H**ypoxia-inducible factor-1α activates transforming growth factor-β1/Smad signaling and increases collagen deposition in dermal fibroblasts**

[Author information omitted]

**ABSTRACT**

Hypoxia occurs during the formation of scar tissues and can result in abnormal wound healing such as keloids. The central areas of keloids can be severely hypoxic and ischemic in comparison to normal scars. Hypoxia-inducible factor-1 (HIF-1), the master regulator of oxygen homeostasis, is a heterodimeric transcription factor that is stabilized in the absence of oxygen and upregulates genes involved in the hypoxia response. Here, we investigated the correlation between hypoxia, transforming growth factor-β1/Smad signaling, and collagen deposition in normal and keloid fibroblasts. Our results show that HIF-1α upregulated TGF-β1, Smad2/3, p-Smad2/3, Smad4, and total collagen under hypoxic conditions in both normal and keloid fibroblasts via HIF-1α. Furthermore, the expression of hypoxia-responsive genes was attenuated by HIF-1α inhibition, with the exception of TGF-β receptor subtype II (TβRII). Silencing Smad4 under hypoxic conditions decreased levels of HIF-1α mRNA and protein, suggesting a role for Smad4 in the regulation of HIF-1α. Finally, we examined the role of the TGF-β1/Smad pathway in collagen deposition and found that the inhibition of TβRII under hypoxic conditions decreased p-Smad2/3 as well as collagen deposition. Likewise, p-Smad2/3, Smad4, and collagen deposition decreased when TβRII was silenced under normoxia conditions. Our findings demonstrate that TGF-β1/Smad signaling in the absence of oxygen is HIF-1α dependent and that both HIF-1α and the TGF-β1/Smad signaling pathway promote collagen deposition in hypoxia, which is an important factor in keloid formation.

**INTRODUCTION**

Trauma causes substantial damage to the vascular network of the skin and increases the metabolic state of cells during inflammation and repair, which leads to ischemic/hypoxic conditions and potentially to the formation of keloid scars [3, 4]. Keloids are common benign fibroproliferative skin tumors that grow beyond the boundaries of a wound, and can be difficult to treat. In keloids, fibroblasts are stimulated to proliferate and deposit excessive extracellular matrix (ECM) proteins, particularly the excessive synthesis and deposition of collagen and mucopolysaccharides [1]. Animal models have confirmed that the oxygen content of local tissues is significantly decreased during scar formation [3]. Expression of the heterodimeric transcription factor hypoxia inducible factor1 (HIF-1) has been shown to be significantly increased during scar formation and wound healing in humans [5–7]. Likewise, keloids are known to exhibit hypoxic conditions, in which the central areas display significantly higher levels of hypoxia-inducible factor-1α (*HIF-1α*) expression and lower vascular density compared to hypertrophic and mature scars, as well as the margins of keloids [2].

HIF-1α—the alpha subunit of HIF-1—is induced by extremely low oxygen concentrations (0–2%) and functions as a major transcriptional regulator of adaptive responses to hypoxia. Under normal oxygen conditions, prolyl hydroxylase (PHD) can hydroxylate HIF-1α and lead to its rapid degradation, whereas hypoxia blocks this process [8]. Schodel et al. [9] found that HIF-1 upregulates connective tissue growth factor (CTGF), which strongly promotes the proliferation of fibroblasts and ECM synthesis, further supporting a key role for HIF-1 in keloid formation. Moreover, Deng et al. [10] reported that levels of transforming growth factor-β (TGF-β) are upregulated in cancer cells during hypoxia.

TGF-β is a multifunctional cytokine, and includes three different isoforms (TGF-β1, TGF-β2, TGF-β3) in mammals. TGF-β activates the membrane receptor serine/threonine kinase complex composed of type II (TβRII) and type I (TβRI) receptors. Upon the binding of the TGF-β complex to receptors, TβRII phosphorylates and activates TβRI, leading to the activation of the TGF-β/Smad signaling pathway. The TGF-β/Smad pathway plays a vital role in cell growth, differentiation, apoptosis, and proliferation [11–13]. TGF-β1 is an important member of the TGF-β family, has been found to be upregulated in keloid tissues, and is reported to stimulate collagen formation and ECM synthesis. Furthermore, TGF-β1 has been shown to decrease extracellular matrix degradation [14, 15] and may be involved in the formation of keloids [16]. Hypoxia has been reported to promote TGF-β1 in gastric cancer [10], while *TGF-β* expression has been shown to be enhanced in scar tissue fibroblasts [17–19]. Moreover, TGF-β has also been reported to cooperate with CTGF to induce sustained fibrosis [20].

Fibroblasts can synthesize, deposit, and remodel the ECM, and play a vital role in wound healing and keloid formation [20]. In the present study, we assessed: 1) the response of human dermal fibroblasts to hypoxia, 2) the effect of TGF-β1/Smad signaling and collagen deposition during hypoxia, and 3) the role of HIF-1α and the TGF-β1/Smad pathway in collagen deposition. Here, we hypothesized that hypoxia promotes the TGF-β1/Smad signaling pathway via HIF-1α. As hypothesized, we observed that hypoxia increased collagen deposition via HIF-1α and the TGF-β1/Smad signaling pathway.

**RESULTS**

**Hypoxia promotes TGF-β1/Smad signaling**

To analyze the effect of hypoxia in fibroblasts, we detected the expression of mRNA and proteins in both human foreskin fibroblasts (HFFs) and human keloid fibroblasts (HKFs) under normoxic (21% O2) and hypoxic (1% O2) conditions. Western blotting revealed that levels of HIF-1α, connective tissue growth factor (CTGF) and vascular endothelial growth factor (VEGF) protein were upregulated after 24 h under hypoxia (Figure 1A). We subsequently investigated whether acute hypoxia stimulated the TGF-β1/Smad pathway…

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