Transforming growth factor beta (TGFβ) and keloid disease

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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.

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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
activity and an elevated fibronectin synthesis, 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. 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.

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. 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.

**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 TGFβ1, β2 and β3, 3 forms of activins and over 20 bone morphogenic proteins (BMPs). 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). The active TGFβ acts on its target cells by combining with transmembrane serine/threonine kinase TGFβ receptors. 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. Each TGFβ isoform (TGFβ1, -β2, and -β3) 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, 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. 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. 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. 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. 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. All of the above signalling pathways help to orchestrate the events of wound healing ultimately leading to accumulation of ECM in granulation tissue. 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.

**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. 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. An animal model by Shah et al. 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. 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.

**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, whereas TGFβ3 has been shown to be either scar inducing or reducing, 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. 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. 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.

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.

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 TGFβ2, especially during the proliferative phase of wound healing, but their rate of collagen overproduction is similar to that by HSFs. 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 TGFβ1 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. Tranilast, an anti-allergic drug, has an inhibitory effect on collagen synthesis in cultured fibroblasts from keloid and hypertrophic scar tissues. This inhibition occurs by release of TGFβ2 from the fibroblasts but not by inhibiting prolyl hydroxylase (the rate-limiting enzyme in collagen synthesis) activity.

TGFβ2 isoform

An in vivo model of human scar xenografts, maintained in congenitally athymic, asplenic “nude” rats, examined endogenous TGFβ2 levels in keloids and burn hypertrophic scars treated with TGFβ2 and TGFβ2 antibodies. The results demonstrated that exogenous TGFβ2 results in a significant increase in endogenous TGFβ2, collagen I, and collagen III production in human proliferative scars. By contrast, exogenous addition of anti-TGFβ2 antibody significantly decreased endogenous TGFβ2, collagen I, and collagen III production. This study supported a causative role for TGFβ2 in the formation of proliferative scars and suggests that anti-TGF β2 antibody may be a new potential anti-scarring agent.

Using a similar in vivo animal model of proliferative scarring, the effects of TGFβ2 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 TGFβ2. 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 TGFβ2 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 TGFβ2 stimulation and the elevated levels of this cytokine more accurately controls the process of keloid and burn hypertrophic scar formation.

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 TGFβ2 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 TGFβ2 antibody.

TGFβ isoform expression was studied in keloids at the protein level using Western blot analysis and showed that TGFβ1 and β2 expression were raised in keloid fibroblast cultures compared with normal human dermal fibroblast cultures. In contrast, the TGFβ3 protein was comparable in both the normal and keloid cell lines. These findings demonstrated increased TGFβ1 and β2 protein expression in keloids, relative to normal human dermal fibroblasts, thus further supporting the roles of TGFβ1 and β2 as fibrosis-inducing cytokines.

TGFβ receptors

While keloid fibroblasts have a unique sensitivity to TGFβ compared to normal fibroblasts, Chin et al. 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. studied the expression of TGFβ1,2,3 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.

Transcription factors

AP-1 transcription factors play an important role in type I collagen production by regulating its signaling pathways and plays a role in TGFβ induced extracellular matrix production. Based on this, Kim
et al.\textsuperscript{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 TGF\textsubscript{β} 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.\textsuperscript{34}

**SMAD intracellular signalling pathways**

Recent studies have laid emphasis on the possibility of TGF\textsubscript{β} intracellular signalling pathways especially the SMAD pathway in the pathogenesis of keloid disease. Phan et al.\textsuperscript{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

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**Figure 2**  This diagram aims to demonstrate potential sites where pathology (e.g. mutations present in TGF\textsubscript{β} family members such as isoforms, receptors, transcription factors or signalling molecules) could result in keloid disease formation. Abbreviations: TGF\textsubscript{β}, transforming growth factor beta; L-TGF\textsubscript{β}, latent TGF\textsubscript{β}; 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 TGF\(\beta\) signalling by the inhibitory SMADs, as the cause of keloid formation.\(^{57}\)

The potential sites in the TGF\(\beta\) 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\(\beta_1\), \(\beta_2\), \(\beta_3\),\(^{27}\) as well as TGF\(\beta\) receptor\(^{40}\) gene polymorphisms with keloid disease. Further studies are required to confirm the role of TGF\(\beta\) and its signalling pathways in the pathogenesis of keloid disease.

**Potential future therapies**

Understanding the mechanisms of TGF\(\beta\) 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 TGF\(\beta\) in keloid scarring. Attempts to reduce pathological scarring include ways to either block or to counter the biological effects of TGF\(\beta\).\(^{62}\) Anti-TGF\(\beta_1\) antibody may show therapeutic potential for treatment of keloids either by inhibition of collagen synthesis by blocking TGF\(\beta_1\) 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 TGF\(\beta\) 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.\(^{13}\) TGF\(\beta\) isoforms have been linked to a wide variety of fibrotic diseases. Importantly, TGF\(\beta\) has been implicated in the pathogenesis of keloid disease. Understanding the actions of TGF\(\beta\) 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**

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