Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/burns

The effect of TLR4/7 on the TGF-β-induced Smad signal transduction pathway in human keloid

Chen Jialiang[a], *Zeng Biwei*[a], *Yao Hangping*[b], *Xu Jinghong*[a,*]

[a] *Department of Plastic Surgery, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang Province, People's Republic of China*

[b] *National Key Laboratory of Infectious Diseases, Institute of Infectious Diseases, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang Province, People's Republic of China*

ARTICLE INFO

Article history:
Accepted 30 July 2012

Keywords:
Keloid
Smad signal pathway
Toll-like receptor
Smad4
Smad7

ABSTRACT

Background: Keloid formation is closely related with transforming growth factor (TGF)-β-induced Smad signal transduction. Recent studies have shown that toll-like receptor4 (TLR4) may mediate liver and kidney fibrosis, and activation of TLR7 has anti-scarring effect. The role of TLR4/7 signalling in keloid formation, however, remains unknown. Our previous tests have found that mute Smad4 inhibited scar. We then speculated that keloid may be affected by TLR4/7 through TGF-β-induced Smad signal transduction.

Objectives: The aim of this study was to evaluate effects of TLR4/7 on the TGF-β-induced Smad signal transduction pathway in human keloid, and provide information for the mechanism and therapy of keloid.

Methods: Normal scar samples with normal fibroblasts (NFs) served as control samples and keloid samples with keloid fibroblasts (KFs) served as experiment samples. Expression of collagen, connective tissue growth factor (CTGF), Smad4 and Smad7 and TLR4/7 were tested by immunohistochemistry, reverse transcription polymerase chain reaction (PCR) (RT-PCR) and Western blotting, respectively.

Results: Expression of collagen, CTGF, Smad4 and TLR4 increased significantly while expression of Smad7 and TLR7 decreased in KFs while compared to NFs in keloid scar group (KFs), which were decreased in the normal scar group (NFs). However, expression of Smad7 and TLR7 decreased in the keloid scar group (KFs) while compared to the NFs.

Conclusions: TLRs participate in fibrosis of scar tissue through the TLRs-TGF-β-Smads signal pathway. Higher expression of TLR4 in keloid increased expression of TGF-β, CTGF and collagen through the Smad4 signal pathway. Activation of TLR7 or Smad7 may inhibit scar formation.

Keloids contain atypical fibroblasts with an overabundance of extracellular matrix (ECM) components, particularly with an excessive deposition of collagen in the dermis and subcutaneous tissue. They grow invasively into the surrounding healthy skin and are not confined to the border of the initial wound. They seldom show a tendency to regress spontaneously [1,2]. The prevalence between the male and female gender is equally distributed. It has been estimated that keloids most frequently occur in 15–20% of Blacks, Hispanic and Asians and less commonly in Caucasians. To date, no

* *Corresponding author at*: Department of Plastic Surgery, The First Affiliated Hospital, College of Medicine, Zhejiang University, 79 Qingchun Road, Hangzhou 310003, Zhejiang Province, People's Republic of China. Tel.: +86 13758229331; fax: +86 057187236628.
E-mail address: xujinghong2003@126.com (J. Xu).

0305-4179/$36.00
http://dx.doi.org/10.1016/j.burns.2012.07.019

keloid formation has been described in albinos [3,4]. There appears to be a genetic predisposition to keloid formation in darker-skinned individuals, but also positive family history is discussed. However, no specific gene has been linked to the development of keloids to date [5].

Smad proteins as intracellular effectors of transforming growth factor-β (TGF-β) signalling, which are the only downstream substrates of TGF-β receptors (TβRs) known so far [6,7], are central to intracellular signalling of TGF-β1 [8–10]. Smads translocate into the nucleus after being activated by receptors to regulate transcription. The Smad family proteins are molecules of 42–60 kDa, which are classified as receptor-regulated Smads (R-Smads: Smad1, 2, 4, 5 and 8), common mediator Smad (Co-Smad: Smad4) and inhibitory Smads (I-Smads: Smad6 and 7) [11]. TGF-βs regulate the phosphorylation of Smad family proteins [12]. Phosphorylated R-Smads form both homomeric and heteromeric complexes with Co-Smad (Smad4), which accumulate in the nucleus and are involved in transcriptional regulation of target genes in co-operation with other transcription factors [13]. Smad7 inhibits TGF-β signalling by preventing activation of Smad2 or Smad3, whereas Smad6 inhibits TGF-β signalling by preventing activation of Smad1, Smad5 or Smad8 [14]. Therefore, an attempt to up-regulate Smad7 may be a promising way to reduce TGF-β1 excretion and inhibit fibrosis.

Toll-like receptors (TLRs) are a group of transmembrane proteins that reside at cell surfaces or in the membranes of intracellular compartments. TLRs are essential to the innate immune system [15]. Severe injury primes the innate immune system for increased TLRs, such as TLR4-induced pro-inflammatory cytokines (interleukin (IL)-1b, tumour necrosis factor (TNF)-a, IL-6), in response to TLR stimuli in macrophages [16,17]. TLRs recognise conserved pathogen-associated molecules such as bacterial lipopolysaccharide (LPS), bacterial lipoprotein (BLP), CpG DNA or viral double-stranded RNA. Each receptor recognises a unique set of ligands. The recent identification of endogenous ligands of TLRs suggests that they function not only to induce defensive antimicrobial immune responses but also as a sensitive detection system to initiate tissue repair after injury [18]. Over 10 TLR members are currently found in animals and humans. TLR4 was the first member of its family to be characterised as a critical factor in the host defence against infection because of its mediation of cell recognition of bacterial LPS. In addition to its role in pathogen recognition, there is an emerging paradigm in which TLR4 plays an important role as a biosensor of tissue damage or sterile inflammatory stimulation [15]. Recently, although the pathogenesis of keloid is not understood, prolonged inflammation is a known contributing factor.

However, it is still unknown whether TLR4/7 exerts its effects on TGF-β/Smads signalling in keloid. Hence, the aim of this study was to investigate the effect of TLR4/7 on the TGF-β-induced Smad signal transduction pathway in human keloid.

1. Materials and methods

1.1. *Patients and tissue specimens*

Informed consent was obtained from all individual subjects for all procedures. The selection of patients was performed by two surgical plastic surgeons undergoing the reconstructive procedure. Fresh tissue specimens were obtained from 10 healthy patients with keloids and five healthy patients with non-pathological scar during reconstructive surgery performed at Plastic Surgery of First Affiliated Hospital of Zhejiang University in China. The study was approved by the Ethics Committee of the University Hospital of Zhejiang, and written consent was obtained from all subjects. A small tissue sample of each resected scar was sent to the pathology laboratory for histologic processing and confirmation of the clinical diagnosis (Table 1 and Fig. 1).

1.2. *Immunohistochemistry analysis*

Samples were frozen in liquid nitrogen for reverse transcription polymerase chain reaction (RT-PCR) or Western blotting analysis. For in vitro analysis fibroblasts isolated from keloids scar and normal scar were cultured in dishes at 37 °C in a 5% CO_2 fully humidified atmosphere and supplemented with antibiotics. The immunohistochemistry for collagen, connective tissue

Table 1 – Source of human keloid and normal fibroblasts.

Patient	Age (years)	Sex	Site of biopsy	Family history	Medical history
KFs	21	F	Ear	–	6 months
	34	F	Ear	+	17 months
	17	F	Ear	–	25 months
	28	F	Ear	+	9 months
	22	F	Ear	–	11 months
	19	F	Ear	+	4 months
	25	F	Chest	+	7 months
	22	F	Forearm	+	6 months
	28	F	Lamosina	–	18 months
	45	M	Shoulder	+	>5 years
NFs	19	F	Abdomen	–	3 years
	27	F	Abdomen	–	2 years
	21	M	Crus	–	7 years
	45	F	Abdomen	–	9 months
	25	F	Forearm	–	10 months