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REVIEW

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The Molecular Mechanisms Involved in the Hypertrophic Scars Post-Burn Injury

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Scar formation is a normal response to skin injuries. During the scar-remodeling phase, scar tissue is usually replaced with normal, functional tissue. However, after deep burn injuries, the scar tissue may persist and lead to contractures around joints, a condition known as hypertrophic scar tissue. Unfortunately, current treatment options for hypertrophic scars, such as surgery and pressure garments, often fail to prevent their reappearance. One of the primary challenges in treating hypertrophic scars is a lack of knowledge about the molecular mechanisms underlying their formation. In this review, we critically analyze studies that have attempted to uncover the molecular mechanisms behind hypertrophic scar formation after severe burn injuries, as well as clinical trials conducted to treat post-burn hypertrophic scars. We found that most clinical trials used pressure garments, laser treatments, steroids, and proliferative inhibitors for hypertrophic scars, with outcomes measured using subjective scar scales. However, fundamental research using human burn injury biopsies has shown that pathways such as Transforming Growth factor β (TGFβ), Phosphatase and tensin homolog (PTEN), and Toll-like receptors (TLRs) could be potentially regulated to reduce scarring. Therefore, we conclude that more testing is necessary to determine the efficacy of these molecular targets in reducing hypertrophic scarring. Specifically, double-blinded clinical trials are needed, where the outcomes can be measured with more robust quantitative molecular parameters.

INTRODUCTION

Burn injuries and the post-burn sequelae is a global problem which leads to increased mortality [1,2]. In the people that survive severe deep burn injuries, the post-burn scar formation persists for a lifetime. The scar tissue can be in the form of hypertrophic scars or contractures across the joints. Hypertrophic scars are raised scars within the boundaries of the original injury. They are reported to arise due to the damage to the deep dermal elements by the burn injury [3,4]. Superficial second-degree burns generally heal without scarring, but deep second-degree burns as well as third-degree burns cause extensive scarring [5]. The scar tissues are usually devoid of the dermal elements such as sweat glands, sebaceous glands, and hair follicles. This results in a scar, which does not have the characteristics of the native skin. The primary functions of skin include thermoregulation, prevention of internal fluid loss, formation of barrier against microorganisms and pathogens, and protection of delicate internal struc-

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Abbreviations: TGFβ, Transforming Growth factor β; PTEN, Phosphatase and tensin homolog; TLR, Toll-like receptors; ECM, extracellular matrix; αSMA, Smooth Muscle Actin; GSK3, Glycogen synthase kinase-3; HUVEC, Human Umbilical Vein Endothelial Cells; Shh, Sonic hedgehog; ADMSCs, adipose tissue derived mesenchymal stem cells; PGE2, Prostaglandin E2; ATAC, Assay for Transposase-Accessible Chromatin; FOXF2, Forkhead Box F2; MKX, Mohawk Homeobox; PBMCs, Peripheral blood mononuclear cells; PLCL, poly (d,l-lactide-co-ε-caprolactone); ATAC, Assay for Transposase-Accessible Chromatin; qRT-PCR, Quantitative Reverse Transcription Polymerase Chain Reaction.

Keywords: Hypertrophic scars, thermal injury, molecular pathways, skin injury, scarring

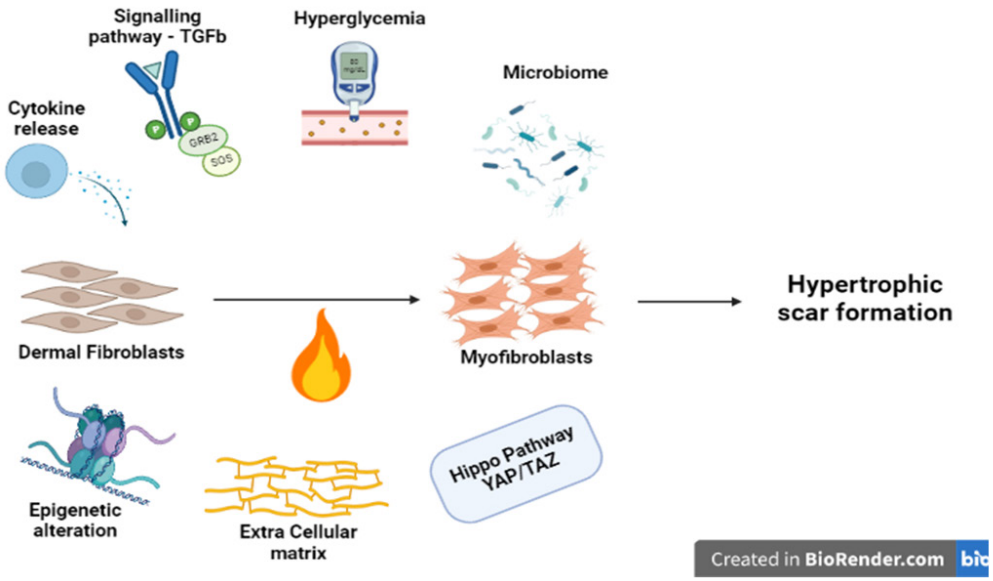

Figure 1. **Various factors that can induce transformation of dermal fibroblasts to persistent hypertrophic scar fibroblasts**. Burn injuries can lead to secretion of inflammatory cytokines such as IL-10 and TGFβ and alter the glucose homeostasis in the body. These along with the trauma injury may alter the activity of epigenetic modulators, lead to excessive extracellular matrix synthesis, dysregulate the mechanobiology and change the original microbiome. Several of these changes together may lead to persistence of hypertrophic scar tissue long after the initial burn trauma injury. The contribution of these and several other aspects needs to be explored.

tures from physical damage [6]. The stem cells residing in the adult hair follicles, sweat and sebaceous glands and epidermis help in re-epithelialization, regeneration of hair, and maintenance of tissue homeostasis [7]. The scar tissue on the other hand is not pliable and lacks the ability to provide an effective barrier against microorganisms due to frequent tissue breakdown and hampered thermoregulation [8,9]. The contractures affect the joint mobility and give rise to deformities and disabilities [10].

It is known that the dermal fibroblasts play a role in the process of wound healing and they have an important role in the formation of hypertrophic scars formation [4,11,12]. There is an upregulation of various proteins like vimentin, collagen, smooth muscle actin, integrin, and heat shock proteins in the hypertrophic scar tissue [11]. Expression of these proteins indicates that extracellular matrix (ECM) proteins are overexpressed in hypertrophic scar tissue, but the molecular mechanisms are not completely understood [13]. Previous reports show hypertrophic scars are prevalent in 32% to 72% [14,15] post-burn injury patients, with the latest report from 2016 [16]. Since the prevalence is reported in percentages, there is variation and it is clear from the epidemiological studies that prevalence rates change with nationality, gender, burn percentage, ethnicity, among others. Another complexity is the definition of hypertrophic scars, since several studies used a visual scar score instead of quantitative blood-based or scan-based diagnostic test; there would be a difference in scars that get classified as hypertrophic scars.

Currently there are a few non-surgical as well as surgical modalities in the armamentarium of a surgeon treating hypertrophic scars. Intralesional steroid injections, pressure therapy, silicone sheet or gel treatment, scar massage, and LASER therapy are some of the non-surgical modalities, which give sub-optimal results [17,18]. The surgical modalities involve scar excision or release followed by replacement of the excised tissue with a skin graft or a flap. Despite these interventions, restoring the skin to native skin post burn is a huge challenge. Mouse models of diseases play a vital role in drug discovery; however, research into hypertrophic scar is handicapped due to lack of a robust animal model. In order to provide better treatment to patients suffering from post-burn hypertrophic scars it is crucial that more research be done on the effect of heat on cell metabolism, intracellular communication via cytokines, mechanobiology, and epigenetic modulators in various cells that are affected (Figure 1).

In this article, we have reviewed articles published on the molecular mechanisms involved in hypertrophic scars and post-burn injury as well as critically evaluated

the clinical trials to reduce hypertrophic scarring.

WOUND HEALING POST BURN

The key stages of wound healing are homeostasis, inflammation, proliferation, and tissue remodeling [19]. The clotting cascade is activated due to any injury and there is initiation of mechanisms to control the bleeding by homeostasis, which leads to a provisional matrix formation. The inflammatory phase proceeds and the wound matrix serves as a scaffold for the migration of various cell types like neutrophils, monocytes and their differentiated form (macrophages), keratinocytes, fibroblasts, and endothelial cells. The next stage of proliferation includes angiogenesis, re-epithelialization, and formation of granulation tissue. The provisional wound matrix is gradually replaced by granulation tissue. Re-epithelialization is brought about by the epithelial stem cells from the skin appendages like sweat glands and hair follicles [20]. The myofibroblasts play an important role in the wound contraction [21]. As the wound heals further, it undergoes a process of remodeling wherein the type III collagen is replaced with the type I collagen that imparts strength to the scar. This entire process is regulated by the interplay of various pathways and any aberration may tilt the delicate balance in favor of the formation of hypertrophic scars or keloids, which are a burden of disease to society.

The depth of the burn injury determines the healing process. In a first-degree burn injury, the basal layer of the epidermis is intact and re-epithelialization occurs over 3-4 days. In second-degree burns, the epidermis is destroyed with different levels of injury to the dermis. The healing takes place depending upon the presence of dermal elements. The re-epithelialization occurs from the epithelial cells lining the dermal hair follicles, sweat glands, and sebaceous glands [4]. The basal keratinocytes are stimulated to migrate into the wound when they encounter the proteins such as fibrin, fibronectin, and Type I collagen [22]. Usually, the second-degree superficial burns do not have scarring. The second-degree deep burns have a lesser number of dermal elements and hence leads to increased scarring. The third-degree burns have loss of dermis as well. These wounds heal by wound contraction and hence more scarring. When the damage to dermis is extensive, there is conversion of dermal fibroblasts to aSMA expressing myofibroblasts and these myofibroblasts lead to contractures [23].

TREATMENT OF HYPERTROPHIC SCAR FORMED AFTER BURN INJURY

For most diseases where conventional therapy is used, animal models such as transgenic mice or rats are used to understand basic molecular mechanisms. However, the field of hypertrophic scar research is hampered due to lack of a widely acceptable animal model due to difference in wound healing between humans and rodents or pigs [24]. Therefore, treatments originated from observations and expertise of clinicians, and not all of them went through the clinical trial path.

The post-burn injury hypertrophic scars are treated currently via surgery, steroid injections, and pressure garment therapy [25,26]. Pressure garments have been partially successful, but surgery alone or corticosteroid injections alone have not been successful [17]. There are several clinical trials underway world over to find new potential treatments for hypertrophic scar formation. Table 1 lists the completed clinical trials registered under clinicaltrials.gov in last 5 years (2017-2022) where we used “hypertrophic scar post burn injury” in search criteria [27]. It is evident from the table that several trials are not addressing the molecular mechanisms that cause hypertrophic scars formation post-burn injury. Moreover, even the trials, which are for post-burn hypertrophic scars, are attempting either pressure garment, steroids, inhibitors, or laser therapy options. No treatment options can differentiate a normal scar tissue or fibroblasts from hypertrophic scar tissue fibroblasts. Thus, the ongoing trials are not very different from current treatment standards. In a country like India, there are only five clinical trials registered with clinical trials registry of India [28]. For a large country like India, which have high incidences of burn related injuries, the number of clinical trials on hypertrophic scars are shockingly low. Burn-related physiological issues seem to be a neglected area among clinicians, major government research organizations, and funding agencies.

MOLECULAR MECHANISMS IN HYPERTROPHIC SCAR FORMATION

In hypertrophic scars the, smooth muscle actin (αSMA) protein leads to formation of stress fibers and contraction of cells, this activation can be triggered due to various signaling pathways such as transforming growth factor β (TGFβ), Notch, WNT, and Hippo signaling pathways. Hypertrophic and keloid scars express higher levels WNT pathway genes such as Glycogen synthase kinase-3 alpha (GSK3α), WISP2, and WNT3a than normal skin on tissue microarray [29]. Activin A and Folistatin receptors have been shown to be expressed on surface of hypertrophic scar tissue derived fibroblasts, but while Activin A promotes cell proliferation, Folistatin suppresses collagen gene expression [30]. USP15 protein enhances the proteasome-mediated degradation of proteins. Hypertrophic tissue derived fibroblasts showed higher expression of USP15, TGFβ1, Smad2, Smad3, αSMA, collagen 13, and collagen1 compared to normal skin. However,

Table 1. List of Clinical Trials Registered with www.clinicaltrials.gov for Hypertrophic Scars. The table lists the clinical trials along with the significant observations from the completed trials

Sr no	Experimental System	Scar type	Significant observations / results and critical observation	Outcome	Ref no/ Clinical Trial No
1	Treatment of Hypertrophic Scars Using Fractional Laser and Fractional Laser-assisted Topical Corticosteroid Delivery	No post-burn hypertrophic scar. Abdominal surgery induced hypertrophic scar were studied	Hypertrophic scars taken from women undergoing abdominal surgery. The hypertrophic scar treated using 0.05% Clobetasol propionate and 2940 nm laser. Skin thickness measured by caliper post laser assisted corticosteroid therapy. No results given. Hypertrophic scar derived post-burn injury not involved in the study.	No results provided hence outcomes cannot be determined	NCT02487212
2	Effect of cryotherapy on hypertrophic scars	Post-burn scar tissue	Experimental group (post-burn injury) patients were given -14°C for 10 mins for 10 weeks with 2 sessions per week. However, no results provided.	No results provided hence outcomes cannot be determined	NCT04532840
3	A Randomized Comparative Study Evaluating the Tolerability and Efficacy of Two Topical Therapies for the Treatment of Keloids and Hypertrophic Scars	Keloid and hypertrophic scar tissue. Not clear if the data was classified based on scar type and how it was generated	Onion gel extract used for hypertrophic scars. Assessment of pain, tenderness, pruritis and scar thickness measured post application of onion gel extract. No statistical difference was reported between control and onion extract group on hypertrophic scars.	Onion gel is effective in controlling post-burn complications such as pain, tenderness and itching	NCT00754247
4	Effectiveness of pressure garment therapy after burn injury	Post-burn hypertrophic scar tissue	The pressure garments to be worn for 23 hours a day with a pressure of 17-24 mm Hg. The trial showed that pressure garments show improvements in terms of softer and thinner skin, however no data on pruritis available.	Pressure garments reduce the skin thickening and allows it to retain pliability	NCT01005732 [70]
5	Safety and efficacy study of PF-06473871 to reduce hypertrophic scars from a recurring post revision surgery	Surgically induced hypertrophic scar tissue	Post-surgical breast scars were considered for the trial and were administered anti sense nucleotide preparation PF-06473871 The trial showed some improvement over placebo. No burn injury generated hypertrophic scar has been screened.	Anti-sense oligonucleotides could be used to treat surgical induced hypertrophic scar tissue	NCT01730339
6	Pain outcomes following intralesional corticosteroid injections	Study included keloids and other scars apart from hypertrophic scars	The outcomes measured by visual analog scale. Surprisingly, corticosteroid with normal saline showed less pain that patients who received corticosteroid and lidocaine. No burn injury scar assessed.	Intralesional corticosteroid injection may be used for reducing pain	NCT03630198

7	Safety and efficacy of EXC001 in subjects who have participated in prior studies of EXC001.	Surgically induced hypertrophic scar tissue	The effect of EXC001 assessed in scar revision patients and not done on burn injury patients. The intradermal injections of EXC001 did not show major improvements in terms of pain, stiffness and color of the scar tissue.	Study may have to be performed with larger number of participants before EXC001 could be used clinically to treat surgical induced hypertrophic scar tissue	NCT01494922
8	Efficacy of pulsed dye laser (PDL) and carbon dioxide (CO_2) laser in conjunction with usual medical care treatment for hypertrophic scars post-burn injury to determine optimal sequence and timing of laser	Post-burn hypertrophic scar tissue	Since the study was withdrawn no data is available	Not applicable	NCT02655211
9	Compare global scar outcomes in those treated with silicone only therapy (SOT) versus silicone pressure garment therapy (SPGT) to prevent hypertrophic scarring in children post traumatic skin injury	Trauma (non-burn) injury induced hypertrophic scar tissue	Observational study where outcomes measured by Vancouver scar scale with 12 randomized patients. Silicone therapy (SOT) to be applied for 23 hours a day per patient for 6 months. SPGT where silicone was applied only for 2 weeks followed by pressure garments with silicone underneath. Patient's perspective on color, pliability, thickness, itching and pain or by caregiver was considered. No data available.	No results provided hence outcomes cannot be determined	NCT01602458
10	Prospective Clinical Trials on Skin Wound Healing in Young and Aged Individuals (RESOLVE)	Not applicable	No data available. The publications emerged from this trial include data from 1999 even though the trial was registered in 2009.	Not applicable	NCT010140104

11	Investigation Into the Scar Reduction Potential of Prevascar (Interleukin-10)	Surgically induced hypertrophic scar tissue	Hypertrophic scar generated via surgical procedure in the upper arm. One arm administered with Prevascar while other arm acted as control. Group 1 participants received Prevascar in the range from 5-1000ng/100μL while group 2 participants received 25-2000ng/100μL dose. The Prevascar was to be injected 24 hours post-surgical injury via intradermal route. The design of the trial was to compare scar reduction in Prevascar treated participants compared to placebo; however, the measurement scale or assay is not available. The publication generated from data given in the clinicaltrials.gov contains data obtained from in vitro mouse studies and does not contain any human clinical data.	No results provided hence outcomes cannot be determined	NCT00984646 [72]
12	The Effect of Intralesian Injection of Umbilical Cord Mesenchymal Stem Cells, Its Conditioned Medium, and Triamcinolone Acetonide on Type 1:3 collagen Ratio and Interleukin-10 Levels in Keloid: A Randomized Controlled Trial	Keloid scar tissue post-surgery and hypertrophic scar tissue and exclusion criteria	The clinical trial was undertaken to study the effect of Intralesian Injection of Umbilical Cord Mesenchymal Stem Cells, Its Conditioned Medium, and Triamcinolone Acetonide on keloid scar tissue. Keloid tissue biopsies were done to measure IL-10 levels, Sirius staining to measure collagen level post administration of umbilical cord mesenchymal stem cells, conditioned media and Triamcinolone Acetonide injected into the keloid scar. Although the study has been completed, no results were available on clincialtrials.gov.	No results provided hence outcomes cannot be determined	NCT05939817

expression of USP15 or suppression by shRNA against USP15 did not significantly affect the proliferation or collagen deposition in hypertrophic scars, contrary to the authors claim [31].

Macrophages play a crucial role on remodeling the hypertrophic scar tissue. In a study done using RBP-J mice, it was shown that Notch signaling regulates expression of Smads, αSMA and collagen along with inflammatory cytokines. Inhibiting Notch signaling in macrophages in RBP-J mice resulted in better wound healing and reduced liver and kidney fibrosis [32]. Notch signaling pathway controls the expression of Smads, αSMA, and collagen to some extent in the fibroblasts derived from hypertrophic scar tissue. However, the hypertrophic scars generated were not due to burn injuries. There are reports on use of traditional Chinese remedies for hypertrophic scars. Oxymatrine derived from the *Sophora flavescens* was shown to provide some benefits in treating hypertrophic scars. However, the effect of oxymatrine was assessed on human umbilical vein endothelial cells (HUVEC) and not cells from the hypertrophic scar tissue [33]. Sonic hedgehog (Shh) signaling pathway is a crucial pathway for regulating development and proliferation of skin tissue and mutations in Shh pathway components can lead to basal cell carcinomas [34-36]. Thus, it might be important if burn injury alters the Shh signaling pathway in dermal fibroblasts and might also serve as therapeutic target.

Overactive Ca^{+2} activated potassium channels leads to severe fibrosis and so a selective inhibitor of the channel can reduce the fibrosis. Fibroblasts derived from hypertrophic scars due to burn injury express higher amounts of collagen I, collagen III, αSMA, and TGFβ1 compared to normal skin. Hypertrophic scar fibroblasts when treated with TRAM-34 (channel inhibitor) reduced the amount of fibronectin, collagen I, collagen III, vimentin, αSMA, and TGFβ1 [37]. However, how the inhibition of potassium channel affects production of ECM proteins, αSMA, and TGFβ1 needs to be investigated to design more specific inhibitors to treat hypertrophic scars. Ears were surgically injured in rats to generate hypertrophic scars. Growth hormone releasing peptide 6 (GHRP6) was topically applied to hypertrophic scars. Ultrasonography revealed reduced hypertrophic scar extent in GHRP6 treated rats compared to vehicle treated rats [38]. It would be interesting to explore the mechanism of inhibitory effects of GHRP6 on hypertrophic scar tissue formed after burn injury. FTY720, a metabolite derived from entomopathogenic fungus *Isaria sinclarii,* was shown to cause cell death of hypertrophic scar fibroblasts while not affecting normal fibroblasts [39]. It remains to be seen whether these results seen in rat hypertrophic scars can be replicated with human hypertrophic scars formed due to burn injury.

Mouse model of burn injury showed that when 100μg BMP7 was applied to post-thermal injury there was decrease in collagen I and collagen III protein level compared to the thermal injury group. However, contrary to the claim of the authors αSMA levels did seem to vary between control and BMP7 treated animals. Also, it was not clear if the dosage of BMP that administered subcutaneously was as per tissue injury or animal weight [40]. Another member of the TGFβ family, Activin B, was found to be differentially expressed between normal and scar fibroblasts obtained from patients undergoing surgery to remove the bile duct. Authors report several other proteins, which were differentially expressed to higher magnitude than Activin B was but were not tested further [41]. This study showed that like previous reports the TGFβ protein family is important in proliferation of scar fibroblasts. Prostaglandin E2 (PGE2) addition *in vitro* to hypertrophic scar fibroblasts showed reduced TGFβ1 cells as well as reduced collagen 1 transcript. It remains to be investigated if PGE2 can also reduce TGFβ1 levels in burn injury derived hypertrophic scar fibroblasts [42]. Hypertrophic scar fibroblasts post severe burn show higher levels of phospho AKT compared to normal skin; overexpression of PTEN led to reduction in levels of phospho AKT [43].

Hypertrophic scars from human tissue were studied for expression of miR101 and EZH2; however, it is not clear what type of injury led to the formation of hypertrophic scar tissue. Inhibition of miR101 using specific chemicals led to higher expression of αSMA, collagen III, and collagen I. Western blot results showed that suppression of EZH2 by siRNA-reduced αSMA, collagen III, and collagen I. It is not completely clear how inhibition of EZH2 leads to reduced αSMA expression and the consequence on hypertrophic scar fibroblasts [44]. EZH2 protein levels could be targeted in fibroblasts of hypertrophic scar tissue to reduce the proliferation of myofibroblasts.

HYPERTROPHIC SCARS: UNRESOLVED ISSUES

Burn injuries that affect the dermal fibroblasts and underlying adipose tissue can result in formation of hypertrophic scars and contractures. The structure, function, and metabolism of keratinocytes, melanocytes, reticular fibroblasts, papillary fibroblasts, hair follicles, and cells of sebaceous glands is affected due to stress, such as burn injury. A search of the literature shows that most published works are review articles or articles on managing scar tissue via LASER treatment, pressure garments, and or grafts. There is a dearth of published work on understanding the mechanisms of hypertrophic scarring that persists several years post-traumatic burn injury, in absence of such data the options for treating hypertrophic

Table 2. List of major studies published from 2013 to 2023 in PubMed and through their results have attempted to decipher the molecular mechanisms of hypertrophic scar formation and persistence

Sr no	Experimental System	Significant results and critical observations	Scar tissue type used	Study endpoint / Assays performed	Ref
1	P75NTR inhibition in human hypertrophic scar tissue	Autophagy factor LC3B II is shown higher in normal while its low in scar tissue fibroblasts 3 and 6 month after burn injury. P75NTR could be a good target to reduce αSMA, collagen I and III protein levels.	Human Post-burn injury hypertrophic scar tissue	*In vitro* endpoint assays - western blot for αSMA, collagen I and III	[52]
2	Effect of tetramethylpyrazine on hypertrophic scar tissue	Tetramethylpyrazine treated hypertrophic scar fibroblasts led to reduced αSMA, collagen I and III protein levels. Higher than 40μM concentration of tetramethylpyrazine lead to cell death. It is not clear if Tetramethylpyrazine can act only on the hypertrophic scar tissue fibroblasts since a normal fibroblasts control was not included. The study did not involve burn injury hypertrophic scar tissue.	Hypertrophic scar tissue obtained from several patients. Authors have not reported whether scar was induced by burn injury or surgery	*In vitro* endpoint assays - protein levels of αSMA, collagen I and III	[69]
3	Role of Notch signaling in wound healing	The study was conducted in RBP-J mice. The results showed that Notch signaling controls expression of Smads, αSMA, collagen I and III to some extent as well as inflammatory cytokines. However, no burn injury was done to generate hypertrophic scars.	No post-burn hypertrophic scar used either from mice or humans	*In vivo* in transgenic mice study with end points being western blot and qRT PCR.	[36]
4	Role of Oxymatrine (OMT) in hypertrophic scar repair	OMT is active ingredient of *Sophora flavescens.* Results do not show change in LC3BII/ LC3BI ratio which is indicative of autophagy. IHC results comparing between groups for various proteins such as p63, αSMA, TGFβ seem from different tissue blocks, hence one cannot easily compare expression of various proteins.	Human fibroblasts used however it is not known if the tissues used were from post-burn injury	*In vitro* end point assay – western blot and Immunohistochemistry	[33]

5	Effect of TRAM-34 in post-burn hypertrophic scar formation	TRAM-34 is an inhibitor of calcium activated potassium channel. TRAM-34 treatment resulted in some reduction in Fibronectin, collagen I, collagen III, Vimentin, TGFβ, αSMA levels in hypertrophic scars derived fibroblasts. Human scar fibroblasts post-burn injury used along with mouse fibroblasts for *in vivo* work.	Normal skin tissue from human obtained from split thickness skin grafting procedure and corresponding burn injury tissue. Mice tissue were also used.	*In vitro* end points – Cell proliferation assay, qRT PCR and western blot. *In vivo* endpoint - histopathology	[37]
6	Effect of P144 on hypertrophic scar model in nude mice	Hypertrophic scars from 30 human samples were transplanted into nude mice and P144 was gel was administered topically daily. Qualitatively moderate reduction in collagen I and III levels in P144 treated mice compared to placebo group.	Hypertrophic scar tissue obtained from human but not apparent if scar was generated due to burn injury	*In vivo* assay where human fibroblasts were transplanted into mice followed by immunohistochemistry assay for collagen protein	[46]
7	Effect of Growth hormone releasing peptide 6 on hypertrophic scarring	Hypertrophic scar generated in rats by ear injury. Hypertrophic scars between control, Growth hormone releasing peptide 6 (GHRP6) and Triamcinolone acetonide (TA) treated rats was done using ultrasonography. The percentage of hypertrophic scar reduced in GHRP6 treated rats. However, it's not clear how GHRP6 exerted its action.	Hypertrophic scar created surgically in rabbits and not due to burn injury	*In vivo* study that used ultrasonography, histopathology proteomic analysis on the hypertrophic scar tissue	[38]
8	Effect of FTY720 on hypertrophic scars	FTY720 is an metabolite isolated from entomopathogenic fungus. Rabbit model used for generation of hypertrophic scars. FTY720 reduced the cell viability of hypertrophic scar fibroblasts but not of normal fibroblasts. However, flow cytometry data shows almost 33% of 20μM FTY720 treated cells underwent cell death.	Normal skin and hypertrophic skin tissue obtained from human subjects, but no mention if the scar was generated due to burn injury	*In vitro* end points – Cell proliferation assay, qRT PCR and western blot. *In vivo* endpoint - histopathology	[39]
9	Toll like receptors role in hypertrophic scar	TLR7 and 4 were expressed more in hypertrophic scar fibroblasts compared to normal skin fibroblasts. This study dhows TLR expression in human burn tissue between 0 to 14 days. The results are important for understanding role of TLR in early wound healing, in absence of data on TLR expression in mature hypertrophic scar, it cannot be proved if TLR regulate hypertrophic scar formation.	Normal skin tissue collected after abdominoplasty and burn tissue collected 14 days after burn injury. No hypertrophic scar tissue	*In vitro* endpoint assay – Expression of TLRs	[70]

10	Role of PTEN in hypertrophic scar tissue	Phosphor AKT levels reported higher in hypertrophic scar derived fibroblasts than those in normal skin fibroblasts. Interestingly, PTEN overexpression reduced levels of phospho AKT in hypertrophic scar fibroblasts. Burn injury may lead to overexpression of PTEN in hypertrophic scar tissue and should be investigated.	Fibroblasts taken from scar tissue formed after severe burn injury	*In vitro* endpoint assays – Western blot, Immunohistochemistry and qRT PCR	[43]
11	Role of mast cell chymase in promoting hypertrophic scar	Immunohistochemistry data showed slightly more CD117 expressing mast cells in hypertrophic scar tissue than normal tissue. Interestingly mast cell chymase was added to hypertrophic scar fibroblasts *in vitro* that led to some increase in mRNA for collagen I, III, Angiotension, TGFβ1 at 12 hrs. Same treatment was not done to normal tissue to establish mast cell chymase only acts on hypertrophic scar fibroblasts. Western blot data pSMA2/3, Smad7, TGFβ1, did not show major variation at different time points for different concentrations of mast cell chymase.	Hypertrophic scars taken between 6 months to 2 years post-burn injury	*In vitro* endpoint assay - Western blot and qRT PCR	[71]

scars are superficial (Table 2).

Burn injuries can lead to significant growth stunting several years after the injury [10]; however, hypertrophic scars persisting after years post injury are not reported. Most of the reports hypertrophic scars generated post-burn injury are from animal models such as rats, rabbit, porcine, and mice or from surgically generated scar tissue in humans [31,38,39,45,46]. Comparison of gene expression profile between normal human fibroblasts and mice post-burn injury has shown that genomic responses are not reproduced in current animal models [47].

ROLE OF INFLAMMATION IN BURN INJURY INDUCED HYPERTROPHIC SCAR

Aberrant overexpression of interleukin-14 (IL-14) and interleukin-13 (IL-13) was reported to contribute to the initiation and continuation of fibrotic skin disease, but it is not known if the same interleukins also contribute to hypertrophic scar formation [48]. Interleukin-10 (IL-10) null mice have been shown to display better wound closure compared to wild type mice, hence this indicated that IL-10 may have a direct role in wound healing [49]. IL-10 overexpressing adipose tissue derived mesenchymal stem cells (ADMSCs) have been reported to prevent hypertrophic scar formation in rabbit models. However, 3 days post-transplantation the IL-10 overexpressing ADMSCs showed reduced levels of αSMA, collagen, and fibronectin, even though the IL-10 secreted generated by ADMSCs had reduced [50]. Fibroblasts derived from hypertrophic scar tissue when treated with LY294002 (PI3K/Akt Inhibitor) had increased expression of collagen I, III, and smooth muscle actin (SMA) even in the presence of IL-10, thus showing that IL-10 inhibits fibrosis associated genes via the PI3k/Akt pathway [51]. The same group recently showed that when hypertrophic scar derived fibroblasts were treated with lipopolysaccharide (LPS) it led to increased expression of collagen I, collagen III, TLR4, αSMA, but when these cells were treated with IL-10 there was some reduction in these markers [52]. In mice, scar formation was induced by performing full thickness excision surgery and IL-17 was injected into these mice subcutaneously. IL-17 led to increased expression of collagen I as compared to control mice and addition of an IL-17 inhibitor reduced the collagen I level. Thus, showing that IL-17 plays a role in promoting fibro-

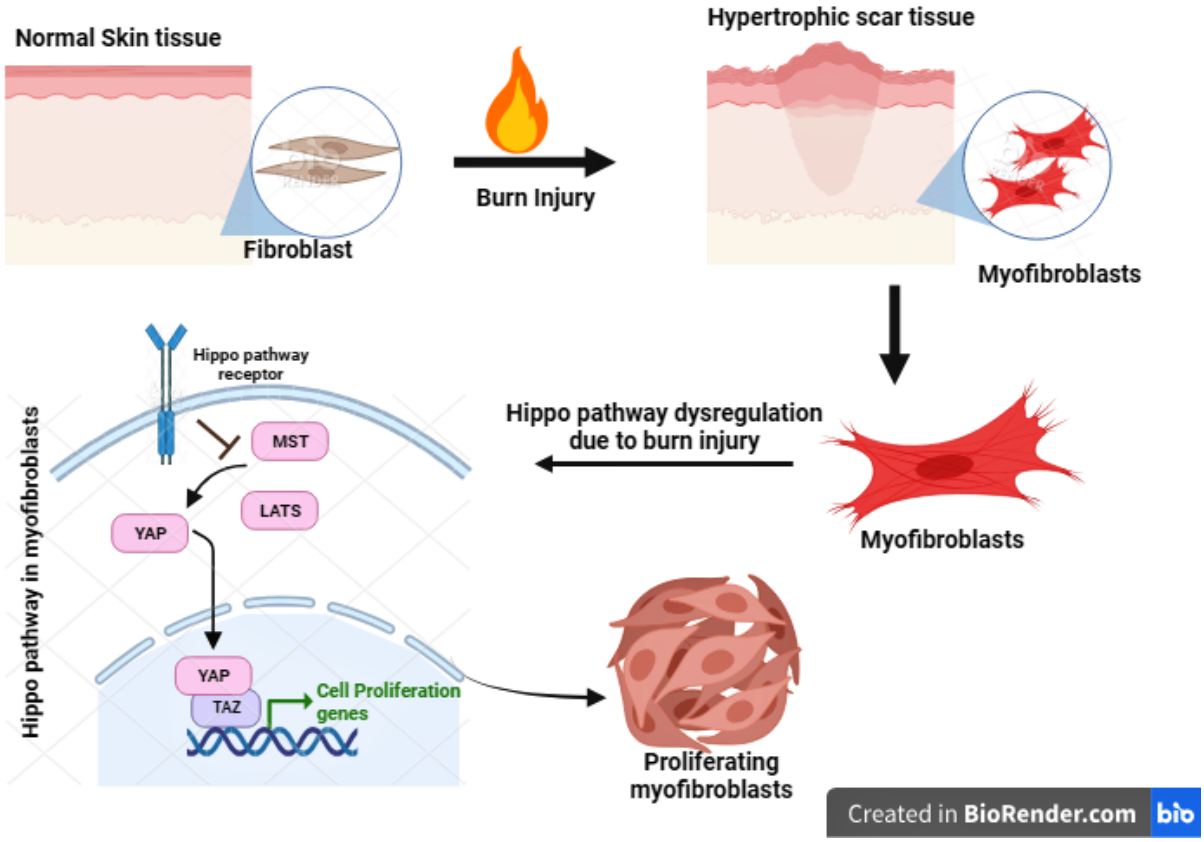

Figure 2. **Effect of Burn injury on Hippo signaling pathway in normal skin tissue**. Second-degree deep burn injuries lead to formation of hypertrophic scars. The burn injury leads to conversion of fibroblasts to myofibroblasts. Within these myofibroblasts, there is dysregulation of Hipposignaling pathway, which does not lead to phosphorylation of MST and LATS proteins, due to non-phosphorylation of these upstream proteins, the YAP protein remains unphosphorylated, and the unphosphorylated YAP enters the nucleus, which leads to activation of cell proliferation genes. The net result is excessive proliferation of myofibroblasts.

sis via increased collagen production [53]. For detailed information on role on IL-10 in organ or tissue fibrosis please refer excellent review article previously published [54]. Hence, it is clear that interleukins, especially IL-10, play an important role in regulating the activity of fibroblasts and thus it can potentially be targeted to control hypertrophic scar formation.

SINGLE CELL SEQUENCING TO UNDERSTAND HYPERTROPHIC SCAR TISSUE

ATAC sequencing (Assay for Transposase-Accessible Chromatin using sequencing) was performed using peripheral blood mononuclear cells (PBMCs) after 4 hours, 24 hours, and 72 hours post-burn injury and was compared to PBMCs from people without burn injury. This study identified a potential epigenetic signature and a transcriptome pattern that can be used for prognosis of burn injury [55]. In an interesting study, from amongst the hundreds of differentially expressed genes and methylated promoters, the most significantly differentially methylated and expressed were *FOXF2* and *MKX*. The scar fibroblasts showed elevated *FOXF2* and its knockdown significantly decreased collagen production [56]. Single cell RNA seq data has identified the specific gene regulation in fibroblasts and keratinocytes in hypertrophic scar tissue compared to normal skin tissue. Curiously, keratin 14 and 5 that are markedly expressed in the basal layer of the stratified layer were highly expressed in hypertrophic keratinocytes compared to normal keratinocytes. The hypertrophic fibroblasts were enriched with transcripts involved in ECM organization, collagen formation, MMPs, epithelial mesenchymal transition (EMT), among others [57].

ROLE OF MITOCHONDRIA IN HYPERTROPHIC SCARS

Using elescomal, an ionophore that targets mito-

chondria, it was shown that fibroblasts collected from circumcision, scar removal surgeries underwent apoptosis. Elescomal activated caspase 3, which led to apoptosis of fibroblasts *in vitro* [58]. Another group used lycorine to test its ability to induce apoptosis in both *in vivo* rabbit ear model and *in vitro* model using human cells. The lycorine treatment led to increased expression of pro apoptotic proteins Bad, Bax, and PARP while reduced the expression of collagen I, III, and αSMA [59]. However, the mechanism by which lycorine only induces apoptosis in scar tissue cells but not the normal cells needs to be explored further. There is dearth of studies on the role of mitochondria in continued proliferation of hypertrophic scar fibroblasts post thermal injury.

EPIGENETIC CHANGES DUE TO BURN INJURY

HUVEC cells overexpressing a long non-coding RNA (linc00174) showed better angiogenesis and proliferation after the cells were exposed to conditions *in vitro* that model burn injury. Several critical transcription factors such as *RUNX1, VEGFA*, and *ZNF24* among others are regulated by H3K27me3 mark, which is catalyzed by a PcG protein EZH2. Bioinformatics analysis showed that the long non-coding RNA linc00174 may regulate EZH2 expression, and so linc00174 overexpressing HUVEC cells exhibited lower H3K27me3 mark at key angiogenic genes [60]. A study found that burn tissue exhibits lower methylation of repetitive elements such as *Alu* compared to their level in normal skin tissue [61]. Using rat models, it was shown that scald injury altered the histone H3 phosphorylation at serine 10; this post translational modifications allows expression of several gene associated with burn injury and the levels of phosphorylated serine 10 increases on histone H3 in specific neurons leading to heat hyperalgesia [62]. Sprague Dawley (SD) rats with 50% total body surface area scald burns showed reduced H3K9 acetylation, elevated VEGF, and vascular permeability. Intraperitoneal injection of valproic acid into these rats partially reversed the effect on H3K9 acetylation [63]. It is clear that much needs to be understood how epigenetic modulators change in response to thermal injury and whether epigenetic modulators drive the persistence of the hypertrophic scar tissue.

ROLE OF BIOMATERIALS FOR TREATING HYPERTROPHIC SCARS

Decorin regulates the collagen fibril assembly and hence a PCL gelatin scaffold was generated to release recombinant decorin. Authors showed that decorin nanofibers targeted myofibroblasts *in vitro* [64]. In a recent study [65], microneedles were used to locally inject corticosteroid to establish proof of concept. However, authors did not show reduced Patient and Observer Scar Assessment Scale score but this strategy could be tested in larger number of patients. Hydrogels containing γ-glutamic acid showed good cell adhesion but slowed the proliferation of fibroblasts and such hydrogels could be implanted in hypertrophic scar tissue to reduce the fibroblast proliferation [66]. Electrospun collagen polyurethane nanofibrous scaffolds reduced myofibroblasts proliferation [67]. The same group in 2015 showed that elastomeric properties are crucial for proliferation of myofibroblasts. Their results suggested that collagen should be incorporated with polyurethane and PLCL based scaffolds [68]. There is dearth of studies where the hypertrophic scar fibroblasts have been studied on various biomaterials to alter their characteristics. Biomaterials could be crucial in reducing the conversion of fibroblasts to myofibroblasts or reduce the ECM proteins secreted by the hypertrophic scar fibroblasts.

CONCLUSIONS AND FUTURE DIRECTIONS

Unlike areas such as cancer, infectious diseases, and cardiovascular diseases there is limited research being carried out on hypertrophic scars generated from thermal injury. Current therapies such as LASER therapy and flap surgery provide short-term cosmetic solutions, but the scars/contractures reappear along with symptoms such as pain and pruritus [17,18]. If the pathomechanisms of hypertrophic scar formation are investigated, we can identify molecular targets in humans. Newer technologies such as single cell RNA sequencing could be done to identify different cell populations within hypertrophic scar tissue. Screening of several anti-fibrotic drugs could be done using hypertrophic scar derived fibroblasts. Scar fibroblasts need to be investigated for the aberrant functioning of the Hippo pathway mediators (see Figure 2), such as Yap/TAZ, LATS, among others, and this know how could be used to design biomaterials of desired stiffness to discourage excessive fibroblast proliferation post thermal injury. It has also been seen that burn tissue completely disturbs the skin microbiota; hence, whether the altered microbiota affects the remodeling of hypertrophic scar could be investigated. Dedicated research projects aimed to explore various aspects of post-burn hypertrophic scar formation; remodeling and persistence will certainly lead to better understanding and hopefully better treatment options. Once these various lines of research are performed, we might get more insights into the molecular mechanisms of post-burn hypertrophic scar tissue, which will help us determine the objective scales to assess outcomes of various interventions in post-burn hypertrophic scar patients.

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