA, Falanga V, Kehrl JH and Fauci AS, Transforming growth factor type β: Rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro*. *Proc Natl Acad Sci USA* **83**: 4167–4171, 1986.
28. Shah M, Foreman DM and Ferguson MWJ, Control of scarring in adult wounds by neutralising antibody to transforming growth factor β. *Lancet* **339**: 213–214, 1992.
29. Border WA, Okuda S, Languino LR, Sporn MB and Ruoslahti E, Suppression of experimental glomerulonephritis by antiserum against transforming growth factor β1. *Nature* **346**: 371–374, 1990.
30. Wolf YG, Rasmussen LM and Ruoslahti E, Antibodies against transforming growth factor-β1 suppress intimal hyperplasia in a rat model. *J Clin Invest* **93**: 1172–1178, 1994.
31. Border WA, Noble NA, Yamamoto T, Harper JR, Yamaguchi Y, Pierschbacher MD and Ruoslahti E, Natural inhibitor of transforming growth factor-β protects against scarring in experimental kidney disease. *Nature* **360**: 361–364, 1992.
32. Segarini PR, TGF-β receptors: A complicated system of multiple binding proteins. *Biochim Biophys Acta* **1155**: 269–275, 1993.
33. Daopin S, Piez KA, Ogawa Y and Davies DR, Crystal structure of transforming growth factor-β2: An unusual fold for the superfamily. *Science* **257**: 369–373, 1992.
34. Schlunegger MP and Grütter MG, An unusual feature revealed by the crystal structure at 2.2 Å resolution of human transforming growth factor-β2. *Nature* **358**: 430–434, 1992.
35. ten Dijke P, Franzen P, Yamashita H, Ichijo H, Heldin C-H and Miyazono K, Serine/threonine kinase receptors. *Prog Growth Factor Res* **5**: 55–72, 1994.
36. Levitzki A, Tyrphostins: Tyrosine kinase blockers as novel antiproliferative agents and dissectors of signal transduction. *FASEB J* **6**: 3275–3282, 1992.
37. Franklin TJ, Gregory H and Morris WP, Acceleration of wound healing by recombinant human urogastrone (epidermal growth factor). *J Lab Clin Med* **108**: 103–108, 1986.
38. Schlessinger J and Ullrich A, Growth factor signalling by receptor tyrosine kinases. *Neuron* **9**: 383–391, 1992.
39. Barker AJ, Davies DH, Brown DS, Green LR, Cartlidge SA, Woodburn JR and Wakeling AE, Inhibition of EGF receptors tyrosine kinase by 4-anilinoquinazolines. *Br J Cancer* **64** (Suppl XXI): 18, 1994.
40. Brilla CG, Reams GP, Maisch B and Weber KT, Renin-angiotensin system and mycocardial fibrosis in hypertension. *Eur Heart J* **14** (Suppl 7): 57–61, 1993.
41. Wolf G, Regulation of renal tubular growth: Effects of angiotensin II. *Exp Nephrol* **2**: 107–114, 1994.
42. Guarda E, Katwa LC, Myers PR, Tyagi SC and Weber KT, Effects of endothelins on collagen turnover in cardiac fibroblasts. *Cardiovasc Res* **27**: 2130–2134, 1993.
43. Peacock AJ, Dawes KE, Shock A, Gray AJ, Reeves JT and Laurent GJ, Endothelin-1 and endothelin-3

induce chemotaxis and replication of pulmonary artery fibroblasts. *Am Respir Cell Mol Biol* **7**: 492–499, 1992.
44. Stein CA and Cheng Y-C, Antisense oligonucleotides as therapeutic agents—Is the bullet really magical? *Science* **261**: 1004–1012, 1993.
45. Rosenbloom J and Harsch M, Hydroxyproline content determines denaturation temperature of chick tendon collagen. *Arch Biochem Biophys* **158**: 478–484, 1973.
46. Pacifici M and Iozzo RV, Remodelling of the rough endoplasmic reticulum during stimulation of procollagen secretion by ascorbic acid in cultured chondrocytes. *J Biol Chem* **263**: 2483–2492, 1988.
47. Tschank G, Brocks DG, Engelbart K, Mohr J, Baader E, Gunzler V and Hanauske-Abel HM, Inhibition of prolyl hydroxylation and procollagen processing in chick-embryo calvaria by a derivative of pyridine-2,4-dicarboxylate. *Biochem J* **275**: 469–476, 1991.
48. Klausner RD and Sitia R, Protein degradation in the endoplasmic reticulum. *Cell* **62**: 611–614, 1990.
49. Anonymous, Lufironil. *Drugs Future* **18**: 704–706, 1993.
50. Clement B, Chesne C, Satie A-P and Guillouzo A, Effects of the prolyl 4-hydroxylase inhibitor HOE 077 on human and rat hepatocytes in primary culture. *J Hepatol* **13** (Suppl 13): S41–S47, 1991.
51. Bickel M, Baader E, Brocks DG, Engelbart K, Gunzler V, Schmidts HL and Vogel GH, Beneficial effects of inhibitors of prolyl 4-hydroxylase in CCl_4-induced fibrosis of the liver in rats. *J Hepatol* **13** (Suppl 13): S26–S33, 1991.
52. Hanauske-Abel HM and Gunzler V, A stereochemical concept for the catalytic mechanism of prolyl hydroxylase. Application to classification and design of inhibitors. *J Theor Biol* **94**: 421–455, 1982.
53. Franklin TJ, Hales NJ, Johnstone D, Morris WP, Cunliffe CJ, Millest AJ and Hill GB, Approaches to the design of antifibrotic drugs. *Biochem Soc Trans* **19**: 812–815, 1991.
54. Prockop DJ, Kivirikko KI, Tuderman L and Guzman NA, The biosynthesis of collagen and its disorders. *N Engl J Med* **301**: 13–22, 1979.
55. Kagan HM and Trackman PC, Properties and function of lysyl oxidase. *Am J Respir Cell Mol Biol* **5**: 206–210, 1991.
56. Poole A, Myllyla R, Wagner JC and Brown RC, Collagen biosynthesis enzymes in lung tissue and serum of rats with experimental silicosis. *Br J Exp Pathol* **66**: 567–575, 1985.
57. Kagan HM, Characterization and regulation of lysyl oxidase. In: *Biology of Extracellular Matrix: A Series. Regulation of Matrix Accumulation* (Ed. Meacham RP), Vol. 1, pp. 321–328. Academic Press, Orlando, FL, 1986.
58. Wakasaki H and Ooshima A, Synthesis of lysyl oxidase in experimental fibrosis. *Biochem Biophys Res Commun* **166**: 1201–1204, 1990.
59. Siegel RC, Lysyl oxidase. *Int Rev Connect Tissue Res* **8**: 73–118, 1979.
60. Kivirikko KI and Majamaa K, Synthesis of collagen: Chemical regulation of post-translational events. In: *Fibrosis: Ciba Foundation Symposium 114* (Eds. Evered D and Whelan J), pp. 34–48. Pitman, London, 1985.
61. Tang SS, Simpson DE and Kagan HM, β-Quinone-directed irreversible inhibitors of lysyl oxidase. *J Biol Chem* **264**: 12963–12969, 1984.
62. Anonymous, Fibromed's focus on fibrosis. *Scrip* **1847**: 23, 1993.
63. Trackman PC, Pratt AM, Wolanski A, Tang SS, Offner GD, Troxler RF and Kagan HM, Cloning of rat aorta lysyl oxidase cDNA: Complete codons and predicted amino acid sequence. *Biochemistry* **29**: 4863–4870, 1990.