High-mobility Group Box Protein-1, Matrix Metalloproteinases, and Vitamin D in Keloids and Hypertrophic Scars

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Summary: Keloids and hypertrophic scars represent excessive wound healing involving high production of collagen by skin fibroblasts. This review focuses on the role of high-mobility group box protein-1 (HMGB-1), matrix metalloproteinases (MMPs), and vitamin D in these conditions. Although the role of HMGB-1 in keloids and hypertrophic scars is unclear, the effect of HMGB-1 on fibroblasts suggests a profibrotic role and a potential contribution to excessive scarring. MMPs contribute extensively to wound healing and characteristically degrade the extracellular matrix. MMP-1 is decreased in keloids and hypertrophic scars. However, other MMPs, including MMP-2, have been found to be increased and are thought to possibly contribute to keloid expansion through peripheral extracellular matrix catabolism. Many novel therapeutic approaches to keloids and hypertrophic scars target MMPs and aim to increase their levels and catabolic activity. The higher prevalence of keloids in darker skin types may partially be due to a tendency for lower vitamin D levels. The physiologically active form of vitamin D, $1,25(OH)_2D_3$, inhibits the proliferation of keloid fibroblasts, and correlations between vitamin D receptor polymorphisms, such as the TaqI CC genotype, and keloid formation have been reported. Additionally, vitamin D may exert an antifibrotic effect partially mediated by MMPs. Here, we critically discuss whether keloid and hypertrophic scar formation could be predicted based on vitamin D status and vitamin D receptor polymorphisms. Specifically, the findings identified HMGB-1, MMPs, and vitamin D as potential avenues for further clinical investigation and potentially novel therapeutic approaches to prevent the development of keloids and hypertrophic scars. (*Plast Reconstr Surg Glob Open 2015;3:e425; doi: 10.1097/GOX.0000000000000391; Published online 19 June 2015.*)

Keloids and hypertrophic scars are abnormalities of wound healing characterized by excessive production of collagen in the skin.

In addition to the cosmetic problems caused by their raised and red appearances, keloids and hypertrophic scars can cause pain, pruritus, and contractures. Keloids are visualized as scars that grow beyond the boundaries of the original wound and rarely regress over time. Keloids are seen in patients

of all races; however, they have a greater occurrence in dark-skinned individuals, with an incidence of 6–16% in African populations.[1] Large keloids can arise from minor injuries to the skin, such as acne and piercings.

Conversely, hypertrophic scars are raised, stay within the boundaries of the original wound, frequently regress spontaneously, and are often located at areas of high skin tension such as flexor surfaces. Incidence rates vary from 44% following surgical wounds to up to 91% following burn wounds, depending on the depth of the wound.[2] These scars are caused by injury to the deep dermis, which can be damaged by traumatic wounds and wounds with prolonged inflammation. Contractures in hypertrophic scars can be caused by excessive contraction of the extracellular matrix (ECM) by wound fibroblasts and can produce severe problems, including range of motion loss (from contracture of a scare over a joint) and body disfigurement.

PATHOPHYSIOLOGY

Keloids and hypertrophic scars develop from an improper balance between deposition and degradation of ECM components, notably collagen.

The excess collagen is produced by malfunctioning fibroblasts due to increased density and activation of growth factor receptors. Indeed, transforming growth factor-beta (TGF-β) has been found to be involved in fibroblast proliferation and chemotaxis, collagen synthesis, and the deposition and remodeling of the new ECM of a wound.[1] Normally, TGF-β activity is turned off when wound healing is completed. In keloids and hypertrophic scars, however, TGF-β levels (especially TGF-β1 isoform) are elevated and persistent.[3]

HIGH-MOBILITY GROUP BOX PROTEIN-1

High-mobility group box protein-1 (HMGB-1) has dual functions. As an intracellular transcription factor, HMGB-1 binds to bent DNA to promote the assembly of nucleoprotein complexes, which is critical in the process of transcription, recombination, replication, and repair. As an extracellular mediator, HMGB-1 acts as a potent inflammatory cytokine.[4] Release of HMGB-1 occurs actively by stimulated monocytes and macrophages and passively by necrotic/damaged cells (Fig. 1).[5,6] HMGB-1 exerts its effects by binding to cell surface receptors, particularly the receptor for advanced glycation end products (RAGE) and the toll-like receptors 2 and 4.[7,8]
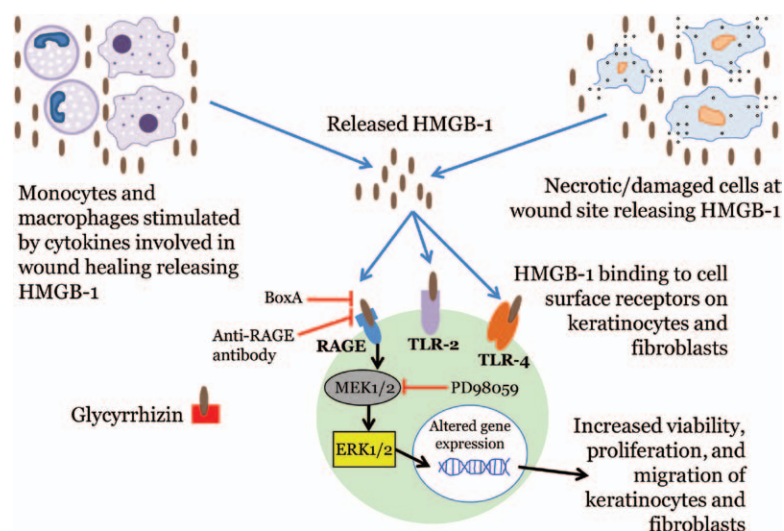
Fig. 1. The role of HMGB-1 in wound healing. HMGB-1 is released actively by stimulated monocytes and macrophages and passively by necrotic/damaged cells.[5,6] It exerts its effects by binding to cell surface receptors (on keratinocytes and fibroblasts), particularly the RAGE and the TLRs 2 and 4.[7,8] HMGB-1 exerts its effects on wound healing by binding primarily to RAGE and activating MEK1/2, which then activates ERK1/2.[9,10] ERK1/2 then translocates to the nucleus, where it presumably alters gene expression, resulting in increased viability, proliferation, and migration of keratinocytes and fibroblasts.[9–13] Inhibitors of HMGB-1 activity, along with their specific points of intervention, are also shown.[9–11] TLRs indicates toll-like receptors.

Interestingly, cells involved in tissue repair are responsive to HMGB-1. Several studies have elucidated a potential role of HMGB-1 in wound healing.[9–11,14,15] Specifically, HMGB-1 accelerates murine wound closure by increasing the viability, proliferation, and migration of keratinocytes and fibroblasts.[9–11] In diabetic skin that has both reduced HMGB-1 levels and altered wound healing, adding HMGB-1 to mice increased fibroblast migration and wound closure rates.[11] In other studies, HMGB-1 exerted its effects on wound healing by binding primarily to RAGE and activating ERK1/2 via phosphorylation. Supporting this was the finding, in both immortalized human keratinocytes and mice, that PD98059, an inhibitor of MEK1/2, abolished HMGB-1 activity (Fig. 1).[9,10] Furthermore, HMGB-1-DNA binding A-box (BoxA), acting as a competitive antagonist by blocking HMGB1 binding to RAGE, and glycyrrhizin inhibited HMGB-1 effects and decelerated murine wound healing.[11] The effects on fibroblasts were also mitigated by an anti-RAGE antibody that blocks HMGB-1 binding in mice.[10] Interestingly, both PD98059 and the anti-RAGE antibody abolished the effects of added HMGB-1, but had no effect when HMGB-1 was not added. Another important observation was that HMGB-1 did not operate as an autocrine factor; normally, it was seen to have a predominantly nuclear localization and was not exported outside murine fibroblasts and immortal human keratinocytes.[9,10] However, induction of necrosis (using H_2O_2) in mouse fibroblasts resulted in HGMB-1 release by necrotic/injured cells at the site of wounding.[10] Straino et al[11] noted that HMGB-1 was present in the cytoplasm of epidermal cells in nonhealing human ulcers. Subsequent stimulation of human skin fibroblasts with tumor necrosis factor-α, an inflammatory mediator, resulted in cytoplasmic translocation of HMGB-1 in these cells.[11] This is supported by findings of other investigators demonstrating that HMGB-1 cytoplasmic relocalization represents a signal for secretion in inflammatory cells stimulated with either lipopolysaccharide or inflammatory mediators, including tumor necrosis factor-α.[14,15]

Although these studies support the role of HMGB-1 as a promoter of wound closure, the role of HMGB-1 in scars remains unclear. By increasing the viability, proliferation, and migration of fibroblasts, HMGB-1 could presumably contribute as a profibrotic molecule to produce collagen. Dardenne et al[16] reported that HMGB-1, when applied to early embryonic (before embryonic day-16) murine skin wounds that normally heal with an absence of scar tissue, induced wound healing with scarring and fibrosis. Additionally, there was a dose-dependent increase in scar size, fibroblast number, and collagen deposition. This correlated with enhanced and prolonged release of HMGB-1 and heal via scar formation in wounds generated beyond murine embryonic day-16. Although the results of this in vivo study are somewhat contradictory to those from another study,[17] which concluded that HMGB-1 decreases in vitro rat fibroblast collagen synthesis, Dardenne et al[16] suggest that fibroblasts could respond differently to HMGB-1 depending on the conditions (in vitro vs in vivo).

The excess production of collagen seen in keloids and hypertrophic scars could result from excess HMGB-1 or increased responsiveness of fibroblasts to HMGB-1, suggesting similar effects of HMGB-1 and TGF-β on the induction of keloid and hypertrophic scar formation. To our knowledge, no studies have yet examined the direct role of HMGB-1 in these conditions. Such studies are warranted to determine the presence and role of HMGB-1 with respect to fibroblast production of collagen and the development of keloids and hypertrophic scars. If HMGB-1 is elevated in these conditions, the therapeutic potential of PD98059, Box A, glycyrrhizin, the anti-RAGE antibody, and other inhibitors of HMGB-1 should be investigated in a clinical scenario.

MATRIX METALLOPROTEINASES

Matrix metalloproteinases (MMPs) are endopeptidases that use a zinc or calcium ion in their active site.[18] Although the main function of MMPs is to catabolize ECM, MMPs also cleave and regulate the activity of many other extracellular bioactive substrates. MMPs are classified into 4 subsets: collagenases, gelatinases, stromelysins, and membrane type (Table 1). The collagenases, which include MMP-1, MMP-8, and MMP-13, cleave types I and III collagens, both

Table 1. Major MMP Subset, the Specific MMPs Included within Each Subset, and the Types of Collagen Cleaved by Each Subset

MMP Subset	MMP Nomenclature	Types of Collagen Cleaved	Reference
Collagenases	MMP-1, MMP-8, MMP-13	I, III	19
Gelatinases	MMP-2, MMP-9	IV, V, VII, X, denatured collagen	22
Stromelysins	MMP-3, MMP-7, MMP-10, MMP-11, MMP-12	IV, V, IX, X	19
Matrix type	MMP-14, MMP-15, MMP-16, MMP-17	None; act on other MMPs	19

of which are present in scar tissue.[19–21] The activity of MMPs is regulated by tissue inhibitors of metalloproteinases (TIMPs), namely TIMP-1, TIMP-2, TIMP-3, and TIMP-4, which inhibit MMPs in a 1:1 inhibitor-to-enzyme ratio. MMPs participate in all 3 phases.[18]

A number of studies have provided insight into the role of MMPs in keloids and hypertrophic scars. Many of the MMPs involved in scar formation are secreted by fibroblasts themselves.[23–29] An imbalance between MMP and TIMP expression could be plausible mechanism for disturbed collagen synthesis and degradation resulting in keloid and hypertrophic scar development.[30–33] In a study on rat fetal wounds, scarless wound healing occurred with a higher ratio of MMP-to-TIMP expression, whereas scar-forming wounds occurred with a smaller ratio.[31]

Specific MMPs have been implicated in keloids and hypertrophic scars. MMP-1 (collagenase), which initiates the degradation of type I collagen, has been found to be decreased, both in mRNA levels and activity, in keloids and hypertrophic scars.[21,33–36] This may be due to TIMP-1, which is increased in human hypertrophic scars compared with normal skin.[32,33] In contrast to MMP-1, MMP-2 increases in keloids and hypertrophic scars compared with normal human skin.[23,25,30,37–39] Even though MMP-2 is thought to be the gelatinase associated with tissue remodeling, which may explain its presence in these abnormal scars, the increase in MMP-2 in keloids and hypertrophic scars may seem counterintuitive considering the characteristic ability of MMPs to degrade collagen.[37,40–42] In keloids, the increased MMP-2 activity, by degrading the ECM on the periphery of the keloid, may contribute to the migration and invasion of keloid fibroblasts into the surrounding (nonkeloid) regions.[23,43] This would lead to keloid expansion. Fujiwara et al[25] reached a similar conclusion in a study that found that MMP-1 (and MMP-2), in contrast to the findings of the aforementioned studies,[21,33–36] was elevated in the medium of human keloid fibroblasts with greater migratory activity of keloid fibroblasts than normal fibroblasts. A dose-dependent decrease in migratory activity (and collagen levels) in the presence of the broad-spectrum MMP inhibitor, GM6001, suggests a direct role of MMPs in this phenomenon, and thus a potential therapeutic role of MMP inhibitors in keloids.[25] Considering the degradative function of MMPs and because keloids have excess collagen, it may again seem counterintuitive to use MMP inhibitors to treat keloids. However, based on the idea that MMPs may contribute to keloid expansion by catabolizing the peripheral ECM, MMP inhibitors may be therapeutic.

Additional studies looking at specific regions (center, periphery/margins) of keloids may explain why some studies find MMPs to be increased while other studies find the opposite. It is possible that MMPs may be decreased in the center (contributing to excessive collagen and scar formation) and increased in the margins (allowing for expansion). Uchida et al[36] briefly discuss this idea and suggest that the variability of results seen in studies of collagenases in keloids may partly be due to the investigation of different regions (central, marginal) of keloid tissue. The accompanying study found that MMP-1 and MMP-8 mRNA and protein expression was lower in human keloid fibroblasts compared with normal fibroblasts, whereas MMP-13 mRNA and protein expression was higher in keloid fibroblasts. More specifically, MMP-13 expression was higher in marginal versus central areas of keloid.[36]

MMP-9 (gelatinase) may also have a role in keloid and hypertrophic scar development. MMP-9 is involved in early tissue repair[37] and could participate in several key areas of wound healing. Neely et al[37] reported decreased activity of MMP-9 in keloids and hypertrophic scars, and a study by Manuel and Gawronska-Kozak[44] found that high levels of MMP-9 were present in the postinjured skin tissue of athymic nude mice (which are unique among mammals in their ability to hear injured skin in a scar-free manner; MMP-9 levels were not elevated in scar-forming mice). High levels of MMP-9 could henceforth contribute to decreased scar formation.

THERAPEUTIC APPROACHES INVOLVING MMPs

Many novel therapeutic approaches aim to increase MMPs in an attempt to decrease excess collagen (Table 2). This may have significant relevance in the development of new methods to treat keloids and hypertrophic scars. For example, hepatocyte growth factor may have therapeutic effects on keloids because of its ability to increase mRNA expression of MMP-1 and MMP-3 in human keloid fibroblasts and increase the activity of MMP-2 in the keloids themselves.[28] In a rabbit ear model, scars treated with indoleamine 2,3-dioxygenase, a rate-limiting enzyme that converts tryptophan into kynurenine (Kyn), were less elevated and had higher MMP-1 expression in fibroblasts.[45] In another study, Kyn increased expression and activity of MMP-1 and MMP-3 and decreased expression of type I procollagen in human dermal fibroblasts.[43] Additionally, significantly less scarring (with less type I collagen and higher MMP-1 expression) was seen in rabbit ear wounds treated with Kyn compared with nontreated wounds.[43] Thus, topical application of Kyn could be an antifibrotic treatment for keloids or hypertrophic scars. Another

Table 2. Novel Therapeutic Approaches That Target MMPs in the Treatment of keloids and Hypertrophic Scars*

Targeted MMPs	Effect on MMPs	Therapy	Theoretical Effect	Reference
MMP-1, MMP-2, MMP-3	Increase levels/ expression	Hepatocyte growth factor	Increased collagen degradation— keloid/hypertrophic scar reduction	28
MMP-1		Indoleamine 2,3-dioxygenase		45
MMP-1, MMP-3		Kynurenine		43
MMP-1		CMC gel		46
MMP-1, MMP-3		Stratifin (14-3-3 σ protein)		34,48–50
MMP-1, MMP-3		CMC gel containing stratifin		46
MMP-1		Fibroblast growth factor-2		21
MMP-1		Quercetin		47
MMP-1		Anti-TGF-β-antibody		25
MMP-1		Oleanolic acid		27
MMP-13		Flash-lamp pulse-dye laser treatment		51
MMP-1		Acetyl salicylic acid (aspirin)		46
MMP-1, MMP-8		Tretinoin		36
MMP-13	Decrease levels/ expression		Decreased collagen degradation— decreased keloid expansion	
MMP-2, MMP-9		GPI-anchored TIMP-1		29,52
Broad-spectrum		MMP inhibitor GM6001		25
MMP-2		TGF-β1 antisense therapy		39
MMP-1, MMP-13	Indirectly inhibits and protects intact collagen	DS-SILY		53

*Listed here are the therapeutic approaches with the MMPs they target, their effect on these MMPs, and their subsequent and theoretical effects on collagen degradation and keloids and hypertrophic scars. Of note is the idea that increasing collagen degradation (by increasing MMP levels/expression) could occur centrally (thus reducing keloid/hypertrophic scar size), while decreasing collagen degradation (by decreasing MMP levels/expression) could occur peripherally (thus reducing keloid expansion). Future studies are needed to determine if MMPs are decreased in the center of keloids and increased in the periphery.
CMC, carboxymethyl cellulose; GPI, glycosylphosphatidylinositol.

approach, using human hypertrophic scar tissue subcutaneously transplanted into mice, determined that treatment with fibroblast growth factor-2, which is released in the first phase of wound healing, increased MMP-1 mRNA and protein expression and caused collagen degradation.[21] MMP-1 levels have also been increased in a fibrotic rabbit ear model by treatment with carboxymethyl cellulose gel, a common hydrogel dressing for wound healing.[46] Antifibrotic effect of quercetin, a component of onion extract, was demonstrated by increase in the expression of MMP-1 in both human skin fibroblasts and, as an ointment, hairless mice.[47]

Regulation of TGF-β and MMPs levels has also been proposed as a potential therapeutic target. Fujiwara et al[25] determined that TGF-β reduced MMP-1 production in human keloids, and an anti-TGF-β-antibody increased MMP-1 production, suggesting its therapeutic potential.[25] The role of TGF-β as a suppressor of MMPs was additionally exploited by a study on hypertrophic scarring in a rabbit ear model, where oleanolic acid, a natural triterpenoid, decreased levels of TGF-β1, increased levels of MMP-1, and decreased levels of type I and type III collagen in hypertrophic scars.[27] Kuo et al[51] determined that flashlamp pulsed-dye laser treatment of biopsied human keloid tissue decreased TGF-β1 expression and increased expression of MMP-13, which may contribute to keloid regression. Aspirin significantly reduces scar formation compared with untreated wounds by inhibiting the infiltration of CD3+ T cells into wounds.[46] Because immune cells, including CD3+ T cells, produce fibrogenic factors such as TGF-β, which suppresses MMP-1, fewer immune cells in a wound would presumably result in less scarring.[25,46]

Additional studies have focused on the ability of MMPs to possibly contribute to fibroblast migration and keloid expansion. Uchida et al[36] found that tretinoin decreased the elevated MMP-13 mRNA and protein expression levels and increased MMP-1 and MMP-8 protein levels and suggested that decreased MMP-1 and MMP-8 contributed to type I and type III collagen accumulation, whereas increased MMP-13 caused remodeling of the peripheral matrix and consequent spread of the keloid. Tretinoin could, therefore, prevent keloid growth (Table 2). In other studies, incorporation of TIMP-1 into the surface of human dermal fibroblasts using a glycosylphosphatidylinositol anchor resulted in reduced secretion of MMP-2 and MMP-9, reduced migration and proliferation of fibroblasts, and reduced expression of profibrotic genes.[29,52] These results, which are logically similar to those caused by MMP-inhibitor GM6001, suggest that TIMPs could be therapeutic, perhaps in the margins of keloids. The peripheral collagen being degraded by MMPs offers another therapeutic target. In dermal scars in rats, a synthetic collagen-binding peptidoglycan therapeutics (DS-SILY) decreased degradation of intact collagen by MMP-1 and MMP-13.[53] Rather than directly inhibiting MMP activity, DS-SILY protected the collagen and reduced the size of the visible scar.

Although the effects of TGF-β could be due to suppression of MMPs, Sadick et al[39] found that TGF-β1 antisense therapy, which selectively impairs TGF-β1 synthesis through the use of oligodeoxynucleotides, decreased secretion of elevated MMP-2 in human keloid tissue, suggesting the role of MMP-2 in keloid expansion. Perhaps more significantly, this study suggests that TGF-β could have different effects on MMPs depending on the region of a keloid. These effects may logically coincide with those of MMPs, such that TGF-β suppresses MMPs centrally and increases MMPs peripherally.

Keratinocytes have also been considered. Tandara and Mustoe[24] found that, in comparison to monocultures, cocultured human fibroblasts and keratinocytes increased MMP levels (including MMP-1) and activity and decreased type I collagen. This suggests that, by affecting the activity of MMPs, paracrine interactions between keratinocytes and fibroblasts may modulate the balance between collagen synthesis and degradation, thus influencing scar formation.[24,54,55] In studies on keratinocyte-releasable stratifin, also known as 14-3-3 σ protein, this factor increases MMP-1 and MMP-3 expression in human dermal fibroblasts, resulting in degradation of accumulated type I and type III collagen (Table 2).[34,48–50] Stratifin may henceforth be released by keratinocytes to regulate the production of collagen by fibroblasts during the remodeling phase.[34] Treatment with stratifin could take the form of a wound insert.[50] Interestingly, wounds treated with carboxymethyl cellulose gel containing stratifin take the appearance of flat, mature scars.[50]

VITAMIN D

Keloids can develop in all skin types, but the risk is 15 times greater in skin containing large amounts of melanin than low-melanin skin, occurring in up to 16% of the black population.[56] The greater incidence of hypertrophic scarring and keloid formation in darker skin types may, in part, be due to a propensity for lower 25(OH)D levels in these individuals.[57] This is plausible considering serum 25(OH)D levels are consistently lower in blacks compared with whites, across all age groups and in both sexes, and in women, who respond 2.5 times less to seasonal changes in sun exposure.[58,59] The production of previtamin D_3 from 7-dehydrocholesterol, the reaction catalyzed by ultraviolet B (UVB) irradiation, 24 hours after exposure to UVB decreases significantly from white to East Asian, Indian, and finally black skin types.[60] Serum 25(OH)D 24 hours after exposure to UVB is also lower in blacks versus whites, but this may be abrogated by consistent exposure to UVB irradiation.[60] Brazerol

et al[61] demonstrated that increases in serum 25(OH)D between blacks and whites after 6 weeks of biweekly UVB exposure were similar.

Levels of the physiologically active form of vitamin D, $1,25(OH)_2D_3$, 24 hours after UVB exposure do not correlate with skin type.[60] This is not surprising considering, with a 4-hour half-life, that levels of serum $1,25(OH)_2D_3$ are relatively labile and mainly reflect metabolism by renal 1α-hydroxylase, which converts 25(OH)D-to-$1,25(OH)_2D_3$, and its role in and relationship to bone and mineral metabolism, and parathyroid hormone levels, respectively.[62,63] Levels of $1,25(OH)_2D_3$ in the skin of keloids and hypertrophic scars would presumably be regulated by extrarenal 1α-hydroxylase, an enzyme that operates well below its Km and, therefore, the activity of which is closely tied to concentrations of its substrate, 25(OH)D.[64] High levels of serum 25(OH)D could then be expected to correlate with high levels of $1,25(OH)_2D_3$ in the tissues receiving nutrients from this blood supply, including the skin.

Evidence for a role of vitamin D in keloid and hypertrophic scar formation was reported by Zhang et al,[65] who examined the relationship using human keloid tissue from a Chinese population. They observed 4 times the vitamin D receptor (VDR) mRNA and more than 2 times the VDR protein expression in fibroblasts from keloids compared with fibroblasts from normal skin. VDR was functional in the cell, as demonstrated by expression of a reporter gene with a VDR response element that was transfected into cells. Incubation with $1,25(OH)_2D_3$ resulted in increased VDR expression, significantly more in fibroblasts from keloids than fibroblasts from normal skin. Perhaps most importantly, $1,25(OH)_2D_3$ inhibited proliferation of keloid fibroblasts without causing cytotoxicity and mediated its effect on TGF-β and MMPs because $1,25(OH)_2D_3$ inhibited TGF-β-induced ECM production and increased MMP-9 activity. Additionally, $1,25(OH)_2D_3$ induced expression of hepatocyte growth factor, an antifibrotic cytokine implicated in the prevention of fibrosis in keloids.

The $1,25(OH)_2D_3$ exerts its effects by binding to the cytoplasmic VDR and translocating to the nucleus to act as a transcription factor.[64] Specific polymorphisms in the DNA encoding the VDR protein have been correlated with human disease, and correlations between certain VDR polymorphisms and keloid formation have been reported.[66] In a Chinese cohort of 261 patients with keloids and 261 controls, the odds of having keloids in patients possessing the TaqI CC genotype compared with the most predominant genotype, TC, approached

but did not reach statistical significance [odds ratio (OR) = 1.280; P = 0.051].[67] However, an increased OR for having keloids was reported for patients possessing the germline TaqI CC genotype compared with the TT genotype (OR = 3.334; 95% confidence interval = 2.042–5.276). When males and females were examined separately, the statistically significant OR remained only for females.[67] The investigators also reported a statistically significant decrease in serum $1,25(OH)_2D_3$ for patients with keloids compared with controls and also in patients possessing the CC genotype.

These findings suggest a connection between VDR polymorphisms, vitamin D status, and keloid scar formation. The proclivity for keloid scar formation in particular racial and ethnic groups may likely be explained by a genetic predisposition combined with environmental factors.[68] To our knowledge, no studies examined the incidence of keloids and hypertrophic scar formation and measured serum 25(OH)D to assess vitamin D status.[69] Additionally, investigating vitamin D metabolism and keloid scar formation in patients with Fitzpatrick skin types IV and V, in whom the propensity for keloid formation and vitamin D insufficiency is increased, has not been done. Connecting epidemiologic and clinical data with tissue analysis including correlations between VDR polymorphisms and tissue expression of VDR also remains a goal and is suggested as a next step.

HMGB-1, MMPs, AND VITAMIN D IN KELOID AND HYPERTROPHIC SCAR PATHOGENESIS

The direct role of HMGB-1, MMPs, and vitamin D in keloids and hypertrophic scars has yet to be elucidated, and a relationship between these 3 factors in the pathogenesis of these conditions henceforth remains unclear. However, findings from current literature suggest a possible interaction between MMPs and vitamin D that could influence scar formation. The aforementioned study by Zhang et al[65] found that $1,25(OH)_2D_3$ inhibited TGF-β-induced ECM production, increased MMP-9 activity, and increased expression of hepatocyte growth factor. $1,25(OH)_2D_3$ may, therefore, exert an antifibrotic effect through a mechanism partially mediated by MMPs. Vitamin D insufficiency would then increase the propensity for excessive fibrosis (Fig. 2). The involvement of HMGB-1 is more obscure. A study by Kao et al[70] found that HMGB-1 drove in vitro hepatic stellate cells toward fibrogenesis and suppressed the activity of MMP-2 (but not MMP-9). These results coincide with the earlier discussion of HMGB-1 as
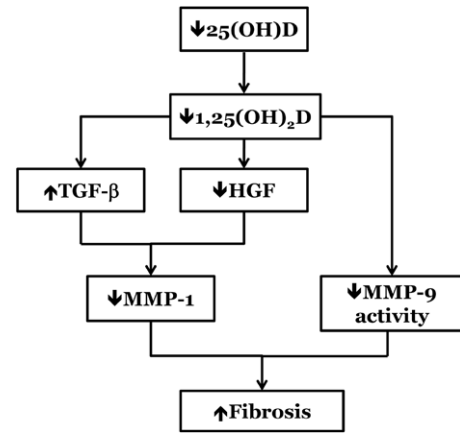
Fig. 2. Vitamin D insufficiency may increase one's propensity for excessive fibrosis. This is due to the ability of $1,25(OH)_2D_3$ to exert a possible antifibrotic effect partially mediated by MMPs. TGF-β was found to decrease MMP-1 production in human keloids,[25] and $1,25(OH)_2D$ was found to inhibit TGF-β-induced ECM production.[65] $1,25(OH)_2D_3$ was also shown to induce expression of HGF,[65] an antifibrotic cytokine that increases expression of MMP-1, MMP-2, and MMP-3 in human keloid fibroblasts/keloid tissue (MMP-2 and MMP-3 not shown).[28] Finally, $1,25(OH)_2D_3$ increased MMP-9 activity.[65] MMP-9 levels were high in the postinjured skin tissue of athymic nude mice, whose skin heals without scar formation.[44] Vitamin D insufficiency (indicated by low 25(OH)D levels) would result in low $1,25(OH)_2D_3$, which would increase TGF-β, decrease HGF, and decrease MMP-9 activity. Increased TGF-β and decreased HGF would result in decreased MMP-1, which, along with decreased MMP-9 activity, would result in increased fibrosis. HGF indicates hepatocyte growth factor.

a profibrotic mediator, but they somewhat conflict with the results of a study by Limana et al,[71] who found that HMGB-1, when injected into the failing, infarcted hearts of mice, reduced collagen deposition, increased MMP-2 and MMP-9 activity, and decreased TIMP-3 levels. It is possible that HMGB-1 has different effects on fibrosis and that these effects are location- and/or organ-dependent. Additional studies investigating the role of HMGB-1 in skin fibrosis and MMP activity are needed.

CONCLUSION

Keloids and hypertrophic scars are not only the cosmetic problems; these conditions can cause pain, pruritis and contractures. HMGB-1 promotes wound healing by inducing the proliferation and migration of fibroblasts. The role of HMGB-1 in keloid and hypertrophic scar formation warrants further investigation. If HMGB-1 is increased in these fibrotic conditions, perhaps this could be a target for developing novel HMGB-1 inhibitors for the prevention and/or reversal of keloid formation and hypertrophic scar. Changes in the expression and activity of several MMPs associate

with the pathophysiology of keloids and hypertrophic scars. It is possible that MMPs are decreased in the center of keloids and increased in the periphery. Future studies are needed for clarification and may provide new opportunities for therapeutic intervention. The increased incidence of keloid formation together with vitamin D insufficiency/deficiency in darker skin subjects suggests that vitamin D may also play a role in the development of keloids and hypertrophic scars. Vitamin D exerts anti-proliferative effect on keloid fibroblasts, and connections between specific VDR polymorphisms, specifically the TaqI CC genotype and keloid formation have been found. Although findings from several studies point to an association between VDR polymorphisms, vitamin D levels, and keloid formation, obviously additional studies are warranted to define this relationship, especially in patients with darker skin, namely Fitzpatrick skin types IV and V.

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