Arch Dermatol Res (2009) 301:259–272
DOI 10.1007/s00403-009-0952-8

MINI REVIEW

Keloid scarring: bench and bedside

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Received: 28 January 2009 / Revised: 25 March 2009 / Accepted: 27 March 2009 / Published online: 10 April 2009
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Abstract Wound healing is a fundamental complex-tissue reaction leading to skin reconstitution and thereby ensuring survival. While, fetal wounds heal without scarring, a normal "fine line" scar is the clinical outcome of an undisturbed wound healing in adults. Alterations in the orchestrated wound healing process result in hypertrophic or keloid scarring. Research in the past decades attempted to identify genetic, cellular, and molecular factors responsible for these alterations. These attempts lead to several new developments in treatments for keloids, such as, imiquimod, inhibition of transforming growth factor beta, and recombinant interleukin-10. The urgent need for better therapeutics is underlined by recent data substantiating an impaired quality of life in keloid and hypertrophic scar patients. Despite the increasing knowledge about the molecular regulation of scar formation no unifying theory explaining keloid development has been put forward until today. This review aims to give an overview about the genetic and molecular background of keloids and focus of the current research on keloid scarring with special emphasis on new forthcoming treatments. Clinical aspects and the spectrum of scarring are summarized.

Keywords Keloid scarring · TGFbeta · SMAD

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Introduction

Keloids are benign dermal fibroproliferative tumors unique to humans. They represent a form of abnormal wound healing in genetically susceptible individuals. The recent identification of regions of the human genome highly correlated with keloid formation underlines the genetic background of keloids. Keloids occur at areas of cutaneous injury and grow continuously beyond the original margins of the scar (Fig. 1). Even the spontaneous development of keloids has been discussed but may be the result of a minor, overlooked trauma [1].

Keloids are defined as scars growing continuously and invasively beyond the confines of the original wound in contrast to hypertrophic scars, which stay within the boundaries of the original wound and after a period of continuous growth slowly regress. The active keloid edge is often erythematous and pruritic. Keloids cause significant cosmetic defects and deformities and may limit joint mobility. They are more common in darker pigmented ethnicities with up to 15% of the population at risk [2].

There is no single effective therapeutic regimen for the treatment of keloids. Numerous treatment options have been described including occlusive dressings, compression therapy, intralesional steroid injections, imiquimod cream, laser and radiation therapy, cryosurgery, 5-fluorouracil, bleomycin, and interferon therapy. Better understanding of the molecular mechanisms behind keloid development led to the development of new promising therapies like the application of recombinant transforming growth factor beta (TGFβ)-3, anti-TGFβ1, interleukin (IL)-10, and mannose-6-phosphate inhibiting the activation of TGFβ1 and TGFβ2.

Unfortunately, in spite of the success that has been seen with these treatment modalities the essential problem of the

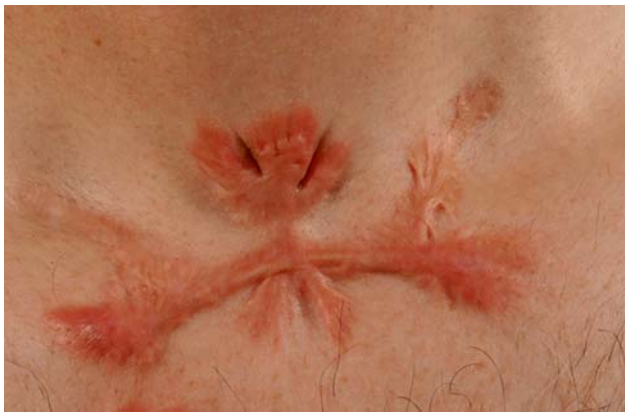

Fig. 1 Typical presternal keloid. The margins are erythematous, growing into the surrounding healthy skin in a claw-like appearance

lack of a clear molecular mechanism explaining the pathogenesis of keloids remains unsolved.

The spectrum of scarring

The clinical appearance of scarring covers a wide spectrum. Wound healing can result in a "normal" fine line scar or in a variety of abnormal scarring including widespread (stretched) scars, atrophic scars, scar contractures, hypertrophic, and keloid scars. If keloids represent one end of the tissue repair spectrum, fetal scarless wound healing represents the other end. Scarless wound healing occurs only in early mammalian embryos and complete regeneration occurs in lower vertebrates, such as salamanders and in invertebrates.

Scarless fetal wound healing

Compared to normal scarring, fetal wounds heal with a minimum of inflammation and the onset of scarring during fetal repair correlates with the presence of an acute inflammatory infiltrate [3, 4]. In adults, wounding stimulates a rapid and persisting increase of the pro-inflammatory cytokines IL-6 and IL-8 which quickly disappear in the fetal wound [5, 6]. This suggests an important function of inflammation during scar formation. The absence of myofibroblasts [7], lower number of neutrophils [8] and platelet-derived growth factor (PDGF) [9], and the decreased expression of homebox genes as HOXB13 [10] are other factors involved in scarless fetal wound healing.

Normal scars

In normal scarring after birth the amount of inflammatory cells, endothelial cells, and fibroblasts decrease as the healing process proceeds. The collagenous matrix becomes more organized into thicker and more cross-linked bundles indicating the development of the mature scar. Proteases and protease inhibitors, such as, plasmin, matrix metalloproteinases (MMPs), hyaluronidase, and elastase are involved in extracellular matrix (ECM) remodeling [11] which is carefully controlled by growth factors, tissue inhibitors of metalloproteinases (TIMPs), and syndecans [12].

Hypertrophic scars

Hypertrophic scars are raised scars remaining within the boundaries of the original wound (Fig. 2). They generally regress spontaneously within month to years after the initial injury [13]. Hypertrophic scars are often red, inflamed, itchy, and even painful. They typically appear after burn injury on the trunk and extremities. Hypertrophic scars are frequently misdiagnosed as keloids. Their gross appearance is similar, although keloids grow beyond wound margins.

Clinical characteristics of keloids

The differential diagnosis of keloids and hypertrophic scars is sometimes difficult. Keloids grow beyond the confines of the original wound, invading the normal surrounding skin and rarely regress over time. They often arise immediately after skin injury and appear as firm nodules which are pruritic and painful. Initially, keloids have a pink or red appearance and telangiectasias may be present (Fig. 1). Keloids continue to grow, unlike hypertrophic scars, which typically reach a certain size and stabilize or regress [14]. Strict clinical and histopathological criteria have been defined to differentiate keloids from hypertrophic scars (Table 1).

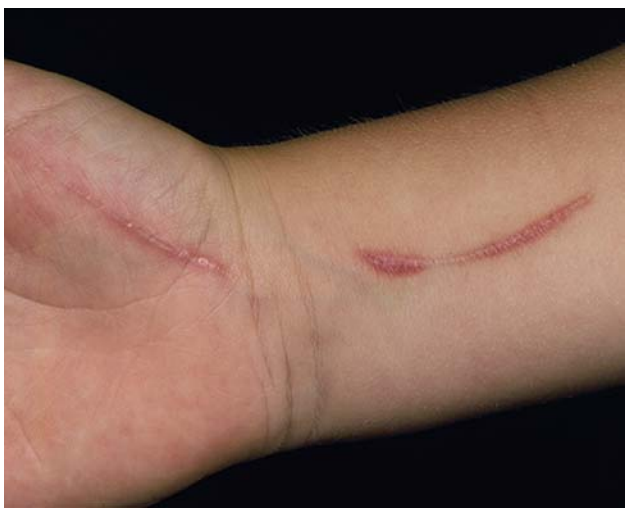

Fig. 2 Hypertrophic scars after injury

Table 1 Clinical and histological criteria's of keloids and hypertrophic scars (modified from [138])

Keloids	Hypertrophic scars
Grow beyond the borders of the original wound	Remain within the boundaries of the original wound
Size varies between a pea and a football; growth may be widespread, vertical, or both	Rarely more than a centimeter in thickness or width
Pruritic and painful	Less pruritic and painful
Appear within several months after initial scar, then gradually proliferate indefinitely	Generally arise within 4 weeks, grow intensely for several months, then regress often within one year
Occur often on the chest, shoulders, upper back, back of the neck and earlobes, rarely on the palms or soles	No predominant anatomical site
Do not regress spontaneously	Regress spontaneously
Larger, thicker and more wavy collagen fibers than normal skin, random collagen fiber orientation, increased ratio of type I to type III collagen	Fine collagen fibers oriented parallel to the epidermis
Increased fibroblast density and fibroblast proliferation rate	Increased fibroblast density
Only few α-smooth muscle actin expressing myofibroblasts	Presence of α-smooth muscle actin expressing myofibroblasts is typical

For unknown reasons, keloids occur more frequently on the chest, shoulders, upper back, back of the neck, and earlobes [15]. This suggests the existence of local populations of abnormal cells or local tissue factors that promote keloid formation. It has been intensively discussed, whether keloids occur primarily in areas of high-skin tension [16]. This might be an oversimplification, since the most commonly affected site, the earlobe [17], is under minimal tension and keloids appear rarely on the palms or soles, where significant skin tension is to be expected. Other local tissue factors might be higher concentrations of melanocytes or sebaceous glands on the chest wall. Keloids could arise from an immune reaction to sebum. Dermal injury exposes the pilosebaceous unit to systemic circulation, and in individuals who retain T-cells sensitive to sebum, a cell-mediated immune response is initiated. Release of cytokines stimulates mast cell chemotaxis and fibroblast production of collagen. Keloids rarely occur on anatomical sites lacking sebaceous glands, such as palms and soles. This sebum reaction hypothesis explains why an individual with two otherwise identical incisions could develop one keloid and one normal scar. The hypothesis also explains why only human beings, the only mammals with true sebaceous glands, are affected by keloid scarring [18]. In the light of the sebum reaction hypothesis it is interesting to point out that Schierle et al. described elevated androgen receptor levels in keloid tissue [19].

Epidemiology

Keloids can develop at every age but have a higher incidence between 10 and 30 years. Mean age of first keloid diagnosis is 22.3 years for women and 22.8 years for men [20]. Hormones have been suggested to influence keloid formation supported by data showing an elevated androgen receptor level in clinically active keloid tissue [19]. Keloids seem to have a higher incidence during pregnancy and puberty, which has been interpreted as related to the hormone profile but other explanations, such as increased neo-angiogenesis in pregnancy are possible [21]. More studies are needed to confirm a correlation between keloid development and hormone profiles.

Although epidemiologic data are limited they suggest differences among racial groups with a higher frequency of keloids in Blacks, Hispanics, and Asians. This may be explained by the sebum hypothesis as in general, Orientals and Blacks have thicker, more seborrhoeic skin than Caucasians [22, 23] and may account for the higher incidence of keloids in these groups.

The incidence of keloids in Caucasians in the United Kingdom is reported to be <1% [24], while the incidence in Blacks and Hispanics varies from 4.5 to 16% [21]. The higher rate for earlobe keloids after piercing may be responsible for a slight female predominance [25].

Genetics

The increased familial clustering in keloids, its increased prevalence in certain races and increased concordance in identical twins suggest a strong genetic predisposition to keloid formation. Identification of genetic markers in candidate genes, such as, the SMAD or TGFβ family may be of significant importance in diagnosis, prognosis, and development of new therapies in the management of keloid scarring.

TGFβ has previously been implicated in keloid pathogenesis. Thus, when tested for an association between

keloid development and polymorphisms within the TGFβ1, β2, β3, and TGFβ receptor (TβR) genes, studies did not reveal a significant association between TGFβ polymorphisms and keloid disease [26–29]. Brown et al. investigated SMAD gene polymorphisms in Jamaican patients with keloid scars. SMADs were previously reported to be involved in fibrotic disorders. The SMAD single nucleotide polymorphisms (SNPs) investigated in their study was not strongly associated with increased risk of developing keloid scarring [30]. Interestingly, a linkage analysis in a Chinese pedigree suggests SMAD to be a possible gene involved in keloid pathogenesis and a susceptibility locus on chromosome 18q21.1 [31]. Some studies suggest that the human leukocyte antigen (HLA) system might be associated with the development of keloids and explain ethnic differences. Lu et al. describe a positive association of HLA-DQA1 and DQB1 alleles and haplotypes with keloids in Chinese population [32] and Rossi et al. proposed an association with the HLA-types, HLA-DR5 and HLA DQw3 [33].

Marneros et al. studied the clinical and genetic characteristics of 14 pedigrees with familial keloids. The ethnicity of these families was mostly African–American but included Caucasian, Japanese, and African–Caribbean. Pedigrees accounted for 341 family members, of which 96 displayed keloids. A female predominance was seen and X-chromosomal linkage was excluded. The pattern of inheritance observed in these families was consistent with an autosomal dominant mode with incomplete clinical penetrance and variable expression [34]. The same group further identified linkage to chromosome 2q23 and 7p11. This could be the first genetic evidence for keloid susceptibility loci and might serve as a basis for the identification of responsible genes [35].

The associations of keloid in certain syndromes like the Rubinstein–Taybi syndrome and Goeminne syndrome supports the hypothesis of a genetic background for keloids [36, 37]. The Rubinstein–Taybi syndrome (OMIM 180849) is characterized by brachydactyly, facial abnormalities, and mental retardation. Dermatologic manifestations include capillary malformations, keloid formation, and pilomatricomas. This syndrome can be caused by mutations in the genes encoding the transcriptional coactivators, CREB-binding protein and EP300. Interestingly, CREB-binding protein and EP300 are coactivators in the SMAD/TGFβ signaling pathway which is suggested to play a key role in keloid development [38]. The Goeminne syndrome (OMIM 314300) is probably inherited as an X-linked trait with incomplete dominance [36]. The syndrome comprises congenital muscular torticollis, multiple keloids, cryptorchidism, and renal dysplasia. Zuffardi and Fraccaro mapped the gene for this syndrome to Xq28, distal to glucose-6-phosphate dehydrogenase (G6PD) [39].

Immunology

Immunological reactions are likely to be involved in keloid etiology. Rossi et al. show a significantly increased concentration of IgG in keloid lesions [33] and IgA and IgM are detected at higher levels in keloids compared to normal skin [40, 41].

The immune cell infiltrate in keloids includes T-lymphocytes (CD3+, CD4+, CD45RO+, and HLA-DR+) and dendritic cells (CD1a+, CD36+, HLA-DR+, and ICAM-1+) [42]. The number of macrophages, epidermal Langerhans cells, and mast cells is increased as well [43, 44]. The number of mast cells and pruritus decrease in keloids after treatment with silicone gel sheeting and mast cells may contribute to an elevated expression of hypoxia-inducible factor 1, alpha (HIF-1α) and vascular endothelial growth factor (VEGF) in keloids [45, 46]. The role of the inflammatory response in the formation of keloids has not been studied in detail and remains to be elucidated.

Quality of life in keloid patients

Common chronic inflammatory skin diseases, such as psoriasis have been shown to impair quality of life (QoL) to the same extent as life threatening diseases [47]. Physical as well as psychological problems have been described in patients with burn scars [48, 49]. Until recently no validated questionnaire for investigation of the impairment of the QoL in patients with keloids has been published. Therefore, a new questionnaire to investigate and measure the quality of life of patients with keloids and hypertrophic scarring was developed [50].

Although other QoL instruments exist they do not include factors known to be associated with pathological scarring. The number of hypertrophic scars and keloids is increasing as decorative piercing is more often practiced particularly in younger-age groups and in visible anatomical sites [51]. Patients suffering from pathological scarring are constantly aware of their skin symptoms and their psychological consequences. Therefore, it can be assumed that the QoL of patients with keloids and hypertrophic scars may be severely impaired.

By using a newly developed questionnaire a prospective study in keloid patients was performed. The results demonstrate for the first time a severe impairment of QoL of life of patients suffering from keloids and hypertrophic scars and suggest that the new questionnaire enables to measure QoL in these patients. Visible scars on the head, lower arms, or lower legs significantly influence the scale "psychological impairment" of the questionnaire implying the role of visible scars as an important stigma. This questionnaire may be useful to document the impact of new

developments in the treatment of pathological scarring on QoL. Future studies in this field should include patients with normal scarring to further support the validity of the scales shown in this study.

Current research

The pathogenesis of keloids is still poorly understood. No keloid-causing gene mutation has been reported so far and the in vitro experiments with keloid-derived cells have revealed multifaceted and intricate pathological alterations in many different aspects of cell behavior.

At present a unifying hypothesis for the pathogenesis of keloid formation has not been put forward. Recent research has focused on the interaction between keloid-derived fibroblasts and ECM, the role of integrins and abnormalities of keloid fibroblasts (KF). In addition, the involvement of proteolytic remodeling of ECM by MMPs, plasmin and their inhibitors have been analyzed and several studies have investigated the expression of cytokines, chemokines, growth factors, and their signaling pathways in keloids.

Teofoli et al. demonstrated an altered TGFβ regulation of proopiomelanocortin (POMC) gene expression in keloid-derived fibroblasts, suggesting that POMC may play a role in the pathogenesis of keloid formation [52]. In this respect, it is interesting to note that so far no human albino with keloids has been described.

Keloids and ECM

The wound healing process requires a complex interaction between different cell types, ECM, and cytokines [53]. A tightly regulated balance between synthesis and degradation of ECM is essential for normal scar formation. If this balance shifts toward increased ECM production or decreased degradation, hypertrophic scars and keloids may occur.

In vitro, keloid fibroblasts show an elevated gene expression for collagen, fibronectin, elastin, and proteoglycan [54–56]. The gene expression of keloid fibroblasts is altered by the interaction with the surrounding ECM via cell surface receptors called integrins [57] and fibroblasts surrounded by densely packed collagen express different genes compared with fibroblasts in a softer matrix [58].

The balance between ECM degradation and synthesis is influenced by proteolytic processes. This remodeling of the granulation tissue in the last phase of wound healing replaces collagen type III with type I, increases proteoglycan production and degrades fibrin and fibronectin. The ECM remodeling is mediated by proteolytic serine proteases (tissue-plasminogen activator (t-PA), urokinase-plasminogen activator (u-PA)), and MMPs produced by fibroblasts. MMP-1 (collagenase-1), MMP-8 (collagenase-2), and MMP-13 (collagenase-3) are capable to cleave collagen type I, II, and III [59].

Another regulator involved in keloid development is the plasmin/plasminogen activator system. U-PA and t-PA activate plasminogen to plasmin which acts fibrinolytic and activates procollagenase [60]. Further, plasmin is involved in activating TGF-β from its latency form [61] and TGFβ induces plasminogen activator inhibitor-1 (PAI-1) and TIMP-1 leading to decreased plasmin and collagenase activity resulting in diminished collagen degradation. Keloid fibroblasts express higher levels of PAI-1 [62] leading to increased accumulation of collagen and fibrin [63]. An alternative or additional reason for elevated PAI-1 expression might be hypoxia and an increased level of the hypoxia marker HIF-1α is found in keloid tissue [64].

Integrins

The contact of fibroblasts with the surrounding ECM is established mainly by integrin receptors. These are transmembrane receptors, which specifically bind different ECM molecules. A group of five integrins, $\alpha1\beta1$, $\alpha2\beta1$, $\alpha3\beta1$, $\alpha10\beta1$, and $\alpha11\beta1$ have been described as binding partners to collagens, with some members also binding to other ECM molecules like laminin ($\alpha1\beta1$) and fibronectin ($\alpha3\beta1$). On fibroblasts, recognition of external collagen is mainly mediated by $\alpha1\beta1$ and $\alpha2\beta1$ integrins. On the collagen side, the binding epitope needs to be in a native, i.e., triple-helical conformation; within the integrin receptor, the major collagen binding site resides in the A-domain (also called I-domain) of the respective $\alpha1$ or $\alpha2$ subunits [58]. Integrin expression is regulated by cytokines as for example TGFβ in an autocrine and paracrine manner [57]. This allows a change in affinity of integrins toward their ligands [65]. Keloid fibroblasts may be able to recognize the changed composition of the surrounding ECM by integrins. This may change the phenotype of keloid fibroblasts promoting keloid development.

Keloids as well as hypertrophic scars show alterations of integrin expression and contain several distinct populations of fibroblasts. One of these populations expresses high levels of $\alpha1$ integrin, and the proportion of these cells is higher in keloids and hypertrophic scars than in normal skin tissues. Integrin $\alpha1$ knockout mice maintain increased collagen synthesis consistent with a role for $\alpha1$ integrin in providing negative feedback on collagen synthesis [66]. These results from mice are controversial. A down-regulation of $\alpha1$ integrin in keloids would be expected but the high expression of TGFβ in keloids maintains high levels of $\alpha1$ integrin and up-regulated collagen production.

Hexadecylphosphocholine (HePC), a topically effective compound, exerts a strong antiproliferative effect on

neoplastic cells. HePC treatment of keloid fibroblasts induced up-regulation of the $\alpha 2\beta 1$ integrins, required for the recognition of external collagen, and a down-regulation of fibronectin synthesis suggesting a possible new therapeutic approach for keloids [67].

Keloid fibroblasts

Most in vitro studies investigated the ECM-producing keloid fibroblasts. Keloid fibroblasts show an increased production of fibronectin and type I procollagen [68]. Their ability to degrade procollagen polypeptides is reduced [69] compared to normal dermal fibroblasts. In addition, keloid fibroblasts have a reduced growth factor requirement for proliferation [70] and the growth response to epidermal-growth factor (EGF) is significantly increased [71]. TGFβ1 stimulation lead to an altered response of the total protein synthesis [72] and induced up-regulated collagen production [73].

Several studies showed a different cytokine expression pattern in keloid fibroblasts. Their expression of TGFβ1 and β2 was elevated [74]. Keloid fibroblasts were more responsive in both chemotactic and mitogenic assays to all three isoforms of PDGF as compared to fibroblasts from normal skin. The enhanced PDGF response of keloid fibroblasts appeared to be mediated by elevated levels of PDGF α receptors, which are 4–5 times higher than those in normal human skin fibroblasts [75]. Wu et al. showed that VEGF is expressed at higher levels in keloid tissues and in keloid-derived fibroblasts compared to normal skin and that VEGF stimulated the expression of PAI-1, but not u-PA in keloid fibroblasts [76]. These findings suggest that VEGF may play an important role in keloid formation by altering ECM homeostasis toward a state of impaired degradation and excessive accumulation. Zhang et al. transfected keloid fibroblasts with siRNA against VEGF. Beside significantly inhibited VEGF expression they found significantly decreased fibroblasts growth and down-regulated PAI-1 expression. Their results suggest that modulation of VEGF production by siRNA may be a potential therapeutic strategy for keloids [77].

Ghazizadeh et al. showed enhanced expression of IL-6 and its receptors in keloid fibroblasts, with a concomitant increase in collagen biosynthesis [78]. Anti-IL-6 antibodies or blocking the IL-6 receptors elicited reduced collagen synthesis, suggesting a role for IL-6 in the regulation of collagen gene expression. These observations imply targeting of the IL-6 signaling pathway as a possible treatment for keloids.

The absence of IL-10 in fetal skin leads to scar formation. The lack of IL-10 may result in continued amplification of the inflammatory cytokine cascade, continued stimulation of fibroblasts, and abnormal collagen deposition. IL-10 is necessary for scarless wound repair [79]. Peranteau et al. showed that overexpression of IL-10 modulated the inflammatory response at an adult wound site to more closely resemble the profile seen in the fetus. In the light of these results, applying human recombinant IL-10 may offer a potential treatment for keloids [80].

There is evidence that keloid fibroblasts failed to undergo physiologically programmed cell death (apoptosis). They were more resistant to Fas-mediated apoptosis [81] and the overexpression of insulin-like growth factor (IGF)-1 receptor inhibited ceramid-induced apoptosis [82]. Sayah et al. demonstrated decreased expression of pro-apoptotic genes (tumor necrosis factor receptor 1-associated protein (TRADD); 19-kDa interacting protein 3 (NIP3); and cytoplasmic dynein light chain 1 (HDLC1)) in human keloid tissue and decreased apoptotic activity in fibroblasts derived from keloids versus those from normal scars [83]. Keloid fibroblasts showed a focal dysregulation of p53 combined with upregulation of bcl-2 which facilitated increased cell proliferation and decreased cell death [84]. Messadi et al. showed that antiapoptotic genes could play a role in keloid pathogenesis. Their results demonstrated higher basal levels of inhibitor of apoptosis (c-IAP-1) in keloids compared to normal skin [85].

Recent studies revealed altered interaction between keratinocytes and fibroblasts in keloids. Co-culture of keloid fibroblasts with keloid keratinocytes induced greater resistance of fibroblasts to apoptosis, increases TGFβ, and collagen type I and III expression compared to keloid fibroblasts from pure cultures [86, 87]. Interestingly, co-culture of normal fibroblasts with keloid keratinocytes lead to increased proliferation of fibroblasts and increased TGFβ and collagen type I and III expression [88]. Lim et al. reported in a current study altered cytokine production in keloid fibroblasts and keratinocytes, either in non-co-culture or co-culture conditions. Cytokine profiling showed angiogenin, oncostatin M (OSM), VEGF, IGF-binding protein-1, osteoprotegerin, and TGFβ2 present in keloid keratinocyte-fibroblast co-culture, but absent in normal keratinocyte-fibroblast co-culture. They demonstrated that only IL-6 and OSM stimulated pY705 Stat3 and cell proliferation in both normal and keloid fibroblasts. This data suggest an altered phenotype of keloid fibroblasts as other cytokines only increase proliferation of keloid fibroblasts and not normal fibroblasts. This underlines the hypothesis that multiple cytokines contribute to keloid pathogenesis and the authors conclude that a combinatorial neutralizing antibody/cytokine therapy may be effective in treating keloids [89].

TGF-β and SMAD

TGFβ family members, which include TGFβ, activin, and bone morphogenetic protein (BMP), are secreted cytokines

that regulate a broad array of cellular responses including proliferation, differentiation, migration, and apoptosis. Dysregulation of their signaling has been implicated in various human diseases including cancer, fibrosis, autoimmune diseases, and vascular disorders. Several growth factors are involved in wound healing but TGFβ appears to play a central role [90]. Most cells implicated in wound healing secrete TGFβ. Three mammalian isoforms of TGFβ exists: TGFβ1, β2, and β3 [91].

TGFβ activity is regulated by TGFβ gene transcription. TGFβ1 itself up-regulates the expression of TGFβ1 mRNA and protein [92]. The active form of TGFβ is non-covalently bound to the latency-associated peptide (LAP) [93] and subsequent activation is required to release TGFβ from its latent form. In addition, the latent TGFβ forms a larger complex with a protein called latent TGFβ-binding protein (LTBP). This complex binds on the cell surface, where the LAP region binds to mannose-6-phosphate (IGF2) receptors. Transglutaminase initiates cross-linking of plasminogen to the cell surface which turns into plasmin and releases active TGFβ [94]. The availability of TGFβ is also regulated by the ECM. Decorin, α-fetoprotein, and biglycan bind and neutralize TGFβ [95, 96] and fibronectin- and thrombospondin-associated TGFβ exhibit biological activity [97, 98].

The TβRs consist of three subtypes, type I, II, and III. They belong to the serine/threonine kinase family of receptors and have an extracellular, a transmembrane and a cytoplasmatic kinase domain. TGFβ1 binds first to TβRII and then TβRI is recruited to the complex [99]. TβRII phosphorylates TβRI to activate it. The affinity of TGFβ2 to TβRII is low and requires TβRIII for assembly but the role of TβRIII is not fully clarified. TβRIII exists also in a soluble form found in serum and in ECM which might act as a reservoir or clearance system for bioactive TGFβ or facilitate TGFβ binding to its receptors [100].

TGFβ is generally considered to be a key player in keloid development although only a few reliable studies describe the expression of TGFβ in keloids. Lee et al. found increased TGFβ1 and TGFβ2 protein expression in keloid-derived fibroblasts compared to normal human skin fibroblasts, whereas TGFβ3 expression did not differ [74]. An increased expression of TGFβ1 mRNA in cultured keloid fibroblasts was recently reported by Fujiwara et al. [101]. Further, an increased expression of TβRI and TβRII protein is described in keloid fibroblasts relative to normal human skin fibroblasts [102]. In human post-burn hypertrophic scar tissue expression of TGFβ1 mRNA and protein is described to be increased [103, 104] and by using immunohistochemistry, Schmid et al. found elevated expression levels of TβRI and TβRII in human hypertrophic scars [105]. Shah et al. injected a neutralizing TGFβ antibody to the margins of healing dermal wounds in adult rats and found wound healing without scar tissue formation [106]. This data provide evidence that an early manipulation of selected cytokines may be a successful approach for the control of scarring. As mannose-6-phosphate (M6P) inhibits the activation of TGFβ1 and TGFβ2 [107], local application of recombinant MP6 is currently under clinical investigation [106].

Inactivation of TGFβ1 may also be achieved by inhibitors of dipeptidyl peptidase IV (DPIV). Thielitz et al. showed that the inhibitors of DPIV-like activity, Lys[Z(NO(2))]-thiazolidide, and Lys[Z(NO(2))]-pyrrolidide, suppressed the proliferation of keloid-derived skin fibroblasts in vitro. They significantly decreased TGFβ1 expression, the secretion of procollagen type I C-terminal peptide, and abrogated collagen synthesis, matrix deposition, and fibronectin expression. In a mouse model of dermal fibrosis, dermal thickening, collagen I, and α-smooth muscle actin expression, was significantly suppressed in the presence of inhibitors. Inhibition of DPIV-like enzymatic activity may therefore be another novel therapeutic approach for the treatment of keloids [108].

Our group demonstrated a combination of increased TGFβ1 and 2, decreased TGFβ3, and increased TβRI/TβRII ratio in keloid fibroblasts [109]. This phenotype may be involved in the development of keloids. The proportion between TGFβ subtype and receptor expression will possibly determine whether fibroblasts have a balanced ECM production or not. As shown in Fig. 3, normal skin fibroblasts exhibit higher levels of TβRII and TGFβ3 together with reduced levels of TGFβ1 compared to keloid fibroblasts. The factors contributing to the different phenotypes seen in keloid and normal skin fibroblasts are still unknown and further studies are needed to identify those factors. Interestingly, TGFβ3 is present even at high levels in developing embryonic skin and in embryonic wounds that heal with no scar and application of human recombinant TGFβ3 shows an improvement in subsequent scar appearance [110] and may be a new treatment option for keloids.

SMAD proteins share two highly conserved domains, Mad-homology domains 1 and 2 (MH1 and MH2) at N- and C-terminal parts of the proteins. Receptor (R)-SMADs transiently interact via their MH2 domains with TβRI and become phosphorylated at their C-terminal [111]. SMAD1, 5, and 8 are involved in BMP signaling. SMAD2 and 3 are restricted to the TGFβ pathway [112].

Upon activation, R-SMADs (SMAD2/3) form heteromeric complexes with Co-SMADs (SMAD4) via their MH2 domains (Fig. 4). This complex accumulates in the nucleus, where it participates in the control of expression of target genes. Inhibitory (I)-SMADs (SMAD6 and 7) prevent the activation of signal-transducing R- and Co-SMADs. SMAD6 seems to preferentially inhibit BMP signaling and SMAD7 seems to be more specific for TGFβ

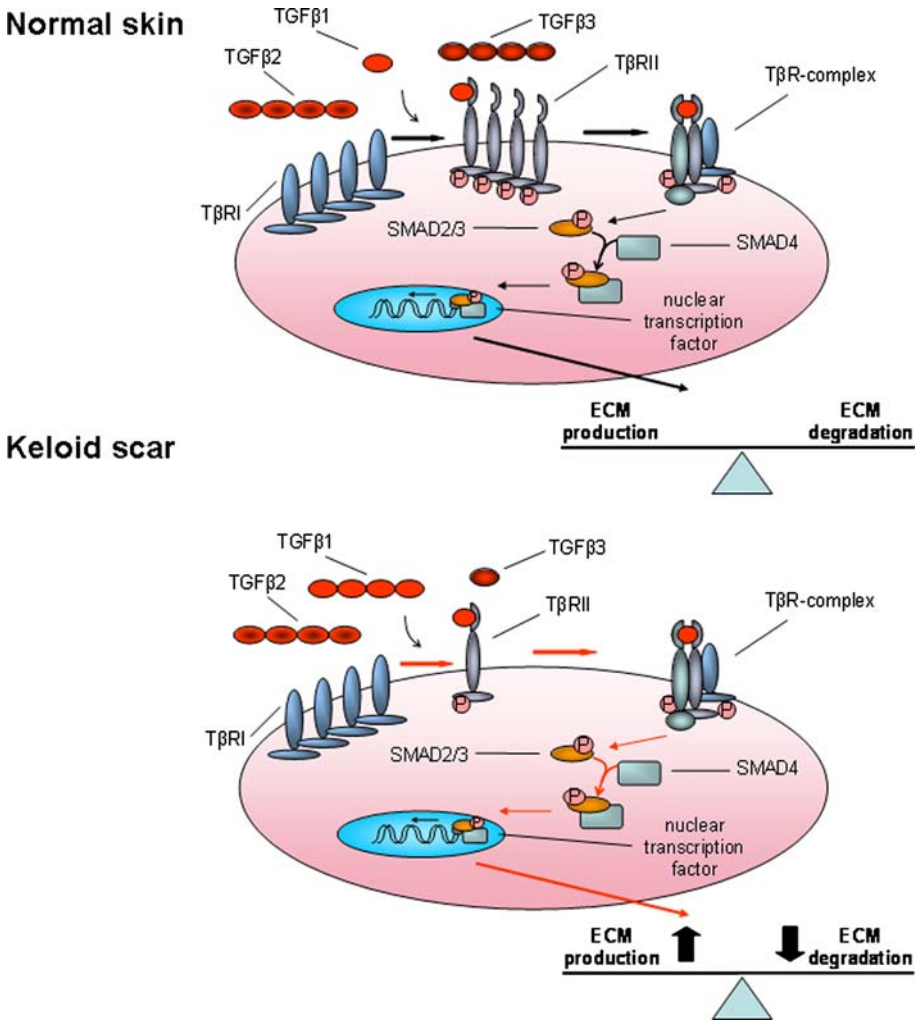

Fig. 3 The relative level of TGFβ subtype and receptor expression determines ECM production (TβR = TGFβ receptor; TβR-complex = complex of TGFβ receptor I, II, and III). TβRI initiate intracellular signaling by phosphorylating specific proteins known as SMAD proteins (for Sma and Mad proteins from *Caenorhabditis elegans* and *Drosophila*, respectively) [139]. SMAD proteins can be divided into three classes: the receptor-activated SMADs (R-SMADs), the common-mediator SMADs (Co-SMADs), and the inhibitory SMADs (I-SMADs)

signaling [112–114]. SMAD6 and 7 interact efficiently with activated TβRI and compete with R-SMADs for binding to the activated TβRI [115]. As the expression of I-SMADs is induced by ligand stimulation [116], they may have a negative-feedback role in signal transduction and thereby forming another regulatory pathway for TGFβ activity.

The expression of SMAD proteins in normal scars has not been analyzed yet and only few studies describe the expression in keloids and hypertrophic scars. Phan et al. found increased basal levels of SMAD2, 3, and 4 proteins in keloid fibroblasts. When these fibroblasts were co-cultured with keloid-derived keratinocytes there was an up-regulation of SMAD3 and phosphorylated SMAD2 protein as well as an enhanced SMAD3 phosphorylation and SMAD2/3/4 binding complex production [117]. Tsujita-Kyutoku et al. showed up-regulation of SMAD2 and 3 protein expression in fibroblasts derived from central parts of keloids [118].

Inhibition of SMAD3 in keloid fibroblasts leads to reduced expression of connective tissue growth factor (CTGF) [119] and silencing SMAD2 by siRNA induce decreased procollagen expression [120] suggesting a functional role of SMAD proteins in keloid development.

Our group demonstrated for the first time decreased SMAD6 mRNA in keloid fibroblasts compared to cells derived from normal scars or control skin. SMAD7 mRNA was found to be significantly reduced in keloids as well as control skin compared to normal scars and its protein to be less expressed in keloids compared to normal scars and control skin [121]. High expression of the I-SMAD6 and 7 may result in an inhibition of TGFβ signaling leading to controlled proliferation and collagen deposition in normal scars. The decreased expression of I-SMADs in keloids may cause a persisting TGFβ signal (Fig. 4). Interestingly, recent studies showed that decreased SMAD7 contributes to cardiac fibrosis [122], to TGFβ hyper-responsiveness in scleroderma [123, 124], and to the development of pulmonary fibrosis [125]. Further, SMAD7 inhibited the expression of ECM genes in hypertrophic scar fibroblasts [126]. We hypothesized that SMAD7 may exert beneficial effects on excessive scar formation and is an interesting target for future treatment strategies.

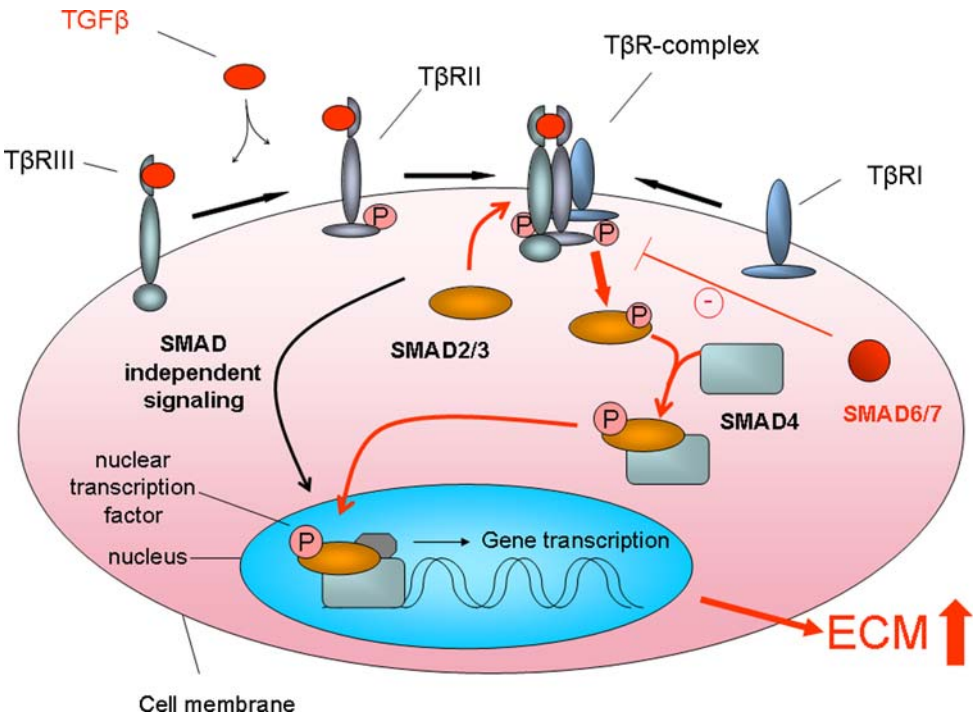

Fig. 4 Model of intracellular TGFβ signaling events. Absence of the inhibiting SMAD6 and 7 in keloid fibroblasts is leading to a persisting TGFβ signal with increased ECM production (*red arrows*). SMAD-independent signaling pathways may contribute to increased ECM production via TGFβ signaling. (TβR = TGFβ receptor; TβR-complex = complex of TGFβ receptor I, II, and III)

Interestingly, there is evidence for the existence of SMAD-independent TGFβ receptor signaling [127, 128]. This has been taken into account when interpreting results of SMAD expression in keloids. Bhattacharyya et al. describe a non-SMAD mechanism of fibroblast activation by TGFβ via the non-receptor protein tyrosine kinase c-Abl [129]. As shown in Fig. 4 TGFβ might by SMAD-independent signaling influence gene expression in keloid fibroblasts initiating a fibrotic response.

Future studies are required to analyze the role of phosphorylated SMAD proteins (p-SMAD) in keloid development as p-SMADs are the biologically active proteins. It is of interest to study the translocation of p-SMADs into the cell nucleus to verify whether gene transcription is induced or not. Further, the interaction of TGFβ/SMAD-signaling with Smurf, an ubiquitin ligase, and SMAD anchor for receptor activation (SARA) needs to be analyzed. Smurf is involved in degradation of R-SMADs and degrades SMAD/TβR complexes [130]. The recognition of R-SMADs by TβRs is facilitated by auxiliary proteins. SMAD2 and 3 are immobilized near the cell surface by SARA [131]. SARA allows more efficient recruitment of SMAD2 and 3 to the receptor for phosphorylation. Finally, it would be very interesting to study the role TGFβ/TβR-complex processing in keloid pathogenesis. The activated TGFβ/TβR-complex undergoes endocytosis via two distinct routes: via coated vesicles to early endosomes for signaling and via caveolae to caveolin-positive vesicles for degradation [132].

Differential gene expression in keloids

There are three previous studies in which gene expression profiles in keloids have been analyzed with microarray technique. In the most recent study the Affymetrix-based microarray included 38,000 genes and revealed 500 regulated genes [133]. The study showed increased expression of several IGF-binding and IGF-binding-related proteins and decreased expression of a subset of Wnt-pathway inhibitors and multiple IL-1-inducible genes. Increased expression of CTGF and insulin-like growth factor binding protein (IGFBP)-3 was observed in keloid fibroblasts only in the presence of hydrocortisone. These findings support a role for multiple fibrosis-related pathways in the pathogenesis of keloids.

Another study analyzed 22,000 genes in keloid fibroblasts compared with normal skin fibroblasts and revealed 43 up- and 6 down-regulated genes [134]. The authors described up-regulation of annexin A2, transgelin, and RPS18 in keloids and they reported for the first time that a few tumor-related genes were overexpressed in keloid fibroblasts. In this study, the age of the participating patients diverge. Of the three patients, the first was an 8-year-old male, the second was 57-year-old and the third with unknown age. Furthermore, the site of the biopsy within the keloid was not presented and the race of the individuals and the reason for keloid development is not recorded.

Chen et al. performed microarray analysis of three keloids after burn injury and three normal skin samples in

Table 2 Comparison of microarray studies

	Satish [134]	Chen [135]	Smith [133]	Seifert [136]
Apoptosis				
p53 Binding protein		+		+
Brain cellular apoptosis susceptibility protein		+		+
Annexin A2	+			+
Matrix				
Proteoglycan 1, secretory granule		+		+
Chondroitin sulfate proteoglycan		+		+
Collagen type I, alpha 1 (COL1A1)	+			+
Collagen, type XI, alpha 1 (COL11A1)		+		+
Collagen, type IV, alpha 1 (COL4A1)		+		+
Collagen, type V, alpha 1 (COL5A1)		+		+
Fibronectin	+	+		+
Collagen, type VIII, alpha 1 (COL8A1)			−	+
Growth factors				
β-Actin	+			+
Insulin-like growth factor binding protein (IGFBP)	+	+	+	+
Heparin-binding EGF-like growth factor		+		+
Transforming growth factor, beta receptor II (TGF-βRII)		+		+
Transforming growth factor, beta 1 (TGF-β1)		+		+
Epidermal-growth factor receptor kinase substrate (EPS)		+		+
Human nerve growth factor (HBNF-1)		+	+	+
Cytokines				
CXCL12			−	−
Other				
Tissue factor pathway inhibitor 2 (TFPI2)			−	+
Tenascin 1 (TNC)			−	−
Syndecan 1 (SDC1)			−	−
MMP3			−	−
Tropomyosin 1	+			+
Mesoderm specific transcript (MEST)			+	+
Signal transducer and activator of transcription (STAT1)			+	+
Disc large homolog 7 (DLG7)			−	+
Coagulation factor II receptor (F2R)			+	+

+ up-regulated genes; − down-regulated genes

Chinese patients [135]. In this study 250 genes were up- and 152 genes were down-regulated. The authors describe differential expression of collagen, fibronectin, proteoglycan, growth factors, and apoptosis-related genes consistent with the published biochemical and clinical observations of keloids and found higher expression of TGFβ1 and nerve growth factor (NGF) in keloids versus normal skin.

Interestingly, comparison of the results shows that there is no overlapping gene expression pattern in these microarray studies. Table 2 gives an overview of some of the regulated genes. Even if most of the genes are regulated in at least two of the four studies, only one (IGFBP) is found to be up-regulated in all studies. Three genes are differently regulated: as Smith et al. found down-regulation of collagen type VIII, alpha 1 (COL8A1), tissue factor pathway inhibitor 2 (TFPI2), and disc large homolog 7(DLG7), our group found an up-regulation of these genes. These results emphasize the need for standardized study conditions.

Our group performed a study comparing for the first time gene expression profiles between different lesional sites of keloids [136]. The microarray chip used in our study covered approximately 47,000 transcripts (38,500 genes). Gene expression patterns in the central part of keloids involve up-regulation of apoptosis inducing genes as a disintegrin and metalloprotease 12 (ADAM12) and ECM degrading genes as MMP19. These genes may contribute to

regression of keloids and are possible future target genes for prevention and treatment of keloids. Overexpression of apoptosis inhibitors as apoptosis caspase activation inhibitor (AVEN) and down-regulation of angiogenesis inhibiting genes as pentraxin-related gene (PTX3) at the active margin of keloids may be responsible for the invasive character of the keloid margin. For the first time there was an important distinction of unique gene expression profiles in different lesional sites of keloids reflecting different stages in the live cycle of keloids. The results of the study support the important role of the biopsy site for research in keloids as these results show that different genes are regulated in different sites of keloids.

Despite the enormous potential of the microarray technique, several issues need a careful approach using this instrument. First, the disease and disease activity have to be defined. Second, heterogeneous cell populations in the keloid sample may confound the results. It cannot be excluded that our results are influenced by the gene expression profiles of subpopulations of keloid fibroblasts and that this profile changes under in vitro conditions. Third, there is a lack of standardization of microarray chips in the numbers, nature and volume of probes (spots), reproducibility of results, and different approaches to data analysis. This makes comparison between published microarray data difficult. Therefore, future microarray studies in keloids need standardization according to Brazma et al. [137]. Their proposal, the minimum information about a microarray experiment (MIAME), describes the minimum information required to ensure that microarray data can be easily interpreted and that results derived from its analysis can be independently verified.

To ensure comparability between keloid studies strict clinical criteria should be used to define keloids. Further, all keloid studies should declare the site of the punch biopsy within the keloid (e.g., active margin) and the body site, where the keloid is located. Beside the ethnical background of the patients, their age, gender, and the cause of the keloid is required.

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