ELSEVIER

REVIEW

Transforming growth factor beta (TGFβ) and keloid disease

Jagajeevan Jagadeesan [a], Ardeshir Bayat [b],*

[a] *Department of Plastic and Reconstructive Surgery, Royal Preston Hospital, Sharoe Green Lane, Fulwood, Preston PR2 9HT, UK*
[b] *Plastic and Reconstructive Surgery Research, Manchester Interdisciplinary Biocentre University of Manchester, 131 Princess Street, Manchester, MI 7ND, UK*

KEYWORDS
Transforming growth factor β;
Keloid disease;
Skin scarring;
Fibrosis

Abstract   Keloids are benign fibroproliferative diseases of unknown aetiology. They occur as a result of derangement of the normal wound healing process in susceptible individuals. Although several factors have been postulated in the aetiopathogenesis of this condition, there has been growing evidence to suggest a role for Transforming Growth Factor beta (TGFβ) family members in its pathogenesis. TGFβ has also been found to be associated with fibrotic diseases affecting different organs of the body including liver, kidney, lung as well as skin. In this review article, we will discuss the morphology and mechanism of action of TGFβ and its isoforms and present the most up to date literature discussing the role of TGFβ isoforms, their receptors, and intracellular signalling pathways (the SMAD pathway) in the pathogenesis of keloid disease. Understanding the role of TGFβ in keloid disease could lead to the development of clinically useful therapeutic modalities for treatment of this condition.
© 2006 Published by Elsevier Ltd on behalf of Surgical Associates Ltd.

Introduction

Keloid scars are fibroproliferative dermal lesions that commonly occur following any form of trauma to the dermis such as surgery or burns.[1,2] Typically, keloid scars extend beyond the margins of the original wound and spread by invasion of the neighbouring skin rather than expansion (Fig. 1). Histologically, keloids are characterised by haphazard deposition of thick hyalinised eosinophilic collagen fibres in a mucinous extracellular matrix (ECM) with abundant lymphocytes and eosinophils and few macrophages.[1–6] A characteristic feature of a keloid disease (KD) is the presence of collagen nodules consisting of dense mass of collagen and fibroblasts.[7] KD shows an elevated collagen mRNA expression,[8] elevated levels of propyl-4 hydroxylase

---

* Corresponding author. Tel.: +44 161 306 5177; fax: +44 161 275 1617.
  E-mail address: ardeshir.bayat@manchester.ac.uk (A. Bayat).

Figure 1 Keloid scar in the posterior aspect of an ear.

activity and an elevated fibronectin synthesis,[9] all of which result in higher level of collagen deposition than normal.

KD is thought to arise as a result of derangement of normal wound healing process. Wound healing is the end result of a group of cellular and humoral events orchestrated with the aim of achieving the normal homeostasis. The classical stages of wound healing include inflammation, epithelialisation, formation of granulation tissue, neovascularisation, wound contraction and scar maturation with ECM reorganisation.[10] A number of growth factors such as transforming growth factor beta (TGFβ), interleukins and insulin like growth factors (IGF) and others have been implicated as important factors involved in the normal wound healing process. These cytokines have all been thought to be involved in scar formation.[11-13]

A central event in dermal repair is the release of cytokines in response to injury. There is overwhelming evidence pointing to TGFβ as a key cytokine that initiates and terminates tissue repair and whose sustained production underlies the development of fibrosis. It induces ECM components, but overproduction of TGFβ can result in excessive deposition of scar tissue and fibrosis.[14] This review

will aim to briefly introduce the TGFβ superfamily and their signalling pathways and then describe in detail the importance of this significant fibrotic cytokine in development of fibrotic diseases such as KD.[15]

Transforming growth factor beta (TGFβ)

TGFβ is a multifunctional natural polypeptide that belongs to a superfamily of cytokines. In mammals, this super family comprises of over 30 proteins including 3 isoforms of TGFβ namely TGFβ$_1$, β$_2$ and β$_3$, 3 forms of activins and over 20 bone morphogenic proteins (BMPs).[16-30] It is released from the alpha granules of platelets by degranulation with thrombin. It is secreted as an inactive latent form called Latent TGFβ (L-TGFβ), which is a large 390–412 amino acid precursor protein and is activated by proteolytic cleavage between amino acids 278 and 279 into active TGFβ and Latency associated peptide (LAP).[20] The active TGFβ acts on its target cells by combining with transmembrane serine/threonine kinase TGFβ receptors.[29] Four different types of TGFβ receptors, present virtually in all parts of the body, have been identified. Types I and II are the most active receptors involved directly with signal transduction and two accessory receptors, namely Type III or betaglycan receptor and Endoglin, which act by presenting the ligands to Type I or II receptors, help in the process of signal transduction.[28] Each TGFβ isoform (TGFβ$_1$, -β$_2$, and -β$_3$) differs in its binding affinity for TGFβ receptors. TGFβ act by binding with type II serine threonine kinase receptor which then transphosphorylates and activates type I receptor. The type I receptor then transduces intracellular signalling pathways by phosphorylating the intracellular signal pathways by interacting with certain intracellular proteins called SMADs in various combinations which in turn stimulates transcription of genes,[31] which affect almost all phases of wound healing. Smads get their name from related genes ''Sma; gene similar to Mothers Against'' and ''Mad; gene Mothers Against Decapentaplegic'' isolated from round worm *Caenorhabditis elegans* and the fly *Drosophila melanogaster*, respectively, both of which were found to be responsible for intracellular signal transmission by TGFβ.[30] The SMAD complex interacts in the nucleus with transcription factors to regulate the transcription of TGFβ responsive genes and mediate the effects of TGFβ at the cellular level.[32] Several different forms of SMADs have so far been identified in mammals and have been classified into different functional classes such as R-Smads or receptor regulated Smads consisting of Smad 1, 2, 3,

5, 8, Co-Smads or Common-partner Smads consisting of Smad 4 and I-Smads, or Inhibitory Smads consisting of Smad 6 and 7.[17] After binding with TGFβ receptor the TGFβ receptor kinase phosphorylates Smad 2 and 3, which are the receptor regulated Smads, which then activate Smad 4 which is the Common partner Smad, which translocates to the nucleus.[36] For transcriptional activation, SMADs require binding of additional transcription factors such as activator protein one (AP-1) and simian virus 40 promoter factor one (Sp-1). These signalling pathways have been shown to regulate type I collagen gene expression.[31–34] All of the above signalling pathways help to orchestrate the events of wound healing ultimately leading to accumulation of ECM in granulation tissue.[35] The negative feedback mechanism which regulates the signal transduction acts in two ways. In the first instance, the continued phosphorylation of R-Smads is competitively inhibited by the Inhibitory Smads; Smad 6 and 7 and the other mechanism is by ubiquitination of Smad 3, which has completed the transcription process by ligases.[36]

TGFβ and fibrotic diseases

Fibrotic diseases are a group of diseases with very limited effective treatment. They are characterised by excessive scarring secondary to excessive production, deposition or contraction of ECM.[36] TGFβ, especially the TGFβ₁ isoform, is a key mediator of tissue fibrosis and has been implicated in many fibrotic diseases like pulmonary fibrosis, systemic sclerosis, keloids and hypertrophic scars, and fibrotic disease of kidney and liver.[14] An animal model by Shah et al.[25] demonstrated the profibrotic activity of TGFβ₁₋₂ and antifibrotic effect of TGFβ₃, and suggested that the way forward for treatment of fibrotic conditions and scarring is either by blocking the effects of TGFβ₁ and TGFβ₂ or administration of TGFβ₃.[25] This profibrotic activity of TGFβ could be due to their ability to induce ECM proteins in mesenchymal cells and also by production of protease inhibitors, which inhibit the enzymatic breakdown of ECM.[37]

TGFβ and keloid disease

TGFβ isoforms

The three TGFβ isoforms identified in mammals; TGFβ₁, β₂ and β₃ are thought to have different biological activities in wound healing. TGFβ₁ and TGFβ₂ are believed to promote fibrosis and scar formation,[38–42] whereas TGFβ₃ has been shown to be either scar inducing[43] or reducing,[25] depending on the study. Due to their profibrotic activity, TGFβ₁

and β₂ isoforms have been proposed to be significant in the pathogenesis of KD. Several studies have demonstrated differences in action of keloid fibroblasts compared to normal dermal fibroblasts as well as the association of TGFβ in KD pathogenesis.

TGFβ₁ isoform

TGFβ₁ stimulates growth and collagen secretion and is thought to be integral to keloid formation.[44] TGFβ₁ results in stimulation of total protein synthesis in normal dermal fibroblasts but not in keloid fibroblasts, suggesting that the TGFβ regulatory pathway is altered in keloid fibroblasts. However, both keloid and normal fibroblasts treated with TGF β₁ exhibit accelerated fibronectin biosynthesis, indicating that keloid cells can respond to TGF β₁. TGFβ₁ induces an increase in fibronectin biosynthesis more rapidly in keloid fibroblasts, suggesting modification of this regulatory pathway. The TGFβ₁-mediated increase in keloid fibronectin production is independent of the steroid regulatory pathway for fibronectin, which accelerates synthesis by means of a post-transcriptional mechanism. Thus, TGFβ₁ stimulation of fibronectin production in keloid cells is likely to involve a transcriptional mechanism and keloid overproduction of ECM components may be due to an inherent modification of the TGFβ regulatory programme.[45] There is a unique sensitivity of keloid fibroblasts to TGF β₁ causing an increase in absolute collagen synthesis in keloid fibroblasts indicating a differential response between keloid and normal fibroblasts. This response occurs at the pretranscriptional level as there is corresponding increase in procollagen type I mRNA levels.[46]

TGFβ₁ protein and mRNA have been detected in areas active in type I and type VI collagen gene expression, indicating that TGFβ₁ gene is transcribed and the corresponding protein is deposited in areas of elevated collagen gene expression, including microvascular endothelial cells of keloid tissue. It is suggested that the initial step in the development of fibrotic reaction in keloids involves the expression of the TGFβ₁ gene by the neovascular endothelial cells, thus activating the adjacent fibroblasts to express markedly elevated levels of TGFβ₁, as well as type I and VI collagen genes.[44]

The effect of TGFβ₁ on the rate of collagen synthesis in keloid (KF), hypertrophic scar (HSF) and normal skin fibroblasts was studied by using fibroblasts cultured in three-dimensional fibrin-gel matrices in the presence or absence of TGFβ or anti-TGFβ neutralising antibody. They demonstrated

that KFs showed a marked sensitivity to TGFβ, especially during the proliferative phase of wound healing, but their rate of collagen overproduction is similar to that by HSFs.[47] Another study demonstrated that keloid fibroblasts showed a 3-fold increase in production of type 1 collagen, 6-fold increase in matrix metalloproteinase one (MMP1), 2.4-fold increase in MMP2, 2-fold increase in production of tissue inhibitor of metalloproteinase one (TIMP1) and 2.5-fold increase in migratory activity compared to normal fibroblasts. Addition of TGFβ$_1$ to the culture media augmented release of type-1 collagen and a decrease in MMPs; in contrast, addition of anti-TGFβ showed the opposite result thus reinforcing the proposed theory for the role of TGFβ in keloid disease.[48] Tranilast, an antiallergic drug, has an inhibitory effect on collagen synthesis in cultured fibroblasts from keloid and hypertrophic scar tissues. This inhibition occurs by release of TGFβ$_1$ from the fibroblasts but not by inhibiting prolyl hydroxylase (the rate-limiting enzyme in collagen synthesis) activity.[47]

TGFβ$_2$ isoform

An in vivo model of human scar xenografts, maintained in congenitally athymic, asplenic "nude" rats, examined endogenous TGFβ$_2$ levels in keloids and burn hypertrophic scars treated with TGFβ$_2$ and TGFβ$_2$ antibodies. The results demonstrated that exogenous TGFβ$_2$ results in a significant increase in endogenous TGFβ$_2$, collagen I, and collagen III production in human proliferative scars. By contrast, exogenous addition of anti-TGFβ$_2$ antibody significantly decreased endogenous TGFβ$_2$, collagen I, and collagen III production. This study supported a causative role for TGFβ$_2$ in the formation of proliferative scars and suggests that anti-TGF β$_2$ antibody may be a new potential anti-scarring agent.[42]

Using a similar in vivo animal model of proliferative scarring, the effects of TGFβ$_2$ on different scars were examined. Proliferative scar specimens were implanted into athymic, asplenic nude rats and isolated in sandwich island flaps based on the superficial inferior epigastric pedicle. After establishment of the transferred flap, the scars were injected with varying doses of TGFβ$_2$. Fibroblasts from the explanted biopsies and the original scars were grown in cell culture. Cell proliferation studies were performed and the results compared. A dose response to TGFβ$_2$ was noticed. Keloid scars demonstrated the greatest cell proliferation kinetics and were significantly faster than non-burn and burn hypertrophic scars. The combination of proliferative scar fibroblast abnormal response to TGFβ$_2$

stimulation and the elevated levels of this cytokine more accurately controls the process of keloid and burn hypertrophic scar formation.[49,50] In another study, using an in vitro fibroblast-populated collagen lattice (FPCL) to evaluate fibroblast activation by measuring contraction of the lattice over time, it was shown that upon exogenous administration of TGFβ$_2$ to fibroblasts from keloid, burn hypertrophic scars and normal skin the fibroblasts from keloid showed a significant increase in contraction and this effect was countered by addition of exogenous TGFβ$_2$ antibody.[51]

TGFβ isoform expression was studied in keloids at the protein level using Western blot analysis and showed that TGFβ$_1$ and β$_2$ expression were raised in keloid fibroblast cultures compared with normal human dermal fibroblast cultures. In contrast, the TGFβ$_3$ protein was comparable in both the normal and keloid cell lines. These findings demonstrated increased TGFβ$_1$ and β$_2$ protein expression in keloids, relative to normal human dermal fibroblasts, thus further supporting the roles of TGFβ$_1$ and β$_2$ as fibrosis-inducing cytokines.[52,53]

TGFβ receptors

While keloid fibroblasts have a unique sensitivity to TGFβ compared to normal fibroblasts, Chin et al.[55] showed that the altered response of keloid fibroblasts to TGFβ is because of changes that occur at the receptor level. They studied the levels of TGFβ receptors as well as SMAD 3 in keloid derived fibroblasts compared to normal human derived fibroblasts and showed that there is an increased expression of TGFβ types I and II receptors in keloid fibroblasts. Bock et al.[54] studied the expression of TGFβ$_{1,2,3}$ as well as types I and II TGFβ receptor mRNAs and showed that the expression of type I TGFβ receptor mRNA was significantly reduced in hypertrophic scars, and that of type II receptor reduced in Keloid scars, thus resulting in an increased TGFβRI/TGFβRII ratio in keloid compared to hypertrophic scars. Expression levels of TGFβ I and II receptors were investigated between keloid and normal human dermal fibroblasts and the results showed that their expression is significantly raised in keloid tissue.[55]

Transcription factors

AP-1 transcription factors play an important role in type I collagen production by regulating its signalling pathways and plays a role in TGFβ induced extracellular matrix production. Based on this, Kim

et al.[34] demonstrated that AP-1 transcription factors play a role in keloid pathogenesis. They studied the characteristics of keloid and normal dermal fibroblasts by blocking the AP-1 transcription factors using AP-1 decoy oligodeoxynucleotides (ODN). Their results showed that TGFβ induced collagen production was significantly suppressed in keloid fibroblasts treated with AP-1 decoy ODN. This is an in vitro study and further studies are required to prove the results in vivo and to prove the efficacy of AP-1 decoy ODN to suppress progression of keloid disease.[34]

SMAD intracellular signalling pathways

Recent studies have laid emphasis on the possibility of TGFβ intracellular signalling pathways especially the SMAD pathway in the pathogenesis of keloid disease. Phan et al.[56] compared the characteristics of SMAD intracellular signalling pathways between keloid fibroblast and normal dermal fibroblast cultures and demonstrated that SMAD3 was over expressed in keloid fibroblasts co cultured with keloid keratinocytes thus suggesting its role in keloid pathogenesis. In contrast, expression of SMAD
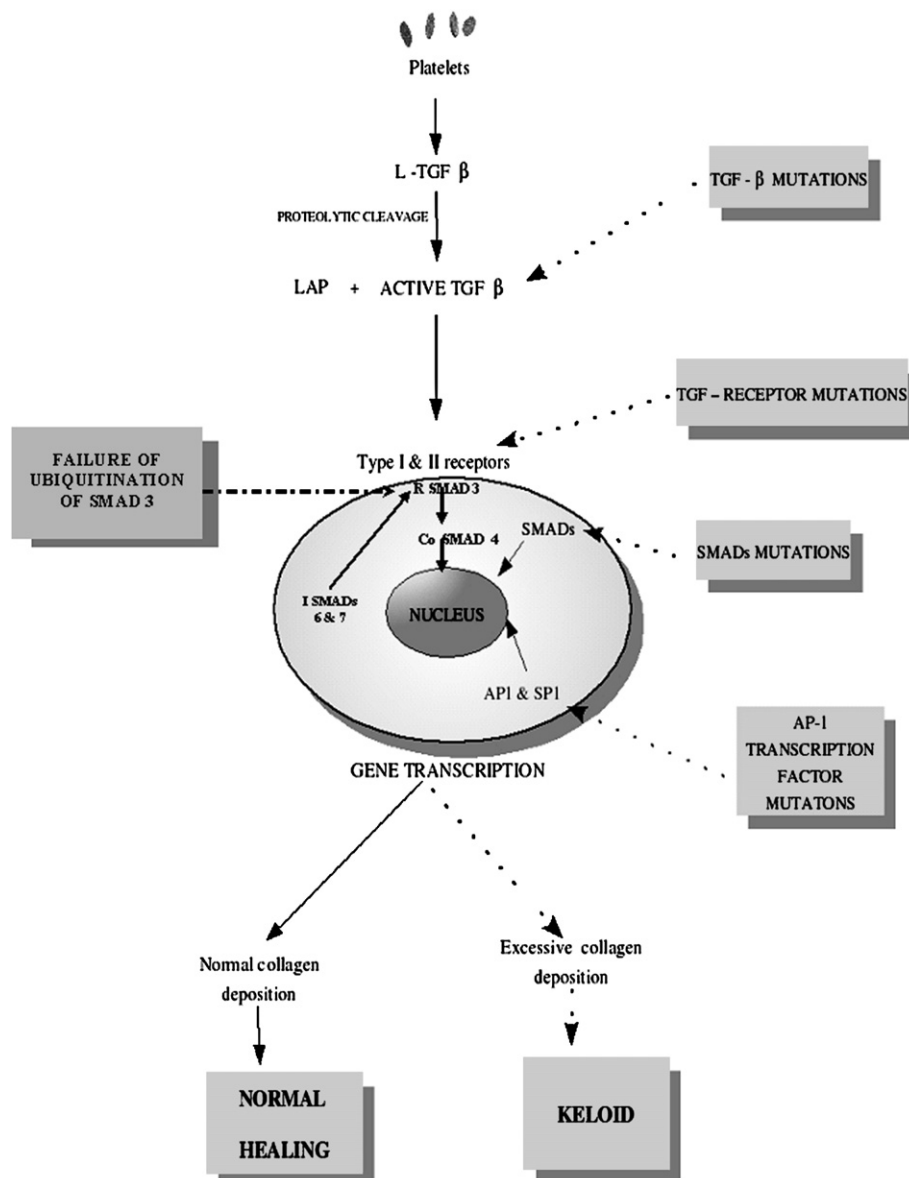

Figure 2 This diagram aims to demonstrate potential sites where pathology (e.g. mutations present in TGFβ family members such as isoforms, receptors, transcription factors or signalling molecules) could result in keloid disease formation. Abbreviations: TGFβ, transforming growth factor beta; L-TGFβ, latent TGFβ; LAP, latency associated peptide; R SMAD, receptor regulated SMAD (Similar to Mothers Against Decapentaplegic/Drosophila gene); Co SMAD, common-partner SMAD; I SMAD, inhibitory SMAD; SP1, promoter specific transcription factor 1; AP1, activating protein 1.

2, 3, 4, 6 and 7 mRNAs in keloid derived fibroblasts compared to normal fibroblasts demonstrated that the expression of SMAD 3 was lower compared to normal skin fibroblasts. The expression of Inhibitory SMADs 6 and 7 mRNA were also reduced. They concluded that the failure of inhibition of TGFβ signalling by the inhibitory SMADs, as the cause of keloid formation.[57]

The potential sites in the TGFβ signalling pathway where mutations in TGF beta family members such as isoforms, receptors, transcription factors or signalling molecules may occur and could result in keloid disease formation is summarised in Fig. 2. Although a number of studies have been carried out with the aim of investigating the genetic basis of keloid disease none to date have shown a significant statistical association between TGF β1,[58] β2,[27] β3,[59] as well as TGFβ receptor[60] gene polymorphisms with keloid disease. Further studies are required to confirm the role of TGFβ and its signalling pathways in the pathogenesis of keloid disease.

Potential future therapies

Understanding the mechanisms of TGFβ mediated up-regulation of collagen gene expression in keloids should provide novel opportunities for treatment of this fibrotic disorder.[61] Several studies to date have suggested a possible role for TGFβ in keloid scarring. Attempts to reduce pathological scarring include ways to either block or to counter the biological effects of TGFβ.[62] Anti-TGFβ1 antibody may show therapeutic potential for treatment of keloids either by inhibition of collagen synthesis by blocking TGFβ1 or accelerated degeneration of types 1 and 3 collagens which are abundant in keloid tissue.[49] Suppression of SMAD3 signalling results in inhibition of keloid fibroblasts which may prove useful in treatment of keloid disease.[56] AP-1 decoy ODN is shown to abolish the TGFβ induced type I collagen gene expression and may also prove relevant in the treatment of keloid disease.[34]

Conclusion

Keloid disease is a benign but progressive form of skin fibrosis and can be a cause of major morbidity. Keloid disease is thought to involve the fundamental processes of wound healing cascade.[13] TGFβ isoforms have been linked to a wide variety of fibrotic diseases. Importantly, TGFβ has been implicated in the pathogenesis of keloid disease. Understanding the actions of TGFβ in keloid disease could lead to the development of clinically useful anti-fibrotic agents. Understanding the processes involved in keloid scar formation is of paramount importance in the treatment of this physically as well as psychologically disturbing disorder.

References

1. Tredget EE, Nedelec B, Scott PG, Ghahary A. Hypertrophic scars, keloids and contractures. The cellular and molecular basis for therapy. *Surg Clin North Am* 1997;**77**:701–30.
2. Bayat A, McGrouther DA, Ferguson MWJ. Medicine, science and future series: skin scarring. *Br Med J* 2003;**326**:88–92.
3. Bayat A, Arscott G, Ollier WER, Ferguson MWJ, McGrouther DA. Description of site-specific morphology of keloid phenotypes in an Afrocaribbean population. *Br J Plast Surg* 2004;**57**(2):122–33.
4. Bayat A, Arscott G, Ollier WER, McGrouther DA, Ferguson MWJ. Keloid disease: clinical relevance of single versus multiple site scars. *Br J Plast Surg* 2005;**58**(1):28–37.
5. Urioste SS, Arndt KA, Dover JS. Keloids and hypertrophic scars: review and treatment strategies. *Semin Cutan Med Surg* 1999;**18**:159–71.
6. Khan S. The pathology of keloid scars. *Surgery* 1983;**71**: 821–5.
7. Kischer CW, Brody GS. Structure of collagen nodule from hypertrophic scars and keloids. *Scanning Microsc* 1981;**3**:371–6.
8. Lee KS, Song JY, Suh MH. Collagen mRNA expression detected in situ hybridisation in keloid tissue. *J Dermatol Sci* 1991;**2**:316–23.
9. Thomas DW, Hopkinson I, Harding KG, Shepherd P. The pathogenesis of hypertrophic/keloid scarring. *Int J Oral Maxillofac Surg* 1994;**23**:232–6.
10. Singer AJ, Clark RA. Cutaneous wound healing. *N Engl J Med* 1999;**341**:738–46.
11. Daian T, Ohtsuru A, Rogounovitch T, Ishihara H, Hirano A, Akiyama-Uchida Y, et al. Insulin-like growth factor-I enhances transforming growth factor-beta-induced extracellular matrix protein production through the P38/activating transcription factor-2 signaling pathway in keloid fibroblasts. *J Invest Dermatol* 2003;**120**(6):956–62.
12. Niessen FB, Spauwen PH, Schalkwijk J, Kon M. On the nature of hypertrophic scars and keloids: a review. *Plast Reconstr Surg* 1999;**104**:1435–58.
13. Tuan TL, Nichter LS. The molecular basis of keloid and hypertrophic scar formation. *Mol Med Today* 1998;**4**:19–24.
14. Border WA, Nobel NA. Transforming growth factor β in tissue fibrosis. *N Engl J Med* 1994;**331**:1286–92.
15. Border WA, Noble NA. Targeting TGF-beta for treatment of disease. *Nat Med* 1995;**1**(10):1000–1.
16. Chin D, Boyle GM, Parsons PG, Coman WB. What is transforming growth factor beta (TGFβ)? *Br J Plast Surg* 2004; **57**:215–21.
17. Goumans MJ, Mummery C. Functional analysis of the TGFβ receptor. Smad pathway through gene ablation mice. *Int J Dev Biol* 2000;**44**:253–65.
18. Sporn MB, Roberts AB, Wakefield Lar M, Crombrugghe B. Some recent advances in the chemistry and biology of transforming growth factor-beta. *J Cell Biol* 1987;**105**: 1039–45.
19. Varga J, Rosenbloom J, Jimenez SA. Transforming growth factor β causes a persistent increase in steady-state amounts of type I and type III collagen and fibronectin mRNAs in normal human dermal fibroblasts. *Biochem J* 1987;**247**:597–604.

20. Khalil N. Transforming growth factor β: from latent to active. *Microbes Infect* 1999;**1**:1255–63.

21. Derynck R, Jarrett JA, Chen EY, Eaton DH, Bell JR, Assoian RK, et al. Human transforming growth factor-β complementary DNA sequence and expression in normal and transformed cells. *Nature* 1985;**316**:701–5.

22. Flanders KC, Burmester JK, Sporn MB. Medical applications of transforming growth factor-β. *Clin Med Res* 2003;**1**:13–20.

23. Assoian RK, Komoriya A, Meyers CA, Miller DM, Sporn MB. Transforming growth factor beta in human platelets; identification of a major storage site, purification and characterization. *J Biol Chem* 1983;**258**:7155–60.

24. Assoian RK, Sporn MB. Type β transforming growth factor in human platelets: release during platelet degranulation and action on vascular smooth muscle. *Cell* 1986;**102**:1217–23.

25. Shah M, Foreman DM, Ferguson MWJ. Neutralisation of TGFβ1 and TGFβ2 or exogenous addition of TGFβ3 to cutaneous rat wounds reduces scarring. *J Cell Sci* 1995;**108**:985–1002.

26. Roberts AB. Transforming growth factor-β: activity and efficacy in animal models of wound healing. *Wound Repair Regener* 1995;**3**:408–18.

27. Bayat A, Bock O, Mrowietz U, Ollier WER, Ferguson MWJ. Genetic susceptibility to keloid disease and transforming growth factorβ2 polymorphisms. *Br J Plast Surg* 2002;**55**:283–6.

28. Bissell DM, Roulot D, George J. Transforming growth factor β and the liver. *Hepatology* 2001;**34**:859–67.

29. Liu B, Connolly MK. The pathogenesis of cutaneous fibrosis. *Semin Cutan Med Surg* 1998;**17**:3–11.

30. Derynck R, Gelbart WM, Harland RM, Heldin CH, Kern SE, Massagué J, et al. Nomenclature: vertebrate mediators of TGF beta family signals. *Cell* 1996;**87**:173.

31. Derynck R, Zhang Y. Intracellular signalling: the mad way to do it. *Curr Biol* 1996;**6**:1226–9.

32. Derynck R, Zhang Y. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* 2003;**425**:577–84.

33. Heldin CH, Miyazono K, ten Dijke P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. *Nature* 1997;**390**:465–71.

34. Kim CW, Suh S, Sung SH, Lee IK, Lee KS. A Transcriptional factor decoy against AP-1 suppresses TGFβ1 induced type I collagen gene expression in cultured keloid fibroblasts. *J Dermatol Sci* 2005;**37**:49–51.

35. Rifkin DB, Mazzieri R, Munger JS, Noguera I, Sung J. Proteolytic control of Growth factor availability. *APMIS* 1999;**107**:80–5.

36. Leask A, Abraham DJ. TGF-beta signalling and the fibrotic response. *FASEB J* 2004;**18**(7):816–27.

37. Verrecchia F, Mauviel A. Transforming growth factor-beta signalling through the Smad pathway: role in extracellular matrix gene expression and regulation. *J Invest Dermatol* 2002;**118**(2):211–5.

38. Kamamoto F, Paggiaro AO, Rodas A, Herson MR, Mathor MB, Ferreira MC. A wound contraction experimental model for studying keloids and wound healing modulators. *Artif Organs* 2003;**27**:701–5.

39. Brown RL, Ormsby I, Doetschman TC, Greenhalgh DG. Wound healing in transforming growth factorβ1 deficient mouse. *Wound Rep Reg* 1995;**3**:25–36.

40. Streuli CH, Schmidhauser C, Kobrin M, Bissell MJ, Derynck R. Extracellular matrix regulates expression of TGFβ1 gene. *J Cell Bio* 1993;**120**:253–60.

41. Shah M, Foreman DM, Ferguson MW. Control of scarring in adult wounds by neutralising antibody to transforming growth factor beta. *Lancet* 1992;**339**:213–4.

42. Wang X, Smith P, Pu LL, Kim YJ, Ko F, Robson MC. Exogenous transforming growth factor beta (2) modulates collagen I and collagen III synthesis in proliferative scar xenografts in nude rats. *J Surg Res* 1999;**87**(2):194–200.

43. Lanning D, Diegelmann RF, Yager DR, Wallace ML, Bagwell CE, Haynes JH. Myofibroblast induction with transforming growth factor-β1 and -β3 in cutaneous fetal excisional wounds. *J Pediatr Surg* 2000;**35**(2):183–8.

44. Peltonen J, Hsiao LL, Jaakkola S, Sollberg S, Aumailley M, Timpl R, et al. Activation of collagen gene expression in keloids: Co-Localisation of type I and VI collagen and transforming growth factorβ1 mRNA. *J Invest Dermatol* 1991;**97**:240–8.

45. Babu M, Diegelmann R, Oliver N. Keloid fibroblasts exhibit an altered response to TGF-beta. *J Invest Dermatol* 1992;**99**:650–5.

46. Bettinger DA, Yager DR, Diegelmann RF. The effect of TGFβ of keloid fibroblast proliferation and collagen synthesis. *Plast Reconstr Surg* 1996;**98**(5):827–33.

47. Suzawa H, Kikuchi S, Arai N, Koda A. The mechanism involved in the inhibitory action of tranilast on collagen biosynthesis of keloid fibroblasts. *Jpn J Pharmacol* 1992;**60**(2):91–6.

48. Younai S, Nichter LS, Wellisz T, Reinisch J, Nimni ME, Tuan TL. Modulation of collagen synthesis by transforming growth factor-beta in keloid and hypertrophic scar fibroblasts. *Ann Plast Surg* 1994;**33**(2):148–51.

49. Fujiwara M, Muragaki Y, Ooshima A. Keloid derived fibroblasts showed increased secretion of factors involved in collagen turnover and depend on matrix metalloproteinase for migration. *Br J Dermatol* 2005;**153**:295–300.

50. Polo M, Smith PD, Kim YJ, Wang X, Ko F, Robson MC. Effect of TGF-beta2 on proliferative scar fibroblast cell kinetics. *Ann Plast Surg* 1999;**43**:185–90.

51. Smith P, Mosiello G, Deluca L, Ko F, Maggi S, Robson MC. TGF-beta2 activates proliferative scar fibroblasts. *J Surg Res* 1999;**82**:319–23.

52. Lee TY, Chin GS, Kim WJ, Chau D, Gittes GK, Longaker MT. Expression of transforming growth factor beta 1, 2, and 3 proteins in keloids. *Ann Plast Surg* 1999;**43**(2):179–84.

53. Chipev CC, Simman R, Hatch G, Katz AE, Siegel DM, Simon M. Myofibroblast phenotype and apoptosis in keloid and palmar fibroblasts in vitro. *Cell Death Differ* 2000;**7**(2):166–76.

54. Bock O, Yu H, Zitron S, Bayat A, Ferguson MW, Mrowietz U. Studies of transforming growth factors beta 1-3 and their receptors I and II in fibroblast of keloids and hypertrophic scars. *Acta Derm Venereol* 2005;**85**:216–20.

55. Chin GS, Liu W, Peled Z, Lee TY, Steinbrech DS, Hsu M, et al. Differential expression of transforming growth factor-beta receptors I and II and activation of Smad 3 in keloid fibroblasts. *Plast Reconstr Surg* 2001;**108**:423–9.

56. Phan TT, Lim IJ, Aalami O, Lorget F, Khoo A, Tan EK, et al. Smad 3 signalling plays an important role in keloid pathogenesis via epithelial-mesenchymal interactions. *J Pathol* 2005;**207**:232–42.

57. Yu H, Bock O, Bayat A, Ferguson MW, Mrowietz U. Decreased expression of inhibitory SMAD 6 and SMAD 7 in keloid scarring. *J Plast Reconstr Aesthet Surg* 2006;**59**:221–9.

58. Bayat A, Bock O, Mrowietz U, Ollier WER, Ferguson MWJ. Genetic susceptibility to keloid disease and hypertrophic scarring: transforming growth factor β1 common polymorphisms and plasma levels. *Plast Reconstr Surg* 2003;**111**:535–43.

59. Bayat A, Walker JM, Bock O, Mrowietz U, Ollier WER, Ferguson MWJ. Genetic susceptibility of keloid disease:

Mutation screening of TGFβ3 gene. *Br J Plast Surg* 2005;**58**: 914—21.

60. Bayat A, Bock O, Mrowietz U, Ollier WER, Ferguson MWJ. Genetic susceptibility to keloid disease: transforming growth factor β receptor gene polymorphisms are not associated with keloid disease. *Exp Dermatol* 2004;**13**:120—4.

61. Uitto J, Kouba D. Cytokine modulation of extracellular matrix gene expression: relevance to fibrotic skin diseases. *J Dermatol Sci* 2000;**24**:S60—9.

62. Parrelli JM, Meisler N, Cutroneo KR. Abrogation of the fibrotic effect of transforming growth factor β in dermal wound healing. *Wound Rep Reg* 1997;**5**:136—40.